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GENETIC DIVERSITY ANALYSIS AND DNA FINGERPRINTING OF TOMATO BREEDING LINES USING SSR MARKERS

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ABSTRACT. There is a need to expand the information on genetic relationships between tomato (*Solanum lycopersicum* L.) lines to improve hybridization breeding. The genetic diversity and relationships among 24 tomato lines were evaluated by simple sequence repeat (SSR) markers. A total of 65 bands were generated with 15 SSR primers, of which 64 bands were polymorphic. The mean polymorphic information content was 0.356. There was a high degree of polymorphism between tomato cultivars. The mean marker index and heterozygosity were 0.045 and 0.454, respectively. Cluster analysis grouped cultivars into 6 main clusters. The cvs. Mo. H. P, 'C. C. Orange', and 'Marb' had the greatest genetic distance from other cultivars and is suitable for hybridization to achieve maximum variability for selection in segregating populations. The data can be used to select appropriate parents in tomato hybridization breeding.

Introduction

Various genetic bottlenecks resulting from self-pollination or artificial selection have occurred during the domestication of the cultivated tomato (*Solanum lycopersicum* L.), resulting in a loss of genetic diversity, especially within commercial cultivars (Foolad, Panthee, 2012).

Resistance, organoleptic properties, and variety have all been systematically favoured by domestication at the cost of high yield and efficiency. Extant diversity among tomato species may be a beneficial opportunity for enriching the genetic pool of planted tomatoes with marginalized alleles that could boost productivity and adaptability to challenges (Gur, Zamir 2004; Bai, 2017).

Despite attempts to improve tomato resistance by traditional and biotechnological breeding, findings have been insufficient due to the complexity of responses to different abiotic and biotic stresses. Landraces are diverse species of cultivated plants with specific eco-geographical backgrounds that have been adapted to local climatic conditions as well as conventional management and uses (Casanas *et al.*, 2017). Typically, landraces have evolved under natural and artificial selection in low-input agricultural systems and represent much of the lost diversity (Terzopoulos, Bebeli, 2010; Corrado *et al.*, 2014). Although pathogen resistance genes are usually absent from landraces, they may represent an important genetic diversity reservoir for traits like abiotic stress tolerance in plant breeding (Sacco *et al.*, 2015).

The phenotypic and molecular diversity of cultivated tomato has been investigated (Jin *et al.*, 2019; Kaur *et al.*, 2019). Parental lines for hybrid breeding are shown. Landraces' use in breeding is also limited by a lack of knowledge regarding phenotypic variation and genetic relationships between them, as well as high phenotyping costs (Corrado *et al.*, 2014). A subset of individuals reflecting the conserved diversity must be created in germplasm collections. Advances in whole-genome sequencing facilities, the abundance of several genomic databases, and the availability of a high-quality reference tomato genome sequence (The Tomato Genome Consortium, 2012), offer new possibilities for the development of highly informative molecular markers, overcoming some limitations associated with phenotypic selection. The low genetic diversity of cultivated tomatoes necessitates the use of modern molecular techniques for the discovery of markers able to detect minor variations within tomato germplasm (Foolad, Panthee, 2012).

Molecular markers have been used in tomato for the identification and characterization of numerous genes and Quantitative Trait Loci (QTL) linked to resistance to late blight, leaf mould or tomato spotted wilt virus (Kim *et al.*, 2017; Panthee *et al.*, 2017; Tseng *et al.*,

2016). The QTL analysis of fruit quality traits, including flavour and aroma (volatiles), firmness, vitamins (especially vitamin C and carotenoids) provide information into the genetic control of complex metabolic pathways that contribute to attributes for the market (Causse *et al.,* 2002; Sun *et al.,* 2012).

Several types of molecular markers, including simple sequence repeat (SSR; microsatellites), amplified fragment length polymorphism (AFLP), sequence characterized amplified region (SCAR), and single nucleotide polymorphism (SNP), has been developed and extensively used for the genetic characterization of tomato germplasm collections and in Marker Assisted Selection (MAS) (Bauchet *et al.,* 2017). SSR markers have long been popular due to their high reproducibility, codominance, and polymorphism; however, SNP markers are becoming more popular due to their cost-effectiveness, precision, and suitability for large-scale genotyping and allelic determinations through technologies including High-Resolution Melting (HRM). The value of SSR (Benor *et al.,* 2008; Mazzucato *et al.,* 2008; Sardaro *et al.,* 2013) and SNP (Sacco *et al.,* 2015; Wang *et al.,* 2016) markers in the study of Solanum genetic variation and the genotyping of promising germplasm have been confirmed.

This study was undertaken to assess genetic diversity in 24 breeding lines of tomato using SSR markers to assist in parental selection for hybridization and to avoid the genetic similarities between hybrid pedigrees in future genetic improvement programs for tomato.

Material and methods

Seed of tomato (*Solanum lycopersicum* L.) breeding lines: 'Rose', 'S. G', 'Wis 55', 'Tas. Ch', 'Bran. 21', 'Glacier', 'Red P.t', 'San II', 'A. Pas.', 'German J.', 'Mo.', 'Nepal', 'Red Pear', 'Amish Pa.', 'Pi. Bee', 'B. B.', 'Fr.', 'Mo. H. P', 'C. C. Orange', 'Marb', 'T100S', 'T120S', T125S', T150S', were provided by the Tomato Genetics Resource Center (TGRC) of the University of California, Davis, USA (http://tgrc.ucdavis.edu). The experiment was conducted in a greenhouse at the horticultural gardens of the Department of Horticulture and Landscape Gardening, College of Agriculture, Diyala of University, Baqubah, Iraq. Ten seed/line were directly sown and germinated in 1 L plastic pots, on 10 January 2020, containing a growth medium composed of 45–50% composted pine bark, vermiculite, Canadian sphagnum, peat moss, perlite, and dolomitic limestone.

Plants were thinned to 5 per pot after emergence. Greenhouse temperature was 20–29 °C with a relative humidity of 75–90%. Light intensity was about 9678 lux. Pots were irrigated once every 2 days with 500 mL of distilled water. Each variety was replicated 3 times. No pesticides or additional fertilizer were used during the experiment.

Four weeks after emergence, leaf tissues were sampled from each plant and used to extract genomic DNA for molecular analyses. Genomic DNA extraction from leaves was according to a modified cetyl trimethyl ammonium bromide method (Hwang, Kim, 2000). About 0.5 g of fresh young leaves were powdered in liquid nitrogen. The leaf powder was transferred to a tube containing 0.6 mL of extraction buffer containing 1% of β-mercaptoethanol added just before use. The extract was incubated for 40 min at 60 °C with occasional swirling, mixed with an equal volume of chloroform: isoamyl alcohol (24:1; v:v), and centrifuged at 16,128 relative centrifugal force (rcf) for 10 min at 4 °C. The aqueous phase was transferred to a new tube and mixed with 2/3 volume of ice-cold isopropanol. The mixture was left at –20 °C for 30 min and again centrifuged at 16,128 rcf for 10 min at 4 °C. The pellet was washed with 70% ethanol and air-dried at room temperature for 20 min; the dried pellet was dissolved in 80 µL TrisEDTA (TE) buffer (Tris–hydrochloride buffer, pH 8.0, containing 1.0 mM ethylene diamine tetraacetic acid (EDTA) and stored at –20 °C.

The quality of total DNA was determined with 2% agarose gel electrophoresis and quantified by spectrophotometry. The concentration of extracted DNA for PCR was adjusted to 50 $ng \cdot \mu L^{-1}$. The PCR reaction for tomato lines was conducted using 15 SSR primers (Table 1). Amplification was carried out in 12.5 µL of reaction mix containing 1.5 µL of genomic DNA (50 $ng \cdot \mu L^{-1}$), 0.6 µL of the primer (10 µM), 1.25 µL of 10× reaction buffer, 1 unit of Taq DNA polymerase (5 $U \cdot \mu L^{-1}$), 0.25 mM of each dNTP, and 2.5 mM $MgCl_2$.

The PCR reactions were conducted in a thermal cycler, model AG (Eppendorf, Germany). Amplifications were conducted with an initial denaturation of 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 1 min, annealing at 35 °C for 1 min, and extension at 72 °C for 1 min; followed by 1 cycle of a final extension at 72 °C for 10 mins. The PCR products were separated by electrophoresis on 2% agarose gel. Gene ruler 100 bp DNA ladder plus SM0321 (Fermentas, Lithuania) was used as the standard to determine the size of polymorphic fragments. The DNA fragments were visualized by staining the gel with ethidium bromide and images documented using Gel Doc (Vilber Lourmat, France).

Group method with arithmetic averages (UPGMA). Combined analysis was performed using the dendrogram and Jaccard's coefficient using NTSYS software (Rohlf, 1998). Polymorphism Information Content (PIC) was calculated according to Roldan *et al.* (2000); PIC refers to the value of a marker for detecting polymorphism within a population. Depending on the number of detectable alleles, and distribution of their frequency, it provides an estimate of the discriminating power of the marker.

The SSR polymorphisms in the tomato accessions were measured in terms of numbers of alleles, gene diversity, and PIC using the Power Marker software ver. 3.23 (Liu, Muse, 2005).

Table 1. Primers used with annealing temperature and amplicon band size

Marker identifier	Primer sequence (5' → 3')	Annealing temperature (°C)	Amplicon band size
SSR47	TCC TCA AGA AAT GAA GCTCTG A CCT TGG AGA TAA CAA CCA CAA	58.4	200–1000
SSR63	CCA CAA ACA ATT CCA TCT CA GCT TCC GCC ATA CTG ATA CG	54.3	200–1200
SSR111	TTC TTC CCT TCC ATC AGT TCT TTT GCT GCT ATA CTG CTG ACA	57.4	200–1000
SSR248	GCA TTC GCT GTA GCT CGT TT GGG AGC TTC ATC ATA GTA ACG	59.4	150–700
SSR304	TCC TCC GGT TGT TAC TCC AC TTA GCA CTT CCA CCG ATT CC	60.5	125–500
SSR603	GAA GGG ACA ATT CAC AGA GTT TG CCT TCA ACT TCA CCA CCA CC	61.1	150–700
T-57	GTG GAC CAT TTC AAG TTC AAC A TGA ATG ACA TCC ATC CAT GA	58.4	125–800
TG12-13	GAA AGA GGT AA ATC GCG GGT CCT TTA CGA TTT CGC CTA CG	59.4	200–300
SLM-6-7	CAA TTG AAG ATT GGG GCT TT AGC AGC TCA CCT CAC GTT TT	54.3	225–1000
STI-0012	GAA GCG ACT TCC AAA ATC AGA AAA GGG AGG AAT AGA AAC CAA AA	57.4	200–1200
TMS-42	AGA ATT TTT TCA TGA AAT TGT CC TAT TGC GTT CCA CTC CCT CT	54.0	100–450
TMS-9	TTG GTA ATT TAT GTT CGG GA TTG AGC CAA TTG ATT AAT AAG TT	54.0	125–1000
AI486387.1	ACG CTT GGC TGC CTC GGA AAC TTT ATT ATT GCC ACG TAG TCA TGA	60.7	300–400
STI003	ACC AAT CCA CCA TGT CAA TGC CTC ATG GAT GGT GTC ATT GG	58.4	125–300
Le-tat002	ACG CTT GGC TGC CTC GGA AAC TTT ATT ATT GCC ACG TAG TCA TGA	62.2	100–1000

Results

The PCR amplification using SSR primers resulted in the generation of reproducible amplification products.

Analysis with 9 SSR primers identified a total of 65 reproducible fragments in the tomato cultivars (Table 2). Most bands were produced by SSR63 and the lowest number of bands was obtained by TG 12–13, and A1486387.1.

The numbers of polymorphic bands ranged from 1 for TG 12–13 to 7 for SSR63. Most SSR primers produced 100% polymorphism, the exception was for TG12-13 primers (50%). Of the 15 SSR markers, the overall PIC value ranged from 0.290889 (TG12-13) to 0.497149 (AI486387.1) with an average of 0.3561703. A higher marker index value occurred for AI486387.1 (0.55875) compared to SSR47 (0.003549). The marker index is a feature of a marker that elucidates the discriminatory power of a marker and was calculated for all the markers.

The maximum heterozygosity values of the AI486387.1 codominant marker were 0.55875 in comparison to SSR47 (0.360725).

The genetic similarity matrix, based on Jaccard's similarity coefficients, indicated that the cultivars were genetically similar (Table 3). The highest genetic similarity was related to the 'C. C. Orange' vs. 'Wis 55' or 'San II' or 'Glacier', and 'Mo. H. P' vs. 'San II' and 'Glacier', followed by 'C. C. Orange', vs. 'S. G', and 'C. C. Orange' vs. 'Tas. Ch'. The lowest similarity was for 'San II' vs. 'Red P.t' followed by 'Mo.' vs. 'German J.' (Table 3). In hybridization, crossing cultivars with greater genetic distances are expected to produce more heterosis and desirable recombinants in segregating generations. The average genetic similarity (Table 3) indicated the existence of high levels of diversity among genotypes.

Table 2. Data on primer polymorphism in the diversity of tomato genotypes

Marker identifier	No. of bands	No. of polymorphic bands	Polymorphism %	Polymorphic information content	Marker index	Heterozygosity
SSR47	3	3	100	0.295664	0.003549	0.360725
SSR63	7	7	100	0.336086	0.005513	0.427438
SSR111	5	5	100	0.351488	0.006635	0.455000
SSR248	4	4	100	0.361526	0.012131	0.473741
SSR304	4	4	100	0.366056	0.011935	0.482422
SSR603	4	4	100	0.369627	0.011682	0.489366
T-57	5	5	100	0.373258	0.009482	0.496528
TG12-13	2	1	50	0.290889	0.011347	0.353299
SLM-6-7	5	5	100	0.323648	0.004794	0.406111
STI-0012	6	6	100	0.362349	0.007702	0.475309
TMS-42	4	4	100	0.361526	0.012131	0.473741
TMS-9	5	5	100	0.374374	0.008971	0.498750
AI486387.1	2	2	100	0.497149	0.558750	0.558750
STI003	3	3	100	0.304688	0.011719	0.375000
Le-tat002	6	6	100	0.374227	0.009808	0.498457

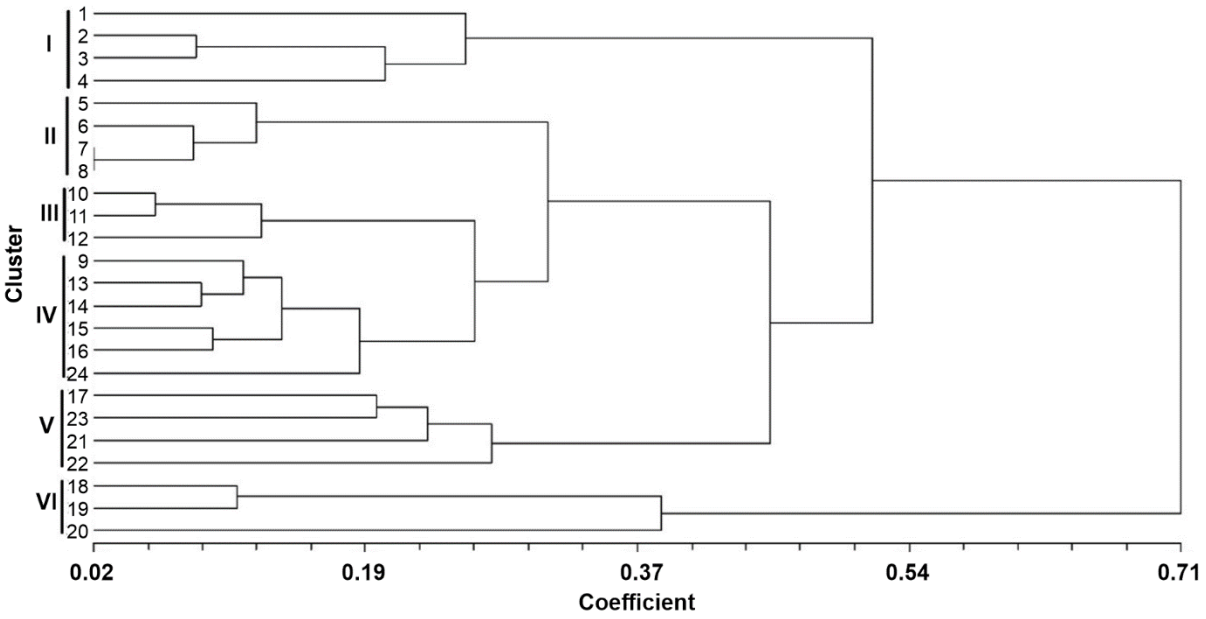
Cluster analysis, based on similarity matrix coefficients using UPGMA, grouped the cultivars into 6 main clusters (Fig. 1). According to the cluster, cvs. 'Rose', 'S. G', 'Wis 55' and 'Tas. Ch' were placed in the same group and cvs. 'Bran. 21', 'Glacier', 'Red P.t' and 'San II' were placed in another group (Fig. 1). The cvs. 'German J.', 'Mo.' and 'Nepal' were placed alone in a separate category and cvs. 'A. Pas.', 'Red Pear', 'Amish Pa.', 'Pi. Bee', 'B. B.', and 'T150S' were placed in

another group. The cvs. 'Fr., T125S', 'T100S', and 'T120S' were placed in the fifth group, and cvs. 'Mo. H. P', 'C. C. Orange', and 'Marb' were placed in a sixth group. Divergent genotypes may have good breeding value. Genotypes in the same cluster may represent members of a single heterotic group.

Table 3. Similarity coefficient matrix of tomato cultivars using SSR markers

Variety	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
2	0.2307																						
3	0.2571	0.0847																					
4	0.2812	0.1698	0.2413																				
5	0.2584	0.3589	0.2771	0.3246																			
6	0.3571	0.4520	0.3589	0.4166	0.1134																		
7	0.2727	0.4026	0.3170	0.3947	0.1287	0.0833																	
8	0.2954	0.4026	0.3170	0.3947	0.1287	0.0833	0.02																
9	0.3580	0.5714	0.5466	0.5652	0.2766	0.2584	0.2258	0.2258															
10	0.5873	0.7692	0.7193	0.8039	0.3947	0.3802	0.4133	0.4133	0.2941														
11	0.5937	0.7735	0.7241	0.8076	0.3766	0.3888	0.3947	0.4210	0.3043	0.0588													
12	0.5625	0.7358	0.6896	0.7692	0.4026	0.3888	0.3684	0.3947	0.2463	0.1372	0.1153												
13	0.3589	0.5522	0.5277	0.5757	0.2527	0.2558	0.2000	0.2000	0.1084	0.2307	0.2121	0.2121											
14	0.3246	0.5151	0.5211	0.5076	0.2666	0.2941	0.2359	0.2359	0.1219	0.3437	0.3230	0.3230	0.0886										
15	0.3600	0.5625	0.5362	0.5873	0.3181	0.3253	0.2413	0.2643	0.1750	0.2580	0.2381	0.2381	0.1168	0.1315									
16	0.4594	0.6190	0.5882	0.6451	0.3103	0.3170	0.3023	0.3023	0.1645	0.2131	0.1935	0.1935	0.1052	0.1466	0.0958								
17	0.4386	0.5652	0.6078	0.5555	0.5428	0.5692	0.4782	0.5072	0.4193	0.4545	0.4666	0.4222	0.3559	0.3448	0.3214	0.3454							
18	0.7674	0.8125	0.8378	0.8064	0.8214	0.8823	0.8181	0.8545	0.7916	0.7333	0.7419	0.6774	0.7777	0.7727	0.7619	0.7561	0.5833						
19	0.8095	0.8709	0.8888	0.8666	0.8545	0.8800	0.8518	0.8888	0.8297	0.7241	0.7333	0.7333	0.8181	0.8139	0.8048	0.8000	0.6521	0.1111					
20	0.5833	0.7297	0.7619	0.7222	0.6721	0.7142	0.6666	0.7000	0.6603	0.5428	0.5555	0.5000	0.6000	0.5918	0.5744	0.5652	0.4482	0.3333	0.4285				
21	0.4098	0.5200	0.5636	0.5102	0.4594	0.4202	0.3972	0.4246	0.3636	0.3750	0.3877	0.3877	0.2698	0.2903	0.3333	0.3220	0.2381	0.7142	0.7037	0.4545			
22	0.5294	0.5500	0.6000	0.5384	0.6562	0.6949	0.6190	0.6507	0.5714	0.6315	0.6410	0.5384	0.5094	0.5000	0.4800	0.4693	0.2500	0.4444	0.5294	0.3913	0.3333		
23	0.5254	0.5416	0.5849	0.5319	0.5555	0.5522	0.4929	0.5211	0.4375	0.4782	0.4893	0.4468	0.3770	0.4000	0.3448	0.2982	0.2000	0.6153	0.6800	0.5483	0.2272	0.2352	
24	0.3600	0.5625	0.5652	0.5555	0.2954	0.3012	0.2413	0.2413	0.2250	0.2903	0.3015	0.3015	0.1428	0.1842	0.1891	0.2054	0.3214	0.7619	0.8048	0.5744	0.3000	0.4800	0.3103

1 = 'Rose', 2 = 'S. G', 3 = 'Wis 55', 4 = 'Tas. Ch', 5 = 'Bran. 21', 6 = 'Glacier', 7 = 'Red P.t', 8 = 'San II', 9 = 'A. Pas', 10 = 'German J', 11 = 'Mo.', 12 = 'Nepal', 13 = 'Red Pear', 14 = 'Amish Pa', 15 = 'Pi. Bee', 16 = 'B. B.', 17 = 'Fr.', 18 = 'Mo. H. P', 19 = 'C. C. Orange', 20 = 'Marb', 21 = 'T100S', 22 = 'T120S', 23 = 'T125S' and 24 = 'T150S'.



Numbers 1 to 24 are 'Rose', 'S. G', 'Wis 55', 'Tas. Ch', 'Bran. 21', 'Glacier', 'Red P.t', 'San II', 'A. Pas.', 'German J.', 'Mo.', 'Nepal', 'Red Pear', 'Amish Pa.', 'Pi. Bee', 'B. B.', 'Fr.', 'Mo. H. P', 'C. C. Orange', 'Marb', 'T100S', 'T120S', 'T125S' and 'T150S' respectively.

Figure 1. Cluster analysis of tomato cultivars using SSR data

Discussion

Sufficient knowledge from the genetic diversity of a crop for the selection of parental materials is essential to maximize genetic improvement. More accurate, and complete, descriptions of genotypes, and genetic diversity patterns, can help determine breeding strategies and facilitate the introgression of diverse germplasm into the current commercial tomato genetic base (Tsivelikas *et al*., 2009).

Molecular markers, which assess genome sequence composition, enable the detection of differences in the genetic information of genotypes and utilize genetic variability for breeding.

The SSRs have been employed to assess genetic diversity within germplasm. In self-pollinating species such as tomato, genetic diversity mainly depends on domestication history and pool size of accessions (Mazzucato *et al.*, 2008). Tomato is generally considered to present low genetic diversity. Landraces

and local populations of tomatoes are thought to have more genetic and phenotypic diversity than commercial cultivars (Park *et al.*, 2004; Mazzucato *et al.*, 2008).

Exploring genetic diversity among tomato accessions is important to breeding and germplasm management (Kanjariya *et al.*, 2017; Gonias *et al.*, 2019). To investigate genetic relationships between the 24 tomato accessions (modern cultivars), 15 SSR loci were selected that have been previously reported to be highly informative in distinguishing tomato genotypes.

Although several different tomato landraces are grown, in the Middle East, exhibiting phenotypic variation, few experiments have been conducted to genetically classify the collections or differentiate them from commercial cultivars. Iraq tomato landraces are believed to have grown in semi-arid environments with low inputs, which may be useful genetic material in productive agricultural systems. Emerging evidence suggests that some of these landraces have high nutritional value. These microsatellites have been verified to be highly polymorphic and able to discriminate different patterns.

The efficiency of a molecular marker system in distinguishing genotypes depends largely upon the polymorphism it can discover (Castellana *et al.*, 2020; Gbadamosi *et al.*, 2020). Based on high polymorphism information content (PIC) values, marker index, and the number of bands (Table 2) the SSR markers used were informative in the assessment of the genetic diversity of tomato accessions. The high PIC values indicate all primers were informative and can be related to high genetic variation among accessions, with similar results previously reported for tomato (Ronga *et al.*, 2018). The variation may have been contributed by gene flow, natural hybridization, propagation by seed and human selection (Choudhary *et al.*, 2018; Gbadamosi *et al.*, 2020).

The heterozygosity and marker index measurements display the distribution and number of alleles (bands) within the genotypes. Bands scored in most genotypes would possess optimal discriminatory power, and with an increase in the number of bands, the heterozygosity of a particular primer pair will be increased (Mazzucato *et al.*, 2008; Ronga *et al.*, 2018; Castellana *et al.*, 2020). Primers with the highest PIC, marker index, and heterozygosity values (AI486387.1) were generally most effective in distinguishing between accessions and could be further used in genetic diversity studies.

Considering time and cost savings, the SSR can differentiate and characterize cultivars useful in tomato breeding. Depending on objectives, potential lines to be selected from different clusters as parents in a hybridization program may be based on genetic distance. The clustering pattern can be used for parent selection for cross-combinations likely to generate the highest possible variability for economic characters.

Conclusions

The highly polymorphic nature of the SSRs used in this analysis was shown, and the existence of a high degree of genetic variation among tomato cultivars was clearly shown.

The discovery of genetic similarities between tomato cultivars makes for more effective germplasm management and use. The results of the SSR analysis showed that each genotype could be distinguished from the others, that the primers were appropriate for tomato germplasm evaluation, and that the SSR marker method was reliable and efficient for identifying tomato cultivars and clonal identification.

The use of well-known divergent genotypes as crossing parents could boost the amount of diversity in a segregating population, which could be beneficial in a tomato development programme.

In the current climate-change scenario, which threatens tomato development, the studied materials could serve as a possible source of genes responsible for widespread adaptation. It may also mean that the observed variety could be exploited by developing a strong crossing programme to produce hybrid cultivars that combine high yield, efficiency, and climate change resilience. The current fingerprint data may be used to build a DNA reference database for the molecular identification of the cultivars in the future breeding program.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

GH – writing a manuscript;
GH, MA – acquisition of data;
GH, AA, MA, NM – analysis and interpretation of data;
GH, MA, AA – critical revision and approve the final manuscript

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IDENTIFICATION OF MATHEMATICAL DESCRIPTION OF THE DYNAMICS OF EXTRACTION OF OIL MATERIALS IN THE ELECTRIC FIELD OF HIGH FREQUENCY

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ABSTRACT. One of the most important stages of the technological process of extraction of target components from oilseeds is extraction. This stage lasts the longest, and therefore, it generally determines the speed and cost-effectiveness of the whole process. A promising direction for effective organization of the extraction process is involving microwave technologies, the use of ultra-high frequency electromagnetic field (EHF) microwave energy directly in the technological process.
The complex nature of the interaction of the factors that determine the intensity of the extraction process in the microwave field does not allow establishing (create) an exact mathematical model of extraction. We propose a method of parametric identification of the mathematical description of the extraction dynamics. This method allows determining the kinetic coefficients of the process from one experimental experiment on the existing installation. A simplified process mechanism was chosen for the study and a general description of the phenomena of heat and mass transfer in a capillary-porous body was used for the known general description. The parameters of the obtained model were identified according to experimental studies. The thus obtained model of non-stationary processes can be used to optimize the parameters and automate the technical means of extraction of oil materials.

Introduction

Analysis of recent research and publications

The main provisions of the theoretical foundations of extraction in the system "solid-liquid" are covered in fundamental works (Romankov, Frolov, 1990; Aksel'rud, Lysjanskij, 1974; Beloborodov, 1999; Perez, *et al.*, 2011), including the use (microwave EMF) of the microwave field (Burdo, 2005; Burdo *et al.*, 2015; Sánchez *et al*., 2019). The theoretical substantiation of the efficiency of the use of the electromagnetic field of ultra-high frequency for the intensification of the intra-volumetric processes of heat and mass transfer, including in the process of extraction of vegetable and oil materials is given in (Burdo, 2005; Sánchez *et al*., 2019; Burdo *et al*., 2017). An analysis of approaches to the mathematical description of the processes of heat and mass transfer in a solid body and under the action of a microwave field is presented in (Sánchez *et al*., 2019; Kotov, Bandura, 2018). Experimental studies of the extraction process of vegetable oil-containing materials confirmed the intensifying effect of the microwave field on the removal of the target component (Sánchez, 2017; Bandura, 2018), which is a significant increase in oil yield. It is established that the physical basis for increasing the yield of the target component is the barofusion component of the process: a sharp increase in the intensity of vaporization and, accordingly, the pressure of the vapour-liquid mixture, which leads to the ejection of the droplet liquid phase from capillaries (microcapillaries) and pores. Thus, it can be assumed that the mechanism of extraction of the target component (oil matter) from the capillary-porous body under the action of the microwave field consists of mass diffusion (which takes into account all types of transfer from capillaries, microcapillaries and pores) and barothermal component (heating, vapor, heat, and steam) and the removal of the vapor-liquid phase from

the capillaries, while the vapor phase condenses on the colder walls of the solid skeleton. Still, analysis of existing model representations showed that even with very simple simplifications, the description in physical phenomena, analytical dependencies that are suitable for practical calculations can only be approximated by experimentally obtained data. So, the most appropriate and promising way of obtaining a mathematical model of the extraction process will be to use the inverse problem method (Alifanov *et al*., 2009) on the basis of experimental information about an object realizable by the Levenberg-McVard algorithm (Burdo, 2010).

Formulation of the problem

Extraction in the dispersed system of vegetable oil materials is widespread in oil production technologies. A promising direction for effective organization of the extraction process is involving microwave technologies, the use of ultra-high frequency electromagnetic field (EHF) microwave energy directly in the technological process.

Due to the imposition of microwave EMF on the interacting phases during the extraction of oil raw materials, it is possible to obtain a concentrated extract, shorten the duration of the technological process and significantly identify it while reducing the specific energy consumption. To determine the rational modes of the process and the parameters of the facilities that implement it, it is necessary to have a mathematical description (mathematical model) that adequately describes the process under study. The complex nature of the interaction of the factors that determine the intensity of the extraction process in the microwave field does not allow establishing (creating) an exact mathematical model of extraction. Therefore, for the study, it is possible to choose a simplified mechanism of the process and to use for its formalization a well-known general description of the phenomena of heat and mass transfer in a capillary-porous body, and to identify the parameters of the obtained model according to experimental studies. The model of non-stationary processes thus obtained can be used to optimize the parameters and automate the technical means of extracting oil materials.

The purpose of the work is to improve the mathematical model of the process of extraction of oil raw materials in the electromagnetic field of ultra-high frequency to intensify its parameters according to experimental studies.

Material and methods

Experimental studies were performed to determine the parameters of microwave processing, which is the highest yield of the target components. Studies on the kinetics of extraction were performed with the rapeseed variety "Ozymyi" (bagasse and whole grain), determining the effect on the process of the following process parameters: the magnitude of the power (N, W) of the pulsed electromagnetic field (IEM field), hydro module extract (the ratio of the mass of the solution to the dry weight of the raw material), temperature (t, ºC), extraction time (τ, s.). The solvents used were C_2H_5OH alcoholic hexane C_6H_{14}. During the experimental studies control devices, equipment and experimental microwave stands, including the development of the authors, were used (Alifanov *et al*., 2009). In a laboratory installation, the power of a microwave amplifier can vary from 0.4 to 1.6 kW. The microwave frequency is 2450 MHz. To study the kinetics of the extraction process, the concentration of the solution was determined. Extraction of the oil from the micelle to determine the concentrations was performed arbitrarily by evaporation of the solvent. To obtain a comparative study of the kinetics of oil extraction from rapeseed, the study was performed in two stages, using the usual method (in the thermostat TS-80) and using microwave radiation. In the usual way, a container with crushed rapeseed bagasse and solvents was placed in a thermostat. The thermostat maintained the temperature inside the vessels at the same level during the extraction process. To study the oil concentration, samples were taken every 10 minutes. They were weighed and placed in a desiccator SPT-200, where the solvent was evaporated from the test sample, and then weighed on an analytical balance VLA-200G-M.

The main elements of the experimental microwave stand (Fig. 1) were the cabinet, as a result of which a microwave field was created and a vessel in which the extraction process itself continued. The rack offered microwave power control. The principle of operation of the experimental stand is as follows: in the vessel with the product 3, the process of extraction was occurring under the influence of a microwave field in the chamber 1.

The extractant vapour enters the reflux condenser 2, condenses and flows back into the reaction vessel together with the test sample and solvent. Using a syringe, five micelles were collected for further study. To ensure the reliability of the results, the experiments were performed in six-fold replication, and in case of significant scattering of results (with a coefficient of variation of more than 15%), the number of repetitions increased to 10. Analytical calculations were performed using software packages for PC: MathCAD 14 M010, Excel 11.0 (Office 2004).

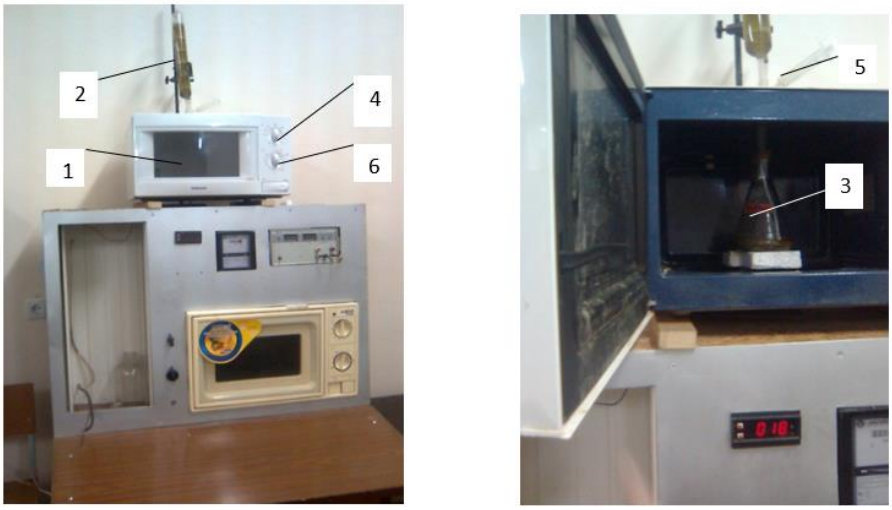

Figure 1. Stand for oil extraction from: 1 – high-frequency microwave camera; 2 – reverse water cooler; 3 – chamber with the product; 4 –power control; 5 – syringe for taking samples; 6 – timer

Results

The total specific mass content of the extractable substance was equal to the sum of the specific mass content of the substance in the liquid phase and as a vapour:

$$u = u_p + u_n, \quad (1)$$

where u – mass concentrations of the extractable substance in the solid (for the liquid phase u_p and a vapour u_n), kg kg_{dm}^{-1}.

As the mass of the extractable substance may have varied due to phase transformation du_f and by transferring the substance by diffusion du_d and due to the transfer of the substance in the droplet in the vapour pressure phase du_p, the following equality holds:

$$du = du_f + du_d + du_n \quad (2)$$

We introduced according to (Burdo *et al.*, 2010) criteria for the phase transformation of the extractable substance ε, ε', according to the ratios:

$$\varepsilon = \frac{du_n}{du}, \varepsilon' = \frac{du_{p-n}}{du} \quad (3)$$

where:

ε – determines the ratio of the substance, which was released in the form of steam, to all the removed substance;

ε' – determines the ratio of the dropping substance, which was removed under the pressure of the formed steam (Lykov, 1971; Burdo, 2010).

There was a relationship between the values ε and ε': $\varepsilon' = (1 - \chi)\varepsilon\ \varepsilon'$ (χ – the criterion of thermomechanical extrusion of the liquid phase).

Then the local rate of change of the mass content of the extractable substance would have been:

$$\frac{du}{d\tau} = \varepsilon\frac{\partial u}{\partial \tau} + \varepsilon'\frac{\partial u}{\partial \tau} + \frac{\partial u_d}{\partial \tau}. \quad (4)$$

Based on the above relations we obtained the differential equation of mass transfer of the extracted substance:

$$\rho_0\frac{du}{d\tau} = a_m\nabla^2 u\rho_0 + \varepsilon\frac{\partial u}{\partial \tau}\rho_0 + \varepsilon'\frac{\partial u}{\partial \tau}\rho_0, \quad (5)$$

where:

ρ_0 – the density of solids, kg m^{3-1};

a_m – the coefficient of mass conduction, $m^2\ s^{-1}$.

In equation (5), the first component of the right part determined the diffusion component of the transfer of the extracted substance, the second was the source of the vapour phase flow and the third was the source of the droplet-liquid flow.

Having transfered the last component to the left part of the Eq. 5 and having replaced the mass concentration with volume ($\rho_0 u = c$) we rewrote equation (5) in the form:

$$\frac{\partial c}{\partial \tau} = D_{ef}\nabla^2 c + \varepsilon\rho_0\frac{\partial u}{\partial \tau}, \quad (6)$$

where c – volumetric concentration of the extractable substance in the solid, kg m^{3-1};

$D_{ef} = D(1 - \varepsilon')$ – effective diffusion coefficient takes into account the transfer of the liquid phase of substances.

Given that the component $\varepsilon\rho_0\frac{\partial u}{\partial \tau} = q_m$ was the source of the flow of matter in the form of steam, Eq. 6 can be written as follows:

$$\frac{\partial c}{\partial \tau} = D_{ef}\nabla^2 c + q_m. \quad (7)$$

The obtained equation determined the dynamics of the extraction process in the presence of an internal source of matter (in the form of steam).

The equation of transfer in a solid capillary-porous body filled with a substance that was extracted in the presence of an internal energy source (in this case, the equation of thermal conductivity of (Eq. 16):

$$\frac{\partial \theta}{\partial \tau} = a_m\nabla^2\theta + r\varepsilon'\frac{1}{c_m}\frac{\partial u}{\partial \tau} + \frac{P_v}{c_m\rho_0}, \quad (8)$$

where:

θ – the temperature of the solid, °C;
r – specific heat of vaporization, J kg^{-1};
c_m – specific heat of the material, J (kg·°C)$^{-1}$;
P_v – capacity of volumetric heat dissipation, W m$^{3-1}$.

By replacing $\frac{du}{d\tau}$ with the obvious equation that followed from the definition of the Kosovich criterion $\left(Ko\frac{rdu}{c_m d\theta}\right), \frac{du}{d\tau} = \frac{c_m}{r}\, Ko\frac{d\theta}{d\tau}$, we wrote Eq. 8 in the form:

$$\frac{\partial\theta}{\partial\tau} = a_e\nabla^2\theta - \frac{q_v}{c\rho_0}, \quad (9)$$

where:
$a_e = a_m(1 - Ko)$;
$q_v = \frac{P_v}{c_m \rho_0}$.

The differential diffusion Eq. 7 in the form:

$$\frac{\partial c}{\partial\tau} = D_{ef}\nabla^2 c + \varepsilon\rho\frac{c_m}{r}\, Ko\frac{\partial\theta}{\partial\tau}. \quad (10)$$

The value $\varepsilon\rho\frac{c}{r}Ko\frac{\partial\theta}{\partial\tau}$ had dimension (kg (m^3 s)$^{-1}$) and characterizes the intra-volumetric steam mass flow initiated by the rate of change of temperature.

To identify the parameters of mass transferred during the extraction process under the action of the microwave field, according to the algorithm (17, 18) it was desirable to have one dependence in the form of the mass conduction equation.

Quantity $q_m = \varepsilon\rho_0\frac{\partial u}{\partial\tau}$ determined the amount of extractable substance vapour emitted per unit time (*i.e.* the rate of pair formation).

The rate of vaporization could have been determined from the equation of thermal balance for the particle mass m_0, which was heated "in contact" with the extractant electromagnetic field of power P. The heat allocated in the volume of the particles of plant material was spent on heating the capillary-porous body, evaporation of the liquid phase and was transmitted by convection to the volume of the extractant:

$$P_m = m_m c_m\frac{\partial\theta}{\partial\tau} + \varepsilon m_0 r\frac{\partial u}{\partial\tau} + \alpha f(\theta - t_p), \quad (11)$$

where:
m_m – the mass of solid fraction, kg;
α, f – coefficient and surface of heat exchange between phases, W (m^2·°C)$^{-1}$ and m^2;
c_m – specific heat of solid phase, J (kg·°C)$^{-1}$;
m_0 – mass of material and solution, kg;
t_p – solvent temperature, °C.

The following equation was written for the volume of the extractant:

$$P_p = V_p\rho_p c_p\frac{\partial t}{\partial\tau} - \alpha f(\theta - t_p), \quad (12)$$

where:
V_p – volume liquid phase, m^3;
ρ_p – density of the liquid phase, kg m$^{3-1}$;
c_p – specific heat of liquid phase, J (kg·°C)$^{-1}$.

Adding Eqs. 11 and 12 we have:

$$P_m + P_p = m_m c_m\frac{d\theta}{d\tau} - m_0 r\frac{du}{d\tau} + m_p c_p\frac{\partial t}{\partial\tau} = N\eta, \quad (13)$$

where:
N – the power source of an electromagnetic emitter, W;
P_m – heat release in the extractable of solid fraction, W;
P_p – heat release in the extractable of the liquid phase, W;
m_m, m_p – mass of solid fraction and liquid, kg;
η – the efficiency of the installation of electromagnetic radiation.

In Eq. 13, the component $m_p c_p\frac{\partial t}{\partial\tau} = P_p'$ was numerically equal to the power of heat dissipation in the volume (mass) of the extractant when neglecting the heat loss to the environment.

Using the replacement of the rate of heating of the body (derivative temperature in time) by the obvious ratio:

$$\frac{d\theta}{d\tau} = \frac{r}{c_m}\, Rb\frac{du}{d\tau}, \quad (14)$$

after the transformations we got:

$$\frac{du}{d\tau} = \frac{Nr - P_p'}{(m_m Rb + \varepsilon m_0)r}, \quad (15)$$

where $Rb = \frac{c_m}{r}\frac{d\theta}{d\tau}$ – Rebinder criteria.

Substituting the value (15) into Eq. 6 we had:

$$\frac{\partial c}{\partial\tau} = D_{ef}\nabla^2 c + \frac{(Nr - P_p')\varepsilon\rho_0}{(m_m Rb + \varepsilon m_0)r}. \quad (16)$$

Thus obtained differential equation of diffusion of the extractable substance in the capillary cell under the action of the microwave field:

$$\frac{\partial c}{\partial\tau} = D_{ef}\nabla^2 c + q_m. \quad (16a)$$

The structure of Eq. 16a was analogous to the equation of thermal conductivity of a body with an internal heat source whose solution is known (Eq. 19).

Setting the initial and boundary conditions of the third kind for the body in the form of an unlimited plate length 2R writing as (if $\tau = 0; \frac{\partial c}{\partial x} = 0; c = c_0; x = R;\ -R < x < +R$) equation:

$$\frac{\partial c(x,\tau)}{\partial\tau} = D_{ef}\frac{\partial^2 c(x,\tau)}{\partial x^2} + q_m,\ -D_{ef}\frac{\partial c(x,\tau)}{\partial\tau} = D_{ef}\frac{\partial^2 c(x,\tau)}{\partial x^2} + q_m, -D_{ef}\left(\frac{\partial c(R,\tau)}{\partial x}\right)_n + \beta(c_p - c) = 0, \quad (17)$$

where β – coefficient of mass transfer, m s^{-1}.

The solution of system (17) if q_m^v = const by analogy with (Eq. 16, 19) was given by:

$$\frac{c(x,\tau) - c_p}{c_0 - c_p} = \frac{Po'}{2}\left(1 - \frac{x^2}{R^2} + \frac{2}{Bi_m}\right) - \sum_{n=1}^{\infty}\left(1 + \frac{Po'}{\mu_n^2}\right) A_n\cos\mu_n\frac{x}{R}e^{-\mu_n^2 Fo_m}, \quad (18)$$

where:

c_0 – initial concentration, kg m$^{3-1}$;

c_p – concentration of the extractable substance in the solvent (extractant), kg m$^{3-1}$;

$Po' = \frac{q_m R^2}{D_{ef}(c_p - c_0)}$ – Pomerantsev criterion, mass transfer;

$Bi_m = \frac{\beta R}{D_{ef}}$ – criterion of Biomass transfer;

$\mu_n = Bi_m ctg\mu_n$ – roots of the characteristic equation;

A_n – coefficient;

$Fo_m = \frac{D_{ef}}{R^2}\tau$ – Fourier criterion.

For the average concentration of the extractable substance:

$$\bar{c}(\tau) = \frac{1}{R}\int_o^R c(x,\tau)\,dx, \tag{19}$$

kinetic dependence was determined by the equation:

$$\frac{\bar{c}(\tau) - c_p}{c_{0-}c_p} = \frac{Po'}{3}\left(1 + \frac{3}{Bi_m}\right) - \sum_{n=1}^{\infty}\left(1 + \frac{Po'}{\mu_n^2}\right) B_n\, e^{-\mu_n^2 Fo_m}, \tag{20}$$

Eqs 18 and 20 indicate:

$$A_n = \frac{2\,sin\,\mu_n}{\mu_n + sin\,\mu_n\,cos\,\mu_n}, B_n = A_n\frac{sin\,\mu_n}{\mu_n}. \tag{21}$$

To determine the change in the average value of the concentration of the extractable substance from the "elementary" particle according to the experiments (extraction kinetics) we used the equation of material balance:

$$V_m(c_0 - \bar{c}) = V_p(c_p - c_{p0}), \tag{22}$$

where:

c_{p0} – initial concentration of the extractable substance, kg m$^{3-1}$;

$V_m = \frac{m_m}{\rho_m}$ – volume of the solid phase, m^3;

$V_p = \frac{m_p}{\rho_p}$ – volume of the liquid phase, m^3.

Using the notion of a hydro module $n = \frac{V_m}{V_p} = \frac{m_m \rho_p}{\rho_m m_p}$ and considering that the initial concentration of the extractable substance (c_{p0}) was zero from Eq. 22 we obtained:

$$\bar{c}(\tau) = \bar{c}_0 - \frac{1}{n}c_p(\tau), \tag{23}$$

where $c_p(\tau)$ was a change in time of concentration of the substance in the extractant, kg m$^{3-1}$.

According to the results of experimental studies, kinetic dependences of the concentration change in the extractant were obtained (Fig. 2).

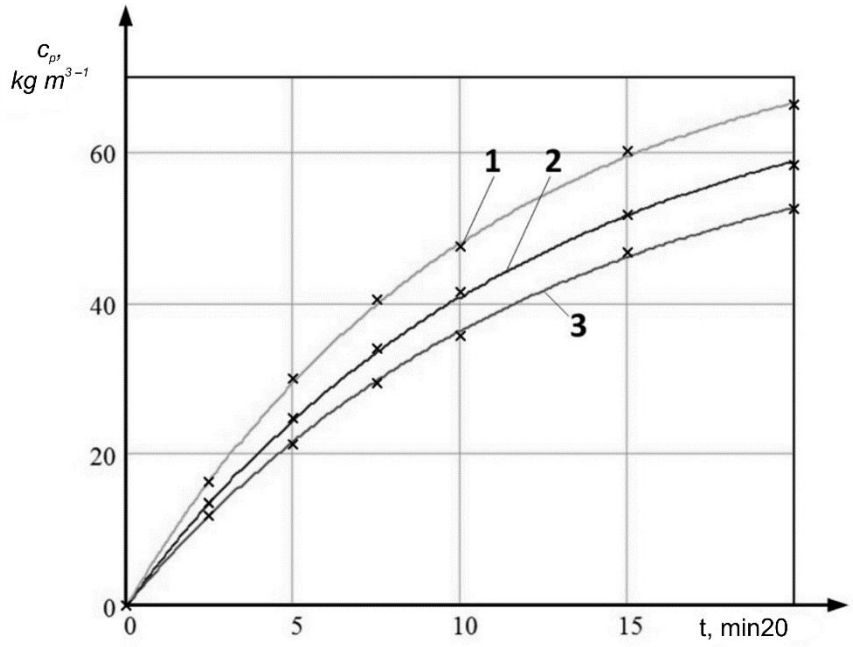

Figure 2. Dependence of oil concentration in solution on time during the extraction of rapeseed meal in the microwave field with power: 1 – 425 W; 2 – 225 W; 3 – 127 W; according to the experiment (x) and the formula (-)

To reduce the influence of random and systematic errors that occur during the experiments on the result of identification (determination of the coefficients of equation (17), (20), the experimental data were approximated by the dependence in the form:

$$\bar{c}_p(\tau) = c_{p\infty}(1 - e^{-k\tau}), \qquad (24)$$

where:

$c_{p\infty}$ was the value of the set (equilibrium) concentration value, kg m$^{3-1}$;

k – extraction coefficient on the extractant, 1 s^{-1}.

The parameters of the experimental kinetics were shown in Table 1.

Table 1. Table of values of coefficients of equation (24)

N, W	$c_{p\infty}$, kg m$^{3-1}$	k, 1 s^{-1}	$t_{p\infty}$, °C
127	66.0	0.080	78.3
225	73.0	0.082	78.3
425	78.3	0.095	78.3

Substituting (Eq. 24) into (Eq. 23), we obtained the equation of the kinetic dependence of the change in the concentration of oil in the seed (rapeseed):

$$\bar{c}(\tau) = c_0 - \frac{1}{n} c_{p\infty}(1 - e^{-k\tau}). \qquad (25)$$

The graphical dependence constructed by the formula 25 is shown in Fig. 3; for microwave field generator power: 127, 225, 425 W.

To identify the diffusion equation with the internal source of the target component according to (18), it was necessary to have information about the initial (approximate) values of the coefficients included in Eqs. 16a and 20. The values of the coefficients D_{ef} and β were also determined from (Eq. 10, 11, 12).

To determine (in the first approximation) the quantitative value of the internal source of liquid phase removal under the action of the microwave field $q_m(N)$ according to the experiment, we used Eq. 20. Restricting ourselves to the first term of the series (as the series converges rapidly (Eq. 19) and making certain transformations according to Lykov (1971), we obtained a simplified equation of the kinetics of the solid body extraction (by the analogy of the drying kinetics) with the source of the liquid phase extraction in the form:

$$-\frac{d\bar{c}(\tau)}{d\tau} = k_e\left(\bar{c}(\tau) - c_p(\tau)\right) - Q_m, \qquad (26)$$

where:

$k_e = \mu_1^2 \frac{R^2}{D_{ef}}$ – extraction coefficient;

$Q_m = q_m \mu_1^2 \left(1 + \frac{3}{Bi_m}\right)$;

$\mu_1^2 = 2.363\, Bi_m - 1.398$;

$\bar{c}_p(\tau) = n(c_0 - c(\tau))$;

$n = \frac{V_m}{V_p}$.

A solution of Eq. 26 under the initial condition: $\tau = 0$; $\bar{c} = c_0$; we got in the form:

$$\bar{c}(\tau) = B - (B - c_0)e^{-k_e(1+n)\tau}, \qquad (27)$$

where $B = \frac{1}{1+n}(nc_0) + \frac{Q_m}{k_e}$.

From the analysis of dependence (Eq. 27) it followed that formally the value was equal to the value of the concentration of the target component of the solid phase in the set mode (finite, equilibrium concentration), the value of which was determined from equation (23):

$$\bar{c}_\infty = \bar{c}_0 - \frac{V_p}{V_m}\bar{c}_{p\infty} = B, \qquad (28)$$

where $\bar{c}_{p\infty}$ – final (established) value of the concentration of the extractant (determined from the graph (Fig. 3) of the kinetics of the extractant), kg m$^{3-1}$.

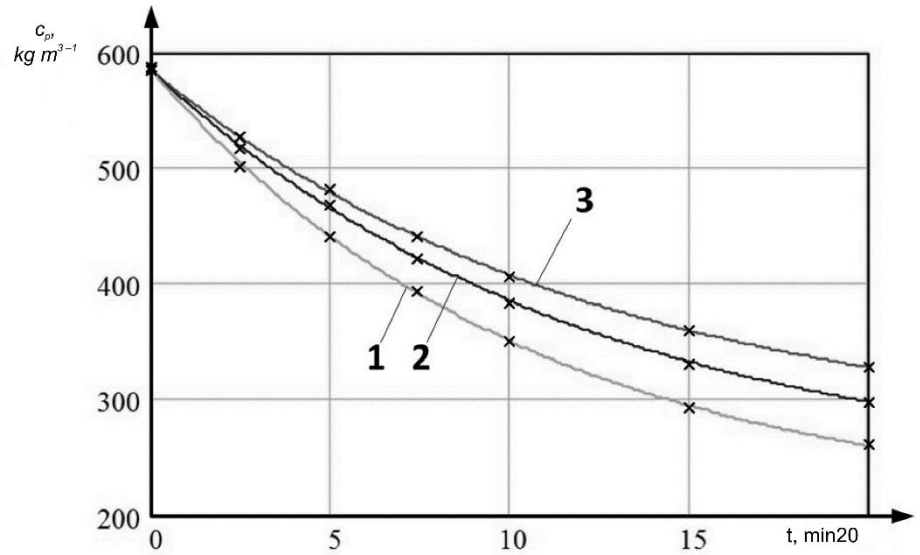

Figure 3. Changing the concentration of rapeseed meal during extraction in alcohol at microwave generator power: 1 – 127 W; 2 – 225 W; 3 – 425 W.

The magnitude $k_e(1+n)$ was the inverse of the transient constant time, which was determined by the exponential curve and could have been estimated from the properties of the exponential (20) (acceleration curves of the dynamic object).

To reduce the influence of random and systematic errors that occur during the experiment on the result of the determination of kinetic coefficients, it was advisable to use the data sets defined by empirical dependencies, in particular (Eq. 24, 25).

To use the Levenberg-McVard algorithm to find unknown mass-exchange coefficients (the value of the internal source of liquid phase removal under the action of the microwave field $q_m(N)$, and the mass-exchange coefficients – β, D_{ef}) in Eq. 20 according to the data obtained from empirical dependence, we first approximated μ_1–μ_5 transcendental characteristic Eq. 21.

For $\mu_1 \ldots \mu_5$, after the numerical experiments with different approximating mathematical structures, the dependence with the highest level of multiple correlations of the form was determined:

$$\mu = b_0 + \frac{Bi_m}{b_1 + b_2 \cdot Bi_m}. \qquad (29)$$

The values of the coefficients of dependence (Eq. 29) were determined in Statistica 10 package and are shown in Table 2.

Table 2. The value of the coefficients of dependence

μ	b_0	b_1	b_2	R (multiple correlation coefficient)
μ_1	0,21	0,8	0,74	0,9998
μ_2	3,12	2,52	0,60	0,9999
μ_3	6,26	4,79	0,57	0,9999
μ_4	9,41	7,33	0,55	0,9999
μ_5	12,55	10,09	0,52	0,9999

Substitute dependence (Eq. 29) instead of Bi_m complex $\frac{\beta R}{D_{ef}}$ and thus determined the dependences $\mu_1 \ldots \mu_5$ substitute for dependence (Eq. 20). Using the built-in genfit function in the Mathcad mathematical package that implements the Levenberg-McWardt algorithm, we determined the values of q_m, β, D_{ef} in which dependence (Eq. 20) would most accurately describe the set of defined data sets by dependency (Eq. 25). The on-screen form of the calculation example was presented in Fig. 4.

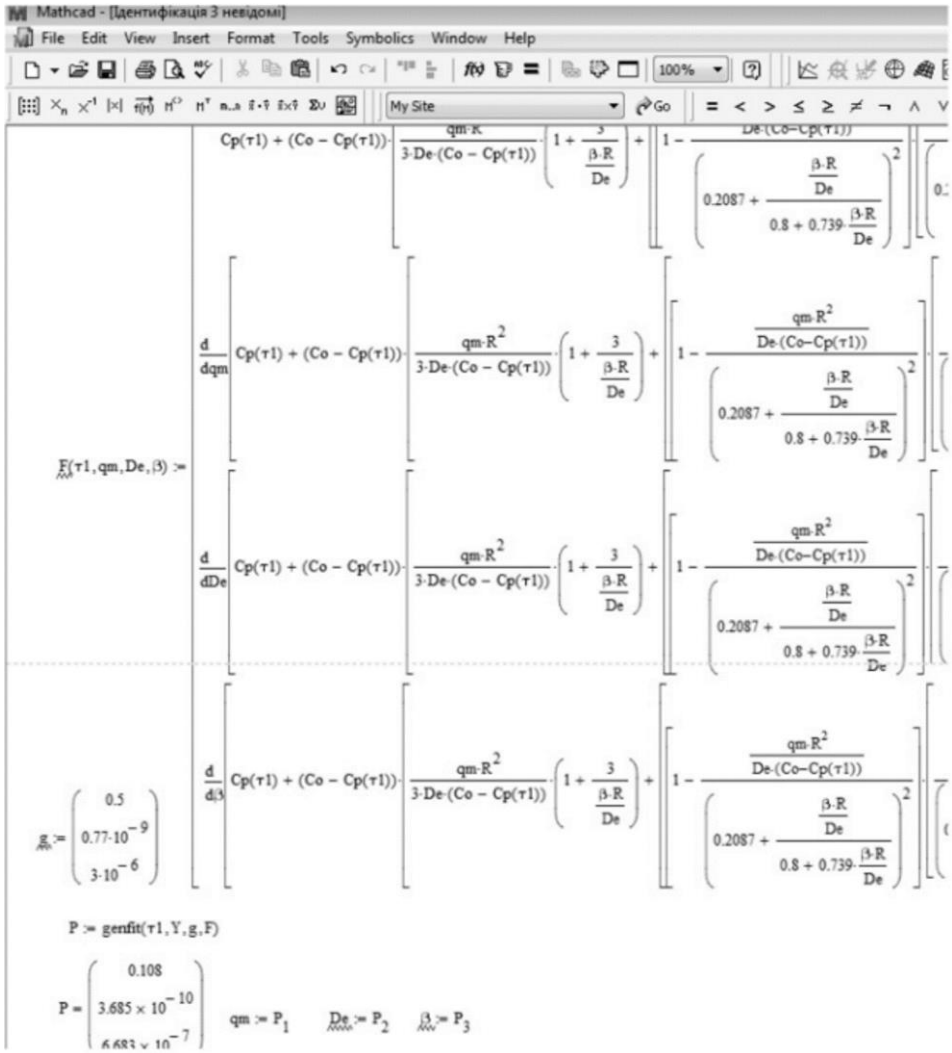

Figure 4. Screen form for determining coefficients in Mathcad mathematical package

In Fig. 5, the points presented were determined by empirical dependence (Eq. 25) and the curves obtained by analytical dependence (Eq. 20) (q_m, β, D_{ef}, determined by the genfit function in MathCAD). In Fig. 6, a comparison was presented.

Thus, the obtained analytical dependence of the change in the concentrations of the substance over time (Eq. 20) in the presence of the values determined as a result of the identification procedure (q_m = 0.108; D_{ef} = 3.685×10^{-10}; β = 6.683×10^{-7}) coincided quite accurately with the experimental dependences (Figs. 4 and 5). This allowed further research to use the known solutions of Eq. 16a with boundary conditions (Eq. 17) to analyze the course of extraction processes in new plants, as well as to determine the rational modes of operation of existing extractors and optimize processes by speed or minimization of energy consumption.

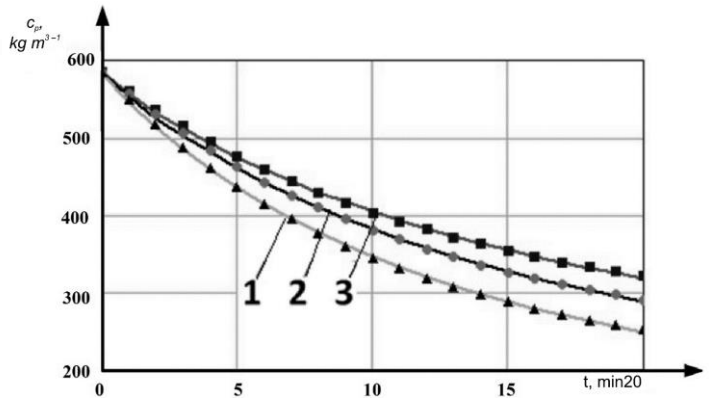

Figure 5. Changing the concentration of rapeseed meal during alcohol extraction at microwave generator power: 1 – 127 W; 2 – 225 W; 3 – 425 W (■, ●, ▲ – empirical points and analytical curves obtained by dependence (20) and identified coefficients)

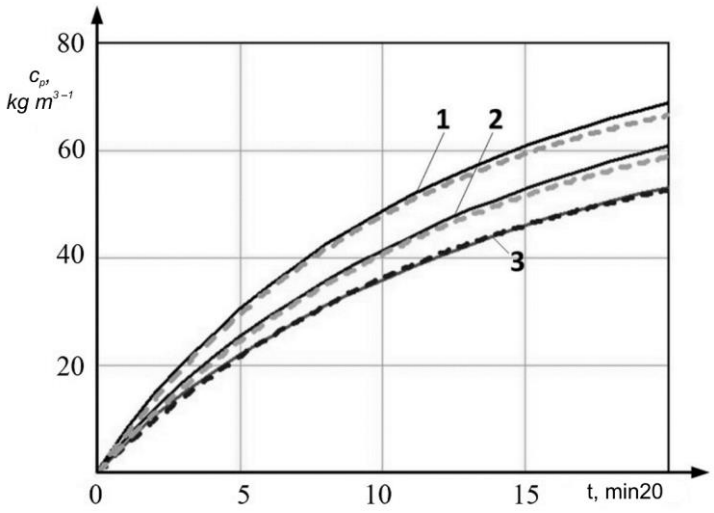

Figure 6. Dependence of oil concentration in solution on time during the extraction of rapeseed meal in a microwave field with power: 1 – 425 W; 2 – 225 W; 3 – 127 W; (-) analytical curves obtained by dependence (20) and the identified coefficients; according to experimental data (- - -)

Conclusions

1. The presented mathematical model of the process of extraction of vegetable raw materials under the action of a microwave field expands the physical understanding of the processes of heat and mass transfer in the system 'solid-liquid', allows quantifying the parameters of the process.
2. The proposed method of parametric identification of the mathematical description of the dynamics of extraction allows determining the kinetic coefficients of the process from one experimental experiment on the current installation.

Conflict of interest

The authors declare that they have no conflict of interest. No funds from the public or private sector were used for this research. The authors covered all expenses.

Author contributions

VB, RK – writing a manuscript;
SG, OL – conducting experimental research;
BK – editing and approval of the completed manuscript;
VG – design and manuscript design.

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KASVUREGULAATORI JA KEVADISE TÄIENDAVA LÄMMASTIK-VÄETISE MÕJU PÕLDTIMUTI (*Phleum pratense* L.) SEEMNESAAGILE JA SEEMNETE KVALITEEDILE

EFFECT OF PLANT GROWTH REGULATOR AND ADDITIONAL NITROGEN FERTILIZATION IN SPRING ON THE SEED YIELD AND SEED QUALITY OF TIMOTHY (*Phleum pratense* L.)

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ABSTRACT. The synergistic effect of the plant growth regulator Moddus 250 EC and nitrogen fertilizer on the seed yield and seed quality of timothy was investigated over a period of four years (2017–2020) in a field trial established with the cultivar 'Tika' in 2016 at the Estonian Crop Research Institute. The trial had three variants: variant 1 – without plant growth regulator (control), variant 2 – plant growth regulator sprayed twice at the rate of 0.4 + 0.4 l ha^{-1} and variant 3 – plant growth regulator sprayed once at the rate of 0.8 l ha^{-1}. In all three variants there were five nitrogen fertilizer rates between N 70–140 kg ha^{-1}. In the trial the lodging resistance of plant cover was monitored, the height of generative tillers was measured, the seed yield was determined by two-phase combine harvesting and the quality of seed was determined. No lodging of timothy was detected in trial variants throughout all trial years. The increase of nitrogen fertilizer rate did not reliably affect the height of generative tillers, the split application of plant growth regulator shortened the height of generative tillers on the average of four years by 7.4%, and one-time spraying by 6.2%. The use of plant growth regulator did not increase the seed yield, the split application of it even reduced the seed yield. The increase of nitrogen fertilizer rate up to N 122 kg ha^{-1} increased the seed yield reliably only in the first year of production, but not in the following years. The use of plant growth regulator slowed down seed maturation, in our trials it was confirmed by bigger amounts of seed in the second harvest phase. The increase of nitrogen fertilizer rate and the use of plant growth regulator did not have any effect on seed germination. In the first production year, the 1000 kernel weight increased under the influence of plant growth regulator, in the later years there was no effect. The germination energy of seed somewhat decreased under the influence of plant growth regulator.

Sissejuhatus

Kõrreliste heintaimede seemnesaagi suurendamise üheks võtmeküsimuseks on lämmastikväetiste kasutamine. Lämmastik omab positiivset efekti fotosünteesile ja sellega kaasnevale taimekasvu produktiivsusele. Suurema lämmastikväetise normiga võib kaasneda generatiivvõrsete pikenemine, mis omakorda võib viia taimiku lamandumisele. Lamandumine on kõrreliste seemnekasvatuses oluline saagi vähenemise põhjus. Selle tõttu võib saamata jääda kuni 60% seemnesaagist (Griffith, 2000). Just lamandumise ohu tõttu on kõrreliste heintaimede seemnekasvatuses kasutatavad kevadised lämmastikukogused limiteeritud. Seisukindluse parandamiseks on hakatud kasutama kasvuregulaatoreid. Kasvuregulaatorite kasutuselevõtt võimaldab lamandumise ohtu vähendada ja lämmastikukoguste suurendamise läbi seemnesaaki tõsta (Young jt, 2007).

Üheks kasvuregulaatoriks, mis kõrreliste heintaimede juures kasutamist leidnud, on Moddus 250 EC, mille toimeaineks on etüültrinexapak. Viimane inhibeerib taimes giberreliinhappe biosünteesi, soodustab juurekava arengut, kõrreseina tugevnemist, kõrre muutumist jämedamaks ja lühemaks (Rademacher, 2000).

Kõrreliste heintaimeliikide ja sortide seisukindlus on erinev. Mida tagasihoidlikum on liigi seisukindlus, seda suurem on üldjuhul preparaadi kasutamisest tulenev positiivne efekt. Austraalias (Trethewey jt, 2010) ja Uus-Meremaal (Chynoweth jt, 2010) saadi katsetes selle preparaadi toel (koos lämmastikväetise lisamisega) 30–50% kõrgem karjamaa-raiheina seemnesaak. Sarnase suurusjärguga edu on kaasnenud Uus Meremaal ka itaalia raiheina seemnekasvatuse katsetes (Trethewey jt, 2016). Tehti kindlaks tugev positiivne korrelatsioon loomisjärgsete, lamandumisele eelnevate päevade arvu ja hilisema seemnesaagi suuruse vahel (Rolston jt, 2010).

Eeltoodutest parema seisukindlusega heintaimeliikidel on jäänud kasvuregulaatori kasutamise positiivne efekt väiksemaks. On kindlaks tehtud, et positiivse efekti ulatus võib liigiti olla erinev (Machač, 2013) või koguni puududa (Aamlid, 2003). Erinev võib olla mõju sama liigi sortidelegi (Rolston jt, 2005; Butler, Affeldt, 2010). Üksikjuhtudel on täheldatud preparaadi toimel seemnesaagi langust (Young jt, 2013). Eestis ei ole seni teaduskatsetes kasvuregulaatori mõju heintaimede seemnekasvatuses uuritud.

Käesoleva uurimistöö eesmärgiks oli selgitada võimalusi põldtimuti seemnepõllul preparaat Moddus 250 EC kasutamisel ja kevadise lämmastikväetise normi suurendamise toel tõsta seemnesaaki.

Materjal ja metoodika

Uurimistöö toimus aastatel 2017–2020 Eesti Taimekasvatuse Instituudis 2016. aasta juulis põldtimuti sordiga 'Tika' mustkesale rajatud lapikatses. Katse külvati normiga 8 kg ha^{-1}, külvik Hege 80, kordusi neli. Tegemist oli leostunud mullaga K_0, mille agrokeemilised näitajad olid järgmised: pH $_{KCl}$ 6,4, C_{org} 1,7%, üldlämmastiku sisaldus 0,19%, ülejäänud toiteelementide sisaldus künnikihist võetud keskmises proovis P 90, K 113, Ca 2041, Mg 116, Cu 1,6, Mn 118 ja B 0,82 mg kg^{-1}. Huumushorisondi tüsedus 23–25 cm, lõimis liivsavi. Katseala sai enne rajamist kompleksväetist EU Fertilizer normiga 300 kg ha^{-1}. Väetise toitainesisaldused: N 21%, P_2O_5 6%, K_2O 11%, S 3,6%. Hilisematel katseaastatel fosfor-kaaliumväetisi ei kasutatud. Kevadeti, vegetatsiooniperioodi algul, sai katseala lämmastikku ammooniumsalpeetrina normiga N 70 kg ha^{-1}, põldtimuti kõrsumise algul väetati taimikuid täiendavalt vastavalt katseplaanile (tabel 2). Väetamine oli katses kombineeritud kasvuregulaatori kasutamisega. Katses oli kolm kasvuregulaatori kasutamise varianti: variant 1 – kontroll, kasvuregulaatorit ei kasutatud; variant 2 – kasvuregulaatoriga pritsiti kahel korral: kõrsumise algul (BBCH 31) normiga 0,4 l ha^{-1} ja loomise algul (BBCH 51) normiga 0,4 l ha^{-1}; variant 3 – kasvuregulaatoriga pritsiti üks kord kõrsumise algul (BBCH 31) normiga 0,8 l ha^{-1}. Variantide valikul lähtuti preparaadi kasutamisjuhistest. Variandid olid ruumiliselt eraldatud 3 m laiuste vaheteedega.

Loomise algul pritsiti katseala timutikärbse tõrjeks preparaadiga Fastac, kulunorm 0,2 l ha^{-1}. Põldtimuti õitsemise järel mõõdeti generatiivvõrsete kõrgused (katselapi kohta kuus mõõtmist), korduvalt hinnati taimikute lamandumist. Seemnesaak koristati kombainiga Hege 140 kahefaasiliselt (v.a 2017. aastal), kahe kombainimise vahel vahe 5–6 päeva. Seemnesaak kuivatati dineesentüüpi kuivatis ja puhastati Kamas-Westrupi firma laboratoorsete masinatega. Seemnete kvaliteet määrati laboratooriumis ISTA metoodika järgi (International..., 1993) esimesel koristusfaasil saadud seemnest kolm kuud pärast kombainimist.

Katseandmete matemaatilisel töötlemisel kasutati arvutiprogrammi AGROBASE 20™.

Katse läbiviimise ajal esines kaks ilmastikuoludelt erandlikku aastat. 2017. aasta oli paljude aastate keskmisele lähedaste sajuhulkadega kuid jahedam (tabel 1). Efektiivse temperatuuri kasvav summa augusti lõpuks oli vaid 1098 °C (norm 1259 °C), mistõttu seeme valmis tavapärasest 10 päeva hiljem – augusti II dekaadi lõpuks. 21. augustil alanud jahe vihmane periood ei võimaldanud saaki õigeaegselt kombainiga koristada. Ülevalminud seemnesaak õnnestus katselappidelt koristada 27. augustil ühefaasiliselt.

Tabel 1. Saagiaastate ilmastikuandmed
Table 1. *Weather data for harvest years*

	Aprill / *April*	Mai / *May*			Juuni / *June*			Juuli / *July*			August / *August*		
	III	I	II	III	I	II	III	I	II	III	I	II	III
					Õhutemperatuur / *Air temperature* °C								
2017	3,2	5,7	9,5	13,2	11,9	14,8	13,4	13,8	14,8	16,1	17,3	17,5	13,1
2018	6	11,1	16,1	16,2	13,4	16,8	14,8	15,6	21,7	23,2	21,1	17,3	15,6
2019	10,6	6,1	12	13,3	18,8	18	16,7	13,9	14,7	18,8	14	16,4	16,6
2020	4,7	9,4	5,7	11,9	14,6	19,3	19,8	15,4	16,1	15,5	17,7	15,9	14,8
Norm	7,4	9,6	10,7	12,4	14	14,2	15,5	16,7	17,4	17,2	17,1	15,6	14,3
					Sademed / *Precipitation*, mm								
2017	46	4	1	4	25	20	33	8	45	4	22	37	34
2018	22	0	3	5	5	7	11	10	5	0,5	31	27	15
2019	0	10	19	21	15	34	6	21	8	5	17	22	10
2020	14	3	23	1	71	27	37	25	6	70	27	5	51
Norm	9	12	18	19	17	34	29	22	27	27	34	26	32

2018. aasta taimekasvuperiood oli eelmisega võrreldes veelgi ekstreemsem. Väga nappide sademetega kaasnes paljude aastate keskmisest kõrgem õhutemperatuur. Efektiivse temperatuuri kasvav summa augusti lõpuks (1547 °C) ületas paljude aastate keskmist 288 °C. Põldtimuti seemnesaak valmis tavapärasest *ca* 10 päeva varem ja koristamist sai alustada 25. juulil. Sügiseni väldanud niiskusepuuduse tõttu oli taimede võrsumine sel aastal takistatud, taimik hõrenes, mille negatiivne järelmõju küündis järgmiste aastate seemnesaakidessegi.

Katseaasta 2019 oli ilmastikuoludelt paljude aastate keskmisele lähedane. Katseaasta 2020 eristus juuli III dekaadi ja augusti I dekaadi rohkete sademetega, mille tõttu seemnete idanevus sel aastal jäi eelmiste aastatega võrreldes madalamaks.

Katsetulemused ja arutelu

Mõju generatiivvõrsete pikkusele ja taimiku seisukindlusele

Põldtimut on keskmise seisukindlusega liik. Katseandmete ja tootmispraktikast pärit kogemuste alusel on liikidel välja kujunenud optimaalsed lämmastikväetise normid, mis on seisukindluse ja seemnesaagi seisukohalt meie ilmastikutingimustes end õigustanud. Põhjamaades (sh Eestis) on põldtimutil õigustanud end kevadel, kasvu algul antava lämmastikväetise norm N 50–70 kg ha^{-1}, millele lisatakse kõrsumise algul veel N 20–35 kg ha^{-1} (Niemeläinen, Järvi, 1995; Aamlid, 1997a, 1997b; Wallenhammar, 1998; Havstad, 2003; Havstad, Aamlid, 2006; Bender, 2016). Meie katses oli üheks eesmärgiks kombata lamandumise ohtu lämmastikväetise normi suurendamisel kombineerides seda kasvuregulaatori kasutamisega.

Põldtimuti generatiivvõrsete pikkus katses sõltus katseaastast ja selle ilmastikuoludest. Jahedal, normile lähedaste sademetega 2017. ja 2020. aastal oli seemnetaimik kõrgeim küündides kontrollvariandis keskmiselt 105–106 sentimeetrini (tabel 2). Sügava põua tingimustes jäid 2018. aastal generatiivvõrsed *ca* 20 cm madalamaks. Kõrsumisfaasi algul antud lisa lämmastikväetise ja isegi selle normi suurendamisega ei kaasnenud generatiivvõrsete pikkuskasvu usutavat suurenemist. Täiendava lämmastikväetise normi suurendamine N 70 kg-ni ha^{-1} põhjustas kontrollvariandis isegi mõningast generatiivvõrsete pikkuse vähenemist. Aastatel 2017 ja 2018 oli vähenemine seejuures ka statistiliselt usutav.

Kasvuregulaatori kasutamine lühendas usutavalt põldtimuti generatiivvõrseid kõigil katseaastail. Mõju generatiivvõrsete pikkusele sõltus katseaastast (tabelid 2 ja 3). 2017. a esimesel taimiku kasutusaastal oli mõju kõige suurem, küündides 9–10 sentimeetrini (8,6–10,1%). Järgnevatel aastatel lühendas pritsimine preparaadiga Moddus 250 EC põldtimuti generatiivvõrseid 5–6 cm (4,7–7,0%). Preparaadi jaotatud (0,4 + 0,4 l ha^{-1}) kasutamine ei omanud ühekordse (0,8 l ha^{-1}) kasutamise ees nimetamisväärset eelist. Mõju erinevus jäi katseaastate keskmisena vea piiresse.

Meie katses põldtimuti seemnetaimik ühelgi aastal üheski katsevariandis ei lamandunud. Nelja katseaasta jooksul esines seejuures kaks ekstreemset juhtu, mis lausa oleks pidanud lamandumist põhjustama. Esimesel kasutusaastal (2017) esines 12. augustil üleeestiline torm, mis lõhkus hoonete katuseid ja murdis puid. Jõgeva piirkond kannatas selle tormi tõttu kahju suhteliselt vähe, väike oli kahjustus ka käsitletaval katsel. Vaid kontrollvariandis olid taimikud tuulekeerise ja sajuhoogude tõttu sasitud ning koolutatud, kuid tõusid kombainimise ajaks püsti tagasi. Taimikut säästis asjaolu, et hilises arengujärgus on kõrs juba puitunud ning tormi mõjudele vastupidavam. Teise, üle Eesti suurt kahju tekitanud tormi elasid katse taimikud üle 11. juulil 2020. Jõgeval olid siis tugevad sajud koos rahega, mis põhjustasid massilist lamandumist teraviljadel ja heinaseemnepõldudel. Põldtimuti taimik oli käsitletavas katses kõikides variantides küll ühtlaselt kooldunud, kuid tõusis pärast saju lõppu jälle endisesse asendisse. Sel korral võis kaasa mõjuda taimiku hõredus, mis oli 2018. aasta suure põua tagajärg. Hõre taimik ongi üldjuhul seisukindlam.

Tabel 2. Lämmastikväetise täiendava annuse mõju põldtimuti generatiivvõrsete kõrgusele, cm
Table 2. *Effect of additional nitrogen fertilizer dose on the height of generative tillers of timothy, cm*

Variant	N kg ha^{-1}	Aasta / *Year*				Keskmine
		2017	2018	2019	2020	*Mean*
Variant 1	N 70	105,5^{a}*	88,5^{a}	101,4^{a}	108,9^{a}	101,1^{a}
Kontroll	N 70 + N 17	105,3^{a}	86,4^{a}	100,4^{a}	105,8^{a}	99,5^{a}
Control	N 70 + N 35	104,3^{ab}	86,0^{a}	96,5^{a}	108,6^{a}	98,9^{a}
	N 70 + N 52	105,0^{ab}	87,8^{a}	100,5^{a}	105,3^{a}	99,7^{a}
	N 70 + N 70	100,7^{b}	81,4^{b}	97,9^{a}	104,3^{a}	96,1^{b}
	PD / *LSD* 0,05	4,4	3,4	6,3	4,9	2,5
Variant 2	N 70	92,3^{a}	84,0^{a}	93,0^{ab}	102,9^{a}	93,1^{a}
Moddus 250 EC	N 70 + N 17	94,0^{a}	80,0^{ab}	91,5^{b}	100,1^{ab}	91,4^{ab}
0,4 × 0,4 l ha^{-1}	N 70 + N 35	93,7^{a}	79,9^{ab}	93,9^{a}	99,5^{ab}	91,8^{ab}
	N 70 + N 52	94,7^{a}	76,7^{b}	92,4^{ab}	97,8^{b}	90,4^{b}
	N 70 + N 70	93,3^{a}	78,5^{b}	94,3	99,8^{ab}	91,5^{ab}
	PD / *LSD* 0,05	2,5	4,2	2,3	3,4	1,4
Variant 3	N 70	96,5^{ab}	77,7^{a}	92,9^{c}	102^{ab}	92,3^{a}
Moddus 250 EC	N 70 + N 17	97,5^{a}	78,8^{a}	98,3^{a}	99,8^{b}	93,6^{a}
0,8 l ha^{-1}	N 70 + N 35	91,5^{b}	81,5^{a}	96,6^{b}	100,7^{b}	92,6^{a}
	N 70 + N 52	92,5^{b}	77,7^{b}	94,3^{c}	104,2^{a}	92,2^{a}
	N 70 + N 70	97,7^{a}	80,8^{a}	96,2^{b}	100,8^{b}	93,9^{a}
	PD / *LSD* 0,05	4,7	4,6	1,6	2,7	2,8

* Samas tulbas sama tähega tähistatud arvandmed ei erine usutavalt (PD test, P>0.05) (siin ja järgnevates tabelites)
** Means followed by the same letter in the same column are not significantly different (LSD test, P>0.05) (Here and in the following tables)*

Tabel 3. Kasvuregulaatori mõju põldtimuti generatiivvõrsete kõrgusele, cm
Table 3. *Effect of growth regulator on height of timothy generative shoots, cm*

Variant	2017	2018	2019	2020	Keskmine *Mean*
Variant 1	104,1[a]	85,9[a]	99,3[a]	106,5[a]	99,0[a]
Variant 2	93,6[b]	79,9[b]	93,0c	100,1[b]	91,5[b]
Variant 3	95,1[b]	79,3[b]	95,7[b]	101,5[b]	92,9[b]
PD / *LSD* 0,05	2,2	2,3	1,9	1,6	2,3

Täiendava lämmastikväetise ja kasvuregulaatori koosmõju seemnesaagile

Esimesel saagiaastal (2017) andis põldtimut kontrollvariandis seemnesaagi vahemikus 537–676 kg ha^{-1} (tabelid 4, 5). Täiendava lämmastikväetise annuse suurendamine kuni normini N 52 kg ha^{-1} suurendas usutavalt seemnesaaki. Edasine lämmastikväetise normi suurendamine viis seemnesaagi langusele. Katsevariandis 2, kus kasutati kasvuregulaatorit jaotatult, lisa lämmastikväetis seemnesaaki ühelgi katseaastal ei suurendanud. Lämmastikulisa N 70 kg ha^{-1} viis ka siin seemnesaagi drastilisele langusele. Parimaid tulemusi saadi 2017. aastal variandis 3. Ühekordne pritsimine kasvuregulaatoriga Moddus 250 EC (norm 0,8 l ha^{-1}) kõrsumise algul võimaldas lämmastiku normi tõstmisega saavutada usutavalt suuremaid seemnesaake, aga selleski variandis kuni normini N 52 kg ha^{-1}. Lämmastikväetise normi edasine suurendamine viis saagi langusele. Ka Soomes põldtimutiga läbiviidud katses on täheldatud sama: kevadisele põhikogusele antud lisa-lämmastikukogus N 60 kg ha^{-1} viis seemnesaagi langusele (Taalas jt, 2011).

Põuasel 2018. aastal jäi täiendava lämmastikväetise mõju tagasihoidlikuks, usutav enamsaak saamata. Põua tõttu seemnetaimikud kõigis katsevariantides hõrenesid. Hõrenenud taimikutel lisa lämmastikväetis 2019. ja 2020. aastal seemnesaagi tõusu ei taganud ja seda mitte üheski katsevariandis. Variandis 2, kus kasvuregulaatorit kasutati jaotatult kahes osas, saadi 2019. aastal lisaväetamisega koguni negatiivne tulemus – seemnesaak vähenes usutavalt.

Tabel 4. Lämmastikväetise täiendava annuse mõju põldtimuti seemnesaagile (esitatud I ja II koristusfaasi summa), kg ha^{-1}
Table 4. *Effect of additional nitrogen fertilizer dose on the seed yield of timothy (presented as sum total of the first and second harvest phases), kg ha^{-1}*

Variant	N kg ha^{-1}	Aasta / *Year*				Keskmine
		2017	2018	2019	2020	*Mean*
Variant 1	N 70	537,5[b]	311,3[ab]	423,7[a]	358,0[a]	407,6[b]
Kontroll	N 70 + N 17	584,8[b]	309,6[ab]	388[ab]	353,8[a]	409,1[b]
Control	N 70 + N 35	630,1[ab]	298,4[b]	383,5[b]	359,0[a]	417,8[b]
	N 70 + N 52	675,8[a]	336,9[a]	420,1[a]	380,0[a]	453,2[a]
	N 70 + N 70	592,7[ab]	333,4[ab]	404,1[ab]	388,2[a]	429,6[ab]
	PD / *LSD* 0,05	88,0	35,0	37,5	51,9	32,0
Variant 2	N 70	600,1[a]	318,7[a]	390,0[a]	356,3[a]	416,3[a]
Moddus 250 EC	N 70 + N 17	609,0[a]	292,3[ab]	337,6[b]	337,0[a]	394,0[ab]
0,4 × 0,4 l ha^{-1}	N 70 + N 35	601,5[a]	255,9[b]	284.0[c]	324,2[a]	366,0[b]
	N 70 + N 52	595,9[a]	250,5[b]	292,1[bc]	306,9[a]	361,4[b]
	N 70 + N 70	446,4[b]	316,4[a]	335,3[b]	303,9[a]	350,5[b]
	PD / *LSD* 0,05	73,2	38,4	51,0	53,7	32,0
Variant 3	N 70	544,6[c]	300,4[b]	347,5[b]	345,3[a]	384,5[b]
Moddus 250 EC	N 70 + N 17	640,1[b]	308,5[b]	353,9[b]	364,8[a]	416,8[ab]
0,8 l ha^{-1}	N 70 + N 35	668,3[ab]	302,7[b]	362,2[ab]	327,2[a]	415,1[ab]
	N 70 + N 52	756,7[a]	307,8[b]	352,9[b]	331,0[a]	437,1[a]
	N 70 + N 70	573,7[bc]	360,2[a]	383,0[a]	342,8[a]	414,9[ab]
	PD / *LSD* 0,05	93,9	43,2	23,5	47,6	38,0

Katseaastatel valitses probleem, et juuni II dekaadi lõpul, mil põldtimut oli jõudnud arenguga loomise algusesse, pärssis taimekasvu põud. Kevadistest hilistest öökülmadest või taimekasvuaegsest põuast tingitud stressis taimikutel aga ei soovitata seda preparaati kasutada. Võib anda negatiivse tulemuse. See võiski olla meie katses 2019. aastal saagilanguse põhjuseks. Ka Soomes on kogetud, et Moddus 250 EC jaotatud andmine (0,4 + 0,4 l ha^{-1}) ei taganud mõnel aastal ühekordse pritsimisega (0,7 l ha^{-1}) võrreldes põldtimutil seemnesaagi lisa (Niskanen, 2011). Junnila (1998) on leidnud, et põldtimuti puhul on Moddus 250 EC kasutamine tulemuslikum, kui ühekordne pritsimine toimub varasemas arengufaasis. Samale järeldusele on jõutud katsetes hariliku aruheina (Junnila, 2004), karjamaa-raiheina (Borm, Berg, 2008) ja punase ristikuga (Anderson jt, 2012, 2015)

Tabel 5. Kasvuregulaatori mõju põldtimuti seemnesaagile, kg ha^{-1}
Table 5. *Effect of growth regulator on timothy seed yield, kg ha^{-1}*

Variant	2017	2018	2019	2020	Keskmine *Mean*
Variant 1	604[ab]	317,8[a]	403,9[a]	367,7[a]	423,4[a]
Variant 2	570,6[b]	286,8[b]	327,8c	325,7[b]	377,7[b]
Variant 3	636,7[a]	315,7[a]	360[b]	342,2[b]	413,7[a]
PD / *LSD* 0,05	38,3	20,9	24,3	23,5	17,1

Varasematest töödest on teada, et preparaat Moddus 250 EC aeglustab taimede arengut ja seemnete küpsemist. Seepärast soovitatakse Soomes pritsitud põldtimuti seemnepõlde koristada pritsimata põldudega võrreldes 2–3 päeva hiljem (Niskanen, 2011). Norras soovitatakse normaalse seemnete idanevuse kindlustamiseks koristusaja vaheks 3–5 päeva (Aamlid, Øverland, 2016). Et neid soovitusi Eesti oludes kontrollida, rakendati katses kahefaasilist koristamist 5–6 päevase vahega. Teise koristusfaasiga saadud seemnesaagi osatähtsus näitab, et kõigil katseaastail

küpses seeme kasvuregulaatorit kasutatud variantides aeglasemalt (tabel 6). Meie katse variantides 2 ja 3 oli II koristusfaasiga saadud seemne osatähtsus kogu seemnesaagist *ca* 7% suurem, kui pritsimata variandis. Variantide 2 ja 3 vahel nimetamisväärset erinevust ei täheldatud.

Tabel 6. Teise koristusfaasi osa seemnesaagis, %
Table 6. *Part of the second harvest phase in seed yield, %*

Variant	N kg ha^{-1}	2018	2019	2020	2018–2020 keskmine / *mean*
Variant 1	N 70	14,7	36,4	18,5	23,2
Kontroll	N 70 + N 17	14,2	39,3	23,4	25,6
Control	N 70 + N35	10,6	42,2	13,3	22,0
	N 70 + N 52	10,7	41,3	26,5	26,2
	N 70 + N 70	20,3	49,5	25,9	31,9
	Keskmine / *Mean*	14,2	41,7	21,8	25,9
Variant 2	N 70	18,1	47,3	25,1	30,2
Moddus 250 EC	N 70 + N 17	21,1	50,0	26,0	32,4
0,4 × 0,4 l ha^{-1}	N 70 + N35	20,3	55,7	25,9	34,0
	N 70 + N 52	22,2	51,8	26,1	33,4
	N 70 + N 70	25,5	56,3	23,6	35,1
	Keskmine / *Mean*	21,4	51,9	25,4	32,9
Variant 3	N 70	18,4	58,2	32,9	36,5
Moddus 250 EC	N 70 + N 17	17,3	51,7	21,6	30,2
0,8 l ha^{-1}	N 70 + N35	19,7	52,2	25,8	32,6
	N 70 + N 52	20,2	51,8	24,3	32,1
	N 70 + N 70	21,9	59,9	25,6	35,8
	Keskmine / *Mean*	19,6	54,8	26,0	33,5

Seemnete kvaliteet

Preparaadi Moddus 250 EC mõju kohta seemnete 1000 seemne massi kohta võib kirjandusest leida vastukäivat informatsiooni. Junnila (1998) andmeil suurendas kasvuregulaatori kasutamine 1000 seemne massi 4–11%, Rønningeni ja Aamlidi (2002) andmeil põldtimuti 1000 seemne mass ei muutunud, sama kinnitavad Butler ja Affeldt (2010) aasnurmika kohta, Machači (2013) järgi oli 11 kõrrelise heintaimeliigiga läbiviidud katses aga enamikul liikidel 1000 seemne mass pritsitud variantides pritsimata variantidega võrreldes madalam (kuigi mitte usutavalt). Andrerson jt, 2015 andmetel vähendab preparaadi Moddus kasutamine punasel ristikul 1000 seemne massi, põuasel aastal on negatiivne mõju suurem (Kirk jt, 2016).

Erinevused katseandmetes võivad tuleneda mitmest asjaolust. Püstises taimikus on lamandunud taimikuga võrreldes 1000 seemne mass seemnete täitumisaegsete paremate toitumisolude tõttu suurem (Boelt, Gislum, 2010), samas kui (nt raiheintel) viljastub preparaadi toimel pähikus rohkem õisi, mis arenevad seemneks (Rolston jt, 2016; Trethewey jt, 2016). See viib 1000 seemne massi alla (Rolston jt, 2019) – seemnesaagi suuruse ja 1000 seemne massi vahel valitseb negatiivne korrelatsioon.

Meie katses põldtimut üheski katsevariandis ühelgi saagiaastal ei lamandunud. Kuna põldtimuti pähikud on üheõielised, ei saanud kasvuregulaator ka seemnete arvu pähikus (ja pöörispeas) suurendada. Kasvuregulaatori mõningane positiivne mõju ilmnes meie katses vaid esimesel kasutusaastal (2017), hiljem enam mitte (tabel 7).

Aastatel 2018–2020 oli analüüsimise all I koristusfaasil saadud seeme, 2017. aastal koristatigi kogu katse (hilinemisega) ühefaasiliselt. Lämmastikväetise normi suurendamisega ei kaasnenud meie katses 1000 seemne massis kindlasuunalisi muutusi. (Andmeid artiklis ei esitata).

Tabel 7. Kasvuregulaatori mõju põldtimuti 1000 seemne massile, g
Table 7. *Effect of growth regulator on timothy 1000 seeds weight, g*

	2017	2018	2019	2020	Keskmine *Mean*
Variant 1	0,472^{b}	0,420^{a}	0,448^{a}	0,514^{a}	0,463^{a}
Variant 2	0,497^{a}	0,424^{a}	0,447^{a}	0,515^{a}	0,471^{a}
Variant 3	0,505^{a}	0,429^{a}	0,447^{a}	0,520^{a}	0,475^{a}
PD / *LSD* 0,05	0,010	0,013	0,003	0,012	0,021

Teadusartiklites tuuakse harva andmeid seemnete idanemisenergia kohta – üldjuhul esitatakse idanemisandmed. Vaid Machač (2013), kes katsetas Modduse kasutamist 11 kõrrelise liigi seemnekasvatuses, tuvastas minimaalse negatiivse mõju nii seemnete idanemisenergia kui idanemisnäidule. 1–2%-list idanevuse langust on kinnitanud Rønningen ja Aamlid (2002), Øverland ja Aamlid (2016) 3,2%-list langust, Niskanen (2011) mõnel aastal kuni 10%-list langust. Idanevuse langust seletatakse preparaadi toimest tingitud taimede aeglasema arenguga, mistõttu soovitatakse kasvuregulaatoriga pritsitud seemnepõlde normaalse idanevuse tagamiseks koristada pritsimata põldudega võrreldes 3–5 päeva hiljem (Aamlid, Øverland, 2016).

On teada, et lämmastikväetise norm seemnete idanemisnäitajaid ei mõjuta. Seda kinnitasid ka meie katseandmed.

Katseandmetest nähtub, et ka kasvuregulaator Moddus ei mõjutanud seemnete idanevust, küll võis täheldada kasvuregulaatori kasutamisega kaasnenud idanemisenergia mõningast langust (tabel 8). Suurim vahe võrreldes kontrollvariandiga ilmnes põuasel 2018. aastal – kuni 24%. Ülejäänud katseaastail oli vahe näitudes 2–4%. Kasvuregulaatori ühekordne ja jaotatud kasutamine mõjusid seemnete idanemisenergiale ja idanemisvõimele sarnaselt.

Tabel 8. Seemnete idanevusenergia ja idanevus, %
Table 8. *Seed germination energy and germination, %*

Variant	N kg ha^{-1}	2017		2018		2019		2020		Keskmine / *Mean*	
		Energia *Energy*	Idanduvus *Germ.*	Energia *Energy*	Idanduvus *Germ.*	Energia *Energy*	Idanduvus *Germ.*	Energia *Energy*	Idanduvus *Germ.*	Energia *Energy*	Idanduvus *Germ.*
Variant 1	N 70	82	99	90	96	86	98	51	89	77	96
Kontroll	N 70 + N 17	86	97	87	97	84	96	47	83	76	93
Control	N 70 + N 35	96	99	82	96	87	96	52	85	79	94
	N 70 + N 52	92	98	87	99	83	94	44	83	77	94
	N 70 + N 70	92	99	79	96	85	97	53	82	77	94
	Keskmine / *Mean*	90	98	85	97	85	96	49	84	77	94
Variant 2	N 70	91	99	65	95	82	95	46	87	71	94
Moddus	N 70 + N 17	90	98	76	97	83	96	48	87	74	95
0,4 ×	N 70 + N 35	91	98	65	93	81	95	45	83	71	92
0,4 l ha^{-1}	N 70 + N 52	80	97	65	95	80	97	48	88	68	94
	N 70 + N 70	79	97	55	88	82	96	44	85	65	92
	Keskmine / *Mean*	86	98	65	94	82	96	46	86	70	93
Variant 3	N 70	89	98	58	98	83	97	50	82	70	94
Moddus	N 70 + N 17	83	99	60	95	82	97	49	84	69	94
0,8 l ha^{-1}	N 70 + N 35	85	99	61	94	80	95	41	84	67	93
	N 70 + N 52	89	99	63	94	80	96	46	85	70	94
	N 70 + N 70	83	98	63	95	83	96	50	85	70	94
	Keskmine / *Mean*	86	99	61	95	82	96	47	84	69	94

Seemnete idanevus oli katseaastati kõigis katsevariantides ja lämmastikväetise foonidel kõrge (94–99%), vaid 2020. aastal 82–89%. Sel aastal oli ka seemnete idanemisenergia näidud ülejäänud aastatest madalamad (41–52%). Põhjuseks võis olla seemnete koristamisele eelnenud pikem sademeterohke periood – juuli III dekaadis ja augusti I dekaadis langes sademeid 97 mm.

Kokkuvõte

Kasvuregulaator Moddus 250 EC kasutamisega põldtimuti seemnepõllul on võimalik kõrre pikkust 6–7% lühendada ja sellega koristusindeksit parandada. Seemnesaagi seisukohalt on tõhusam kasvuregulaatoriga ühekordne pritsimine taimede kõrsumise algul. Kasvuregulaator aeglustab taimekasvu ja nihutab optimaalset koristusaega mõne päeva võrra hilisemaks. Põldtimuti seemnete 1000 seemne massi ja idanevust kasvuregulaator ei mõjuta, küll aga vähendab seemnete idanemisenergiat. Kevadisele, kasvu algul antavale lämmastikväetise normile N 70 kg ha^{-1} võib heas seisus põldtimuti seemnetaimikule kõrsumise algul kasvuregulaatorit kasutades anda täiendavalt lämmastikväetist normiga N 35–52 kg ha^{-1}. Hõrenenud seemnetaimikul kasvuregulaatori ja lisa lämmastikväetise kasutamine end ei õigusta.

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Effect of plant growth regulator and additional nitrogen fertilization in spring on the seed yield and seed quality of timothy (*Phleum pratense* L.)

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Summary

With the application of plant growth regulator Moddus 250 EC to the seed field of timothy it is possible to shorten the stem height by 6–7% and thus improve the harvest index. Having in mind the seed yield, it is more effective to spray the plant growth regulator once in the beginning of stem elongation with the application rate of 0.8 l ha^{-1}. The plant growth regulator slows down the growth and postpones the optimum harvesting time by a couple of days. The 1000 kernel weight and germination of timothy is not affected by the plant growth regulator, but it can reduce the germination energy of seeds. In addition to the nitrogen fertilizer rate N 70 kg ha^{-1} that is applied in the beginning of growth in spring, another dose of N 35–52 kg ha^{-1} may be applied to the timothy stand that is in good condition in the beginning of stem elongation. The use of plant growth regulator and additional nitrogen on a sparse timothy stand is not justified.

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REVIEW: THE INFLUENCE OF GENOTYPIC AND PHENOTYPIC FACTORS ON THE COMFORT AND WELFARE RATES OF COWS DURING THE PERIOD OF GLOBAL CLIMATE CHANGES

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ABSTRACT. The study of the influence of weather phenomena on behavioural and physiological processes plays an important role in the development of highly effective methods of dairy farming management. Climate and weather factors have important signification in the system of interaction "organism-environment". One of the main factors of cows' comfort improvement in different types of premises, on ground runs and pastures is the creation of such indicators of microclimate that would best meet the biological needs of dairy cows, depending on the season and productivity. Due to the constant metabolic processes, the body of cattle is very hurtable to ambient temperature. This is especially felt during periods of prolonged low or high-temperature shocks. Disorders of metabolic and thermoregulatory processes directly affect the duration and nature of behavioural and physiological reactions and cause stress in animals. Prolonged temperature stress is the reason for fluctuations in productivity, quality of milk and problems with reproduction and together significantly affect the profitability of production. To reduce the impact of temperature stress on the body of dairy cows, scientists have proposed management strategies during periods of high and low-temperature shock. These strategies are divided into genotypic: the selection of heat-resistant individuals of different breeds and phenotypic: the use of microclimate control methods and modernization of feeding management methods. The effect of temperature stress on the body of dairy cows can be minimized due to genotypic (breeding of heat-resistant breeds) and phenotypic factors (water irrigation systems, ventilation, and the use of shade shaded shelters in summer and insulation of side curtains in winter), or a combination thereof. The purpose of this article is to summarize existing knowledge about the effects of temperature stress on the health, productivity and comfort rates of cows and to discuss management strategies that would mitigate the effects of these factors.

Introduction

Adaptation of livestock breeds to local climatic conditions is an important feature of modern agriculture, as it helps to reduce the influence of temperature stress to which animals are exposed, and leads to increased livestock production. Cattle under the influence of evolutionary factors (migratory movements together with human populations, as well as during the periods of natural migration to domestication) underwent a long natural selection and has adapted to various environmental conditions from equatorial Africa and America to central and northern Siberia (Upadhyay *et al*., 2017). Domestication has resulted in more than 1000 existing breeds with varying levels of productivity, product quality, feed conversion, and other economically important features (Berman, 2011; Zhang *et al*., 2013; Scheu *et al*., 2015).

In recent decades, the trend towards global warming has continued, which is already felt significantly at the regional and local levels (WMO, 2018; Hempel *et al*., 2019). The main direct effects of climate changes that have a negative influence on animal physiology, welfare, health and reproduction are rising air temperatures. The number of days with thermal stress caused by an increase in the temperature-humidity index (THI) increased by 4.1% during the period from 1973 until 2008 in Central Europe (Solymosi *et al*., 2010). Data from Novak *et al*. (2009) shows that in this region there are already more than 90 hot days a year. This affected the benefit of milk production at the stages from feed production to reproduction.

Along with the increase of the average annual temperature, the indicators of relative air humidity, amount of precipitation, as well as the direction and strength of the wind change (Herbut *et al*., 2013). Seasonal shifts and changes in the frequency and intensity of weather indicators affect most economic phenomena in agriculture (Nardone *et al*., 2010). Peculiarities of natural processes cause quite a frequent recurrence of unfavourable weather phenomena for agriculture, such as showers, hail, strong winds, dust storms, dry winds, droughts, touches of frosts, icy spots, etc. According to the Food and Agriculture Organization (FAO), approximately 26% of all losses and damages related to climate and weather calamities drop on such sectors of agriculture as crop science, farm animal production, fisheries, aquaculture and forestry (FAO, 2017).

The topic of the influence of climate changes on farm animal production is becoming increasingly urgent and relevant (Vitt *et al*., 2017). Adverse climatic conditions for farm animals lead to deterioration of their health, impaired thermoregulatory traits, growth and development, reduction of productivity and product quality, reproductive traits, metabolic status of animals and their resistibility (Broucek *et al*., 1991; Angrecka, Herbut, 2015). Thermoregulatory characteristics of cows are partly an individual feature and depend on body surface area, skin thickness, density and length of hair and fluffy, as well as the presence of dirt on animal hair (Herbut *et al.*, 2020).

The term climatic stress (*i.e*. heat and cold stress) means metabolic changes in farm animals in an attempt to adapt to changing weather conditions. This includes physiological and behavioural changes (Galán *et al*., 2018) and is caused by various combinations of air velocity, temperature, humidity, atmospheric pressure, and solar insolation (Mader *et al*., 2006).

Johnson (2018) identifies three strategies for managing and reducing the effects of temperature stress on the body of dairy cows: breeding heat-resistant breeds (genotypic factors), the use of microclimate control and the modernization of feeding management methods.

The purpose of this article is to summarize existing knowledge about the effects of temperature stress on the health, productivity and comfort rates of cows and to discuss management strategies that would mitigate the effects of these factors.

Genotypic factors

Breeding is one of the ways to reduce the influence of climate changes on dairy cows. The ability of dairy cattle to maintain body temperature during periods of excessively high and low-temperature stress is a feature that has recently been actively included in breeding programs (Kim *et al*., 2013; Kim *et al*., 2015). Currently, the development of DNA of animal bases with bio informative analysis of adaptation traits of certain breeds, lines and families to temperature stresses is becoming relevant (Srikanth *et al*., 2017; Liu *et al*., 2020). The use of such approaches leads to the correction of genes responsible for thermoregulatory processes and thus to the development of breeding strategies for breeding cows with good thermoregulatory traits.

Dikmen *et al*. (2008) indicate the attempts to improve the thermoregulatory traits of Holstein cattle by genetic means. To do this, the animals are injected with a smooth hair gene (SLICK). This gene is responsible for the length and density of the hair, which regulates heat input by evaporation. However, this method has not been widely used, because the breeding of shorthaired animals is relevant only in those regions where the average annual temperature does not fall below +15 °C.

Bernabucci *et al*. (2010) in their studies report that animals with lighter and shorter hair colour tolerate high temperatures better than animals with dark colour and long hair. This trait is characteristic of tropical cows of the Senepole breed in which the dominant gene is associated with increased sweating intensity, lower values of rectal temperature and respiratory rate (Mariasegaram *et al*., 2007).

Studies executed with African aboriginal cattle indicate that genes such as HSPA4 and SOD1 are responsible for adapting animals to hot housing conditions (Kim *et al*., 2017).

Heat stress genes have been identified and used as markers in the selection of thermotolerant bulls. The main heat shock proteins Hsp are Hsp100, Hsp90, Hsp70, Hsp60, Hsp40 and micro Hsps (so-called Hsp size below 30 kDa). HSPs play a crucial role in the recovery of cells from the effects of stress factors, as well as perform the function of cytoprotection. Hsp-gene expression during changes in temperature stress includes (i) activation of heat shock transcription factor (HSF1); (ii) increase of the expression of Hsp genes and decrease of the expression of the synthesis of other proteins; (iii) increase of the glucose and amino acids oxidation and decrease of fatty acids metabolism; (iv) activation of the endocrine system under stress; and (v) activation of the immune system through extracellular Hsp secretion. If stress persists, these changes in gene expression lead to a change in a physiological condition called acclimatization, a process that is largely controlled by the endocrine system (Collier *et al*., 2008).

Charoensook *et al.* (2012) noted the association of single nucleotide polymorphism (SNP) in Hsp genes in response to temperature stress. The association of Hsp90AB1 polymorphisms with heat resistance has been reported in studies of Thai aboriginal cattle and Sahival breed, and genes: HSF1, HSP70A1A, and HSBP1 at Chinese Holstein cattle (Li *et al*., 2011a; Li *et al.*, 2011b; Charoensook *et al*., 2012 Wang *et al*., 2013). Also, it has been found that genes that are not rated as Hsp genes fall for expression in response to temperature stress. These single nucleotide polymorphisms can be used as markers in the selection of heat-resistant animals (especially bulls) at an early age.

Feeding factors

Among the feeding strategies that can provide appropriate means to alleviate heat stress, the most important is the use of dietary fats, minerals, trace elements, vitamins, fibre, microbial ingredients (yeast), plant extracts and other additives that improve antioxidant and immune function (Min *et al*., 2019; Shan *et al*., 2020). Besides, bicarbonate, potassium, zinc, vitamins C, E and B_3 in feed rations should be increased during heat stress (Kadzere *et al*., 2002).

West (2003) reports that during periods of high temperatures, the protein content in the diets of dairy cows should not exceed 18% in terms of dry matter of feed.

Adjusting rations by increasing the proportion of concentrated feed or adding vegetable fats may contribute to lower milk losses during low temperatures (Kadzere *et al.*, 2002). However, these methods are not always effective in animals of other sexes and ages. Studies conducted in South Korea dealing with the effect of low temperatures (average daily temperature – 6.4 °C) on the growth rates of young cattle showed that the group of bulls fed with the bypass fat supplement did not differ from the group with a mixed diet (Kang *et al*., 2019). Ghasemi *et al*. (2017) in their studies conducted during the cold period (average daily temperature 5 °C) in Iran was divide sixty Holstein calves (3 days of age; 39.7 ± 3.8 kg of body weight) into 5 starter diets supplemented with (1) no fat or oil source (control), (2) 3% palm fat, (3) 3% soybean oil, (4) 3% tallow and (5) a 3.2% mixture of palm fat, soybean oil and fish oil. Feeding supplemental soybean oil tended to improve average daily gain and final body weight.

Holstein high-yielding cows are more prone to heat stress in comparison with less productive counterparts because they dissipate more metabolic heat (Spiers *et al*., 2004). During the period of thermal stress in the body of animals, there is an increase in the basic metabolism caused by the activation of the thermoregulatory system.

Climatic conditions have a direct influence on the health of cattle and can exacerbate or inhibit the development of diseases caused by temperature fluctuations. In addition, climatic conditions have a direct influence on the formation of immunity and the normal functioning of the endocrine system (Das *et al*., 2016). Climatic influence on the health and productive characteristics of cows occurs during periods of high temperatures when the feeding behaviour of animals changes significantly (there is an increase in concentrate consumption while reducing the total feed intake), which in turn contributes to acidosis, which causes lameness. In addition, reducing feed intake in high-yielding cows increases the risk of subclinical or clinical ketosis during the summer months (Lacetera *et al*., 1996; Rojas-Downing *et al*., 2017). The short period of heat stress during the final phase of embryonic development can have a significant impact on the health, growth and development of calves (Laporta *et al*., 2017). Fabris *et al*. (2019) indicate that cows exposed to heat stress during the dry period decreased productivity, protein, and lactose content in milk within the next lactation

Sunil Kumar *et al*. (2010) found in their studies conducted in India with adult buffaloes during periods of dry and wet heat loads that the addition of sodium bicarbonate, potassium carbonate and ascorbic acid polyphosphate to the diet prevents oxidative stress and increases immunity at the cell level.

The use of modern feeding approaches increase milk production per cow by 2–3% per year, but this leads to additional costs for veterinary measures, increased incidence of metabolic diseases and culling rates (von Keyserlingk *et al*., 2009). Bruno *et al*. (2009) report the effectiveness of the use of dry yeast culture (*Saccharomy cescerevisiae*) of 30 g per day during the period of high temperatures in the diets of adult cows. Milk productivity of such cows was 1.2 kg per day higher compared to cows that were not fed with dry yeast.

Gonzalez-Rivas *et al*. (2018) in their studies conducted in Queensland (Australia) during periods of high temperatures, divided lactating cows of the Holstein-Friesian breed into three groups: the first was fed with a total mixed ration (TMR) + flattened wheat; the second group TMR + flattened wheat grain treated with 2% starch solution, and the third group TMR + flattened corn grain. As a result, cows of the second and third groups had higher productivity compared to the first one, and cows of the third group had a lower rectal temperature than animals of the first and second groups.

Studies executed in New Jersey (Tinek Township) with a crossbreed of Holstein cows and Gir cows showed a positive effect thanks to the use of Omnigen-AF feed additive (1 kg of the additive: 650 g of bentonite, 250 g of purified diatomaceous earth and 100 g of dry brewer's yeast) during high-temperature shock. The experimental cows had higher rates of dry matter consumption (per 7%), fattening on the 56th day of the study (per 11%) and the average concentration of insulin in the serum (per 35%) in comparison with analogues from the control group. Thus, Omnigen-AF improves hyperthermia, appetite and immune parameters of the mammary glands in lactating dairy cows that were under the influence of heat stress (Leiva *et al.*, 2017).

Microclimate factors

Climate change, including global warming, and its consequences has a significant impact on productive and reproductive traits, and the well-being and health of cows. However, the system and method of animal housing play a fundamental role.

Among the weather factors that affect the functioning of dairy cattle, the greatest influence has an ambient temperature. Knizkova *et al*. (2002) found that thermoneutral for the body of dairy cattle is a temperature in the range from –5 to +25 °C. Most breeds are quite sensitive to higher and lower temperatures in this range. Gregory (1995) reported that dairy cattle could produce milk at temperatures up to –30 °C under conditions limiting the effects of wind and precipitation. Yurchenko *et al*. (2018) report that meat and dairy Yakut cattle are found above the Arctic Circle and can adapt to very low temperatures (up to –50 °C).

The effect of air temperature on dairy cattle should be considered in combination with relative humidity. The influence of heat stress on dairy cows is determined by the temperature-humidity index (THI) (Bouraoui *et al*., 2002; Dikmen, Hansen, 2009).

Ambient temperature (from 25 °C to 26 °C) or critical limit of THI (THI = 72, respectively 28 °C at a relative humidity of 50%) is critical, at which dairy cows can maintain a stable body temperature without increasing metabolic cost (Berman, 2011). An increase in the number of hot days with temperatures above the upper critical limit of THI aggravates the superventions of heat stress. Nardone *et al*. (2010) believe that the influence of global warming on animal productivity and their welfare and health will lead to adjustments in housing technology elements in many parts of the world.

The system of keeping animals is a set of zootechnical, technological, veterinary and organizational measures that take into account natural and economic conditions and ensure the flow of production processes. Animal housing systems differ in the degree of intensity of animal use, the type of feed production, the level of mechanization of production processes and indicators of comfort and well-being of maintenance (Ruban *et al*., 2020).

More than 83% of dairy cows in the EU use yard housing, in winter – indoors, and during spring-autumn – at feedlots or pastures. These combinations of housing do not only reduce the workforce but also meets animal welfare requirements (Zähner *et al*., 2004).

Von Keyserlingk *et al*. (2009) considers that measures to reduce the influence of global warming in Central and Eastern European countries can be adopted through the experience of livestock management in hotter regions and countries (Israel, Mexico and Brazil). Various technological approaches have been used to mitigate the adverse effects on productivity, reproductive efficiency, health, and comfort of cows (West 2003). First, these are systems of mechanical ventilation and cooling of animals (fans and irrigation systems), use of rest mattresses with the pumping of cooled water through them, use of walking areas with tents for rest and feeding, as well as their combinations. Ventilators, which accelerate air movement and increase convection, have been used to reduce ambient temperature and alleviate heat stress by reducing the respiratory rate, rectal temperature, and increase dry matter intake of animals.

The use of air temperature cooling systems at the beginning of the dry period affects the total milk yield, and cooling throughout the dry period helped to increase productivity by 7.5 kg per day in subsequent lactation, compared to cows exposed to heat stress (Dahl *et al*., 2017).

Keeping dairy cattle in pastures is considered more comfortable than keeping them indoors, as the animals spend most of the day in the wild (von Keyserlingk *et al*., 2009). However, high temperatures and humidity adversely affect dairy cattle while on pasture. Legrand *et al*. (2009) reported that yard-housing cows in the indoor enclosure with attached pasture preferred to stay at pasture in the evening, at night, and in the morning, while staying indoors during the day. Behavioural activity during heat stress at pasture housing differs from indoor activity (de Palo *et al*., 2006). Due to the longer distances between waterers, animals spend more time walking and cooling themselves than keeping them indoors.

Provision shade to cows during the period of heat stress is an important component of managing the thermal energy of the animal body, which leads to an increase in the proportion of animal's food consumption (from 19 to 24%) (Blackshaw, Blackshaw, 1994), an increase of productivity (West 2003), and lowering of body temperature compared to animals in unshaded areas (Kendall *et al*., 2006). The use of tents that provide shade at grounds, feedlots (the plots for fattening) and pastures is an effective method of heat stress reduction.

Eigenberg *et al*. (2005) reported that keeping animals under tents reduced respiration rate, heart rate, and body temperature during peak periods of temperature stress. In addition, the use of tents decreased the average vaginal temperature and increased daily milk yield (Kendall *et al*., 2006). The use of tents is less effective than irrigation systems in terms of reduction of body surface temperature and respiration rate of dairy cows. However, in a study by Schütz *et al*. (2011) most cows (65%) during periods of peak temperatures preferred to stay and rest under tents rather than walk through irrigation systems.

The use of shaded areas by cattle is not only associated with higher temperatures but also is more noticeable during intense insolation (Brown-Brandl *et al*., 2005). Tucker *et al*. (2008) reported that cows, which were provided with rest areas with different levels of shading for protection from sunlight, were in more shaded areas for a longer period and had a lower minimum body temperature because their level of

protection increased in comparison to animals, which were in less shaded or unshaded areas.

The use of irrigation systems reduces the air temperature and at the same time increases its humidity. In a study by Smith *et al*. (2006a), irrigation systems increased humidity by 22%. Wet aeration helped to reduce rectal temperature and respiration rate (Khongdee *et al*., 2006) and increased milk and milk protein yield in experimental Holstein cows (Broucek *et al*., 2006). US farms use high-pressure irrigation systems, which are injected with fans, or low-pressure sprinkler systems, which completely wet the cows by soaking their hair. West (2003) indicates that both of these systems increase feed activity, have a positive effect on reproductive traits, and reduce the severity of calving and rectal body temperature. Another way to reduce heat stress in cows is to install irrigation systems with an element of self-control, *i.e*. animals pass through systems of pressure-sensitive sensors mounted on the floor of the passages. This system has the advantage that it reduces the overall use of water (Legrand *et al*., 2011).

The orientation of rooms and ground runs depending on geographical location can also help to alleviate heat stress by reducing insolation and surface temperature of structures, which in turn increases heat transfer from the cow's body to the environment. Angrecka and Herbut (2016) conducted studies dealing with the effects of solar insolation during the summer in shade built with different geographical locations of longitudinal walls from north to south; from east to west; and with a 30° – deviation from north to south. They found that the use of the location of longitudinal walls: from north to south has the best effect on reducing the level of solar insolation during the summer.

Kendall *et al*. (2007) investigated the reduction of thermal capacity using three cooling systems: shaded shelters, irrigation systems and their combination. The use of shaded shelters reduced the respiration rate by 30% compared to the control group (without cooling systems), while the use of irrigation systems and a combination of both options reduced the respiration rate by 60% and 67%, respectively. Meyer *et al*. (2002) compared three ventilation systems in their studies. The productivity of cows was the highest in the room with the placement of fans (0.9 m fan blade) above the feeding passage (40.1 kg per day), compared with the option of placing longitudinal fan tubes above the cubicles (37.6 kg per day) or with ceiling (1.4 m blades) fans (37.1 kg per day). Also, with the option of placing fans above the feeding passage, the respiration rate constituted 75.3 times per minute, compared to the system of ceiling fans (83.5 times per minute) and longitudinal fan pipes above the cubicles (82.3 times per minute).

Combinations of different cooling systems have been extensively studied in investigations that took place in Israel using automated irrigation system (30 s) with the following ventilation (4.5 min) for 30-minute periods (Her *et al*., 1988; Wolfenson *et al*., 1988). The results showed that this combination of cooling was effective and helped to reduce heat stress in cows, as well as improved their heat balance, productivity and reproductive performance, lowered body temperature and met the recommended duration of behavioural responses.

Studies in the United States indicate a successful combination of tunnel ventilation and irrigation to reduce heat stress and improve milk production during dairy feeding (Smith *et al*., 2006b). Compared to traditional cooling technologies (cooling by fans and irrigation systems; cooling by shaded shelters and fans), the use of tunnel ventilation in combination with irrigation has reduced the effect of heat by 84%. The respiration rate and rectal temperature of cows cooled by this combination were reduced (Smith *et al*., 2006a). In addition, the combination of tunnel ventilation and irrigation had a positive effect on feed consumption (+11...12%), productivity (+2.6...2.8 kg per day), reduced the content of somatic cells in milk, while the quality of milk remained unchanged (Smith *et al*., 2006b).

The main disadvantages of irrigation and sprinkler systems are the consumption of large volumes of water (depending on the climatic characteristics of the region up to 215 litres cow^{-1} day^{-1} together with water consumption for milking, cleaning and watering). This in turn brings not only economic but also environmental consequences and is particularly irrational in regions (or countries) with limited freshwater reserves (von Keyserlingk *et al*., 2013). Besides, although the use of sprinklers significantly reduces the frequency of respiration and the influence of animals' distraction on insects (tail movements, shifting from foot to foot, twitching of the skin and throwing the head), their use also leads to increased cases when animals avoided irrigation and lowered the head at the time of the first stressful hit of water jets on the body (Schütz *et al*., 2011; Chen *et al*., 2016).

Avendano-Reyes et al. (2006) showed that the use of efficient cooling systems, *i.e*. fans in combination with irrigation systems in the calving pen during the period from 10.00 to 18.00 compared to cooling only by fans inhibits the reduction of productivity and milk fat content, improves calves growth and shortens the number of days open in cows.

The sense of heat from solar radiation could be partly reduced by changes in air velocity, which influence the convection cooling of cattle (Herbut *et al.,* 2020). The recommended air velocity for dairy farms in the United States during periods of high ambient temperatures is 1.8 to 2.8 m s^{-1} (Bailey *et al*., 2016). However, the airflow rate in naturally ventilated farms is not very uniform (Wu *et al*., 2012; Herbut *et al*., 2013) and depends not only on the characteristics of the internal layout of the room but also on such details as the presence of animals standing in the way of airflow and thus change its direction for other animals that lie or are at a lower or higher level (Berman, 2019).

The rate of air movement significantly affects the thermal balance of animals' body, providing a cooling

effect and lowering the body temperature of animals (Yi *et al*., 2019). Increased air velocity at low humidity and high temperatures cause hypothermia and can lead to lung diseases. In winter, during the long-term stay of animals on feedlots (feeding grounds) at air velocities of 5–7 m s^{-1} and air temperature even up to –20 ºC, there are cases of frostbite of certain parts of the animals' body (Nusinovici *et al*., 2015; Rong *et al*., 2015).

Heat insulation of light and ventilation curtains in winter is important when keeping cows in light-duty premises in countries with temperate-continental climates. It has been established that the use of curtains heat insulation with the use of polyvinyl chloride can extend the permissible norms of wind speed indoors for 13 days and protect animals from the environment more effectively at different categories of wind speed, as well as reduce indoor airspeed by 11.68–21.74% compared with uninsulated rooms of different configurations and heights of longitudinal walls (Borshch *et al*., 2021).

Prolonged precipitation in the form of rain during the spring period when keeping animals on pastures of different types (with shaded shelters and without them) at average daily temperatures of 12.1 °C and below affects the daily energy expenditure spent on basic metabolism and heat exchange, as well as on indicators of rest in the lying position (Borshch *et al*., 2020).

There is a dependence of the influence of litter material during periods of low-temperature shock (–11.8 °C and below) on the indicators of metabolic energy expenditure for heat exchange and behavioural reactions in cows (Borshch *et al*., 2019). Thus, with the use of deep straw litter, the total energy consumption for heat production was 2.95 and 2.43 MJ lower, compared to the housing of sawdust and compost manure on the litter. Besides, with this variant of bedding material, the indicator of the duration of rest in the lying position was higher by 38 and 25 minutes, respectively.

Conclusions

Analyzing the data already established, we tried to systematize the results of scientific research and discoveries of scientists on the effects of temperature stress on the health, welfare and productivity of dairy cattle. Given the processes of global climate change, combating temperature stress in animal husbandry is becoming very relevant, because it has a direct impact on food security. The effect of temperature stress on the body of dairy cows can be minimized due to genotypic and phenotypic factors, or a combination thereof. Phenotypic factors, which include the use of microclimate control (water irrigation systems, ventilation, and the use of shade shaded shelters in summer and insulation of side curtains in winter), are more effective due to the speed of commissioning but carry inevitable depreciation, which will affect cost and profitability of all production. Due to these factors, the number of days (or hours of the day) with thermoneutral temperature increases and the well-being, productivity and reproductive characteristics of cows improve. The application of feeding strategies with the use of feed additives, which contribute to better resistance of the body to temperature stresses, will not have a full effect without the simultaneous action of technological solutions. In addition, it requires periodic costs that will affect the cost of feed. Genotypic factors, which are the breeding of heat-resistant breeds, are long and currently not conceptually studied in terms of differentiation of adaptive traits of animals in different continents, latitudes and climatic zones. The most promising in terms of impact on the health and welfare of cows is a strategy that combines all these factors. Further comprehensive research should include engineering, genetic and feeding solutions, primarily to minimize the negative effects of climate change on animal health.

Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this paper.

Author contributions

All authors made equal contributions in literature analysis and writing a manuscript.

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RESPONSE OF ONION GROWTH AND YIELD GROWN IN SOILS OF SEMI-ARID REGIONS TO FOLIAR APPLICATION OF IRON UNDER WATER STRESS CONDITIONS

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ABSTRACT. Iron (Fe) is one of the major micronutrient crucial for plant growth, yield and quality. A field experiment was conducted in Fallujah, Iraq during the autumn season in 2019 to study the effects of foliar application of iron and irrigation levels on growth characteristics and yield of onion (*Allium cepa* L.). A two-factorial experiment arranged as a randomized complete block design with three replications was conducted in loamy sand soil. The two factors were water stress (50, 75, and 100% consumptive use of water) and iron concentrations (0, 100 and 200 mg L^{-1}). The results show a significant decrease in plant height, number of bulbs, total chlorophyll content, dry mass, iron content in the leaves, the average weight of bulbs and total yield of bulbs by reducing irrigation levels from 100 to 50% of the water supply. Application of iron by foliar spraying significantly increased most of the aforementioned traits. The interactions between iron and irrigation levels were significant in most of the measured traits. The interaction between 100% water supply and 100 mg L^{-1} of iron achieved the highest total yield value (4 332 Mg ha^{-1}) while the combination of 75% of water supply and 100 mg L^{-1} of iron gave the highest value of water use efficiency (84.7%).

Introduction

Water scarcity is one of the constraining factors for crop production, it plays a primary role in reducing the crop production more than the reduction resulting from all other environmental factors, hence irrigation has the priority in influencing the characteristics and quality of the crop through its effect on the formation of plant parts and its growth (Sharp *et al.,* 2004; Al-Shammari *et al.,* 2018). Consequently, limits the photosynthesis rate thereby cells partition, growth, and biogeochemical processes (Su, Shangguan, 2019; Abood *et al.,* 2019). This consistent with the scarcity of water in arid and semi-arid regions that suffer from low precipitation and deep groundwater. Therefore, improving the understanding of soil moisture dynamics is very important to precisely determine the soil moisture status (Wang *et al.,* 2013), which requires a revision of water resources management for optimal use of water, by rationalizing its consumption in the agriculture sector by controlling the number of irrigation events per season, or supplying the sufficient amount of water through irrigation scheduling (El-Siddig *et al.,* 1998). Besides water deficiency, the low availability of nutrients often controls the crop growth and production potential because most crops are sensitive to water and nutrients shortage during different growth stages. Microelements that applied to the soil are often dissolved too quickly and then drain into deeper layers of the soil profile, making nutrients unavailable to plants. Iron functions are very important in plant growth and development, such as take part in the biosynthesis of chlorophyll, respiration, chloroplast development and improves the performance of photosystems. Also, an iron element is an essential part of many enzymes (Barker, Pilbeam, 2007). Moreover, it participates in the oxidation

process that releases energy from sugars and starches, and convert nitrate to ammonium in the plant, iron plays a vital role in nucleic acid metabolism (Havlin, 2014). Water available status in the soil profile determines the nutrient distribution in the soil solution, with limited soil water content, the nutrients absorption and the diffusion of nutrients between the soil and root decrease due to the low soil water potential (Marschner, 2011). Recent studies have pointed out the effect of iron in reducing the negative effect of water deficiency. In this regard, iron foliar spraying caused increasing in soybean yield when exposing the plant to water stress (Kobraee *et al.,* 2011). In the same direction, the iron foliar spraying for sesame has increased the yield components and reduced the effect of water deficiency at the same time (Heidari *et al.,* 2011). However, iron availability depends on soil moisture content, where the iron is more available under wet conditions due to its transformation to more soluble forms (reduced). Therefore, supply iron under dry soil conditions may give adverse results (Havlin, Beaton, 1999). Onion is a major bulbous crop among vegetables, falls in order of 15 among vegetables classified by FAO, onion next to tomato in terms of total global production (Pathak, 2000). It has moderate amounts of protein, fat, fibre and good amounts of calcium, phosphorous and potassium, vitamin C and B6 (Mitra *et al.,* 2012). Therefore, around 175 countries cultivate onion (FAO, 2019). This study aimed to determine the effects of the irrigation levels and iron concentration applied using the foliar method in some growth characteristics and yield of onion.

Material and methods

Study area description

The study area was agricultural (33°17'26.5"N 43°48'56.1"E) located 8 km south of Fallujah, Iraq. It is a semi-arid area with a big difference in day and night temperature variation and the humidity is low. July and August are the hottest months with temperature rises to 49 °C and the temperature drops to 9 °C in January. The wind is northwesterly and southwesterly, sometimes with a maximum speed of up to 21 m s^{-1}. The average annual rainfall is less than 250 mm. Therefore, agriculture in the study area depends mainly on the irrigation water from the river Euphrates.

Field preparation and experimental design

A field experiment was conducted during the autumn season in 2019. The soil was classified as loamy sand. Soil samples were randomly collected and mixed to prepare a represented sample to be used in the analysis to determine the chemical and physical soil properties (Table 1). The percentage of sand, silt, and clay at 30 - cm depth were recorded by using the pipette method after oxidizing soil organic matter with hydrogen peroxide. While pH and EC were measured using pH and EC meter from the suspension of 1:1 soil water ratio (Black *et al*., 1965). The potassium was determined after treating the sample with ammonium acetate buffer (NH_4OAC) at pH 7.0 using 500801 PFP7/C jenway flame photometer, while phosphorus was determined according to (Olsen *et al.* 1982), the total nitrogen was measured using Kjeldahl, where the sample was digested in sulfuric acid, in the presence of a catalyst ($CuSO_4/TiO_2$) that helps to convert the amine nitrogen to ammonium ions, which subsequently converted into ammonia gas, then heated, distilled and titrated with a standard solution for the required calculations. The micronutrients such as iron, zinc, manganese were extracted according to NH_4HCO_3-DTPA (dientilenetriaminepenta acetic acid as an extractant) modified method (Soltanpour *et al.* 1979) and the elements were measured using Atomic absorption spectroscopy (AOAC, 1999). Before planting, the field preparation involved ploughing, levelling and dividing the field into plots. A two-factorial experiment of randomized complete block design (RCBD) with three replications was conducted. The two factors were water stress (W_1 = 50; W_2 = 75 and W_3 = 100% of union water consumptions) and iron concentration (Fe_0 = 0, Fe_1 = 100, and Fe_2 = 200 mg L^{-1}), where the iron source is Fe-EDTA 15%. The plots area were 3.6 m^2, during the period of vegetative growth. The T-Tape irrigation system was used, where three irrigation lines were used with the discharge of 1 L h^{-1}. The distance between irrigation lines and adjacent drippers were 0.75 and 0.1 m, respectively. Before planting, the field was irrigated liberally to prepare the plots for cultivation. The onion seeds (Texas Early Grano) were sowed on 30.11.2019 on both sides of drip irrigation lines (T-Tape). The application rates of fertilizer were 140, 60 and 120 kg ha^{-1} for nitrogen, phosphor, and potassium respectively. The manually weeding was conducted when needed. The data was analyzed statistically at P = 0.05, by using Genstat software (GenStat, 2005). The Least Significant Difference (LSD) was used to compare the averages of measurements.

Calculations of supplied water

After the seeds have been germinated, the supplied water was calculated according to (Al-Janabi, 2005). The water amount was divided based on the number of irrigation events from the germinated to the maturity stage. A valve was fixed at the beginning of each drip line to control the amount of supplied water. The supplied water for each plot was calculated according to Eq. 1:

$$Supplied\ water, m^3 = \frac{Water\ irrigation\ depth}{1000} \times Area, ha \quad (1)$$

The plot area used in this equation was 3.6 m^2. The operation time for each drip line was calculated according to Eq. 2.

$$Operation\ time, h = \frac{Supplied\ water\ to\ plot,\ L * No.\ of\ plots}{No.\ of\ drippers * Dripper\ discharge} \times 100 \quad (2)$$

The onion was harvested on 02.03.2020 when the onion bulbs reached the marketable size (2–3 cm diameter).

The traits under study

Five plants were randomly chosen in each plot to determine the plant height at the end of the season, the measurements were from the contact point between the

stem and soil to the highest point of the plant using measurement tape. The total yield was counted according to Eq. 3.

$$Total\ yield, kg\ ha^{-1} = \frac{Yield\ per\ plot * 10\ 000}{Plot\ area} \quad (3)$$

The number of leaves on the main stem was accounted manually. At the end of the season, five plants were randomly collected from each plot, then the roots and bulbs were removed before drying. The vegetative parts were dried at 70 ºC for 72 hours until the constant weight is reached. Ten plants were randomly chosen to take the average reading of the total chlorophyll. The measurements were conducted by using a Chlorophyll meter (SPAD-502). The weight of the bulb was calculated after removing the vegetative part according to Eq. 4.

$$Weight\ of\ bulb, g = \frac{Total\ yield\ per\ plot}{Number\ of\ plants\ per\ plot} \quad (4)$$

The iron content of leaves was determined based on Jones *et al.* (1991), where extracts were made of plant samples, then iron content was measured using Atomic absorption.

Water use efficiency (kg m^{-3}) was calculated according to Wright (1988) Eq. 5.

$$Water\ use\ efficiency = \frac{Yield,\ kg}{Supplied\ water,\ m^3} \quad (5)$$

Table 1. Chemical and physical properties for the soil under study

Chemical properties			Physical properties		
Parameter	Value	Unit	Parameter	Value	Unit
EC 1:1	7.4	ds m^{-1}	Sand	83	%
pH 1:1	7.35		Silt	9	%
Potassium	165.97	mg kg^{-1}	Clay	8	%
Phosphor	13.44	mg kg^{-1}	Soil texture: loamy sand		
Nitrogen	35.26	mg kg^{-1}			
Iron	2.79	mg kg^{-1}			
Zinc	1.72	mg kg^{-1}			
Manganese	2.67	mg kg^{-1}			

Results

Characteristics of vegetative growth

The obtained results showed a reduction in the average of plant height associated with water content decline, where supplying 50% of water supply (W_1), caused a significant reduction in the average of plant height reached 26.37 cm compared to 100 and 75% of water supply that gave 29.18 and 30.09 cm respectively Table 3. Probably due to water stress causes many physiological and chemical changes in the plant that leads to growth limitation. In addition to water stress also led to a decrease in the frequency of plant cell division and elongation (Sharp *et al.* 2004; Zheng *et al.*, 2013). Furthermore, the direct effect of dehydration lies in the expansion of the plant cell wall, where elongation involves cell wall to stretch under the influence of fullness effort, an effort decline as a result of the imbalance of the water plant content leads to decline or even to stop growth completely underwater deficiency (Cosgrove, 1989).

The plant height was significantly increased when the foliar feeding has been used, wherein the level of 200 mg L^{-1} (Fe_2) had achieved the highest value of plant height reached 30.70 cm, with the increasing rate of 13.45 and 10.15% compared to 0 and 100 mg L^{-1} respectively, increasing of plant height was achieved as a result of iron spraying on the onion vegetative part that reflected positively on the studied traits owing to the role of iron in the process of cell division thereby formation of many cytochromes and viroxin compounds which has great importance in photosynthesis and respiration processes, thereby increases the plant efficiency to absorb nutrients and then increase in most growth traits (Rout,Sahoo, 2015). Also, the interaction effect between water supply and iron fertilizer was significant in the aforementioned trait, where the highest value of plant height was 33.84 cm for the combination of W_1Fe_2 whilst the lowest value was 21.40 cm for the combination of W_1Fe_0 with the increasing rate of 58.13%.

The results showed a significant reduction in the average number of leaves, reached to 4.21 and 4.71 leaf $plant^{-1}$ for the treatments 50 and 75% of water supply respectively, with a reduction rate 21.75 and 45.12% compared to 100% of water supply, which achieved 5.38 leaf $plant^{-1}$. Also Table 2 shows a significant difference in terms of dry weight when reducing the water supply of onion, where the treatments W_2 and W_3 gave the lowest value of dry weight reach 5.69 and 5.63 g respectively, compare to the treatment W_1 that gave 9.66 g $plant^{-1}$. The evidence indicated the soil moisture content was insufficient to supply the needs of the onion plants consequently, reduced growth and the development of smaller bulbs (Kandil *et al.*, 2011; Tolossa, Yildiz, 2020). On the other hand, the evidence showed that the iron foliar spraying significantly differenced the average of dry weight, where the highest value was obtained from the treatment Fe_2 reach 7.74 g $plant^{-1}$ and did not differ significantly from Fe_1, but achieved an increasing rate reached 27.72% compare to Fe_0 (6.06 g $plant^{-1}$). Due to the role of iron in the physiological process which subsequently leads to an increased dry weight matter (Kim *et al.*, 2006).

Table 2. The plant height, leaves number, dry weight and total chlorophyll content of the onion receiving different combination of irrigation water and iron concentration levels

Water supply	Iron levels	Plant height, cm	Number of leaves $plant^{-1}$	Dry weight, g	Total chlorophyll, mg g^{-1}
W_1	Fe_0	21.40	5.10	6.07	26.03
	Fe_1	32.31	5.43	11.43	34.38
	Fe_2	33.84*	5.60*	11.47*	35.17*
W_2	Fe_0	31.88	4.10	6.23*	25.50
	Fe_1	26.86	4.77	5.20	25.31
	Fe_2	31.52*	3.77	5.63	25.43
W_3	Fe_0	27.91	5.43*	5.87	26.47
	Fe_1	24.44	4.00	4.90	23.07
	Fe_2	26.75*	4.70	6.13	24.63
LSD(0.05)		2.986	1.077	1.285	3.388

W_1 – 100, W_2 – 75 and W_3 – 50% represent the water supply. While Fe_0, Fe_1 and Fe_2 represent the iron concentration (0, 100 and 200 mg L^{-1}, respectively).

The interaction between water supply and iron fertilizer has significantly superior in the plant leaves trait, where the highest number of leaves was obtained from the combination of W_1Fe_2 reached 5.60 leaf plant^{-1} while the lowest number of leaves was 3.77 leaf plant^{-1} obtained from the combination W_3Fe_2 with increasing rate 48.54%, the evidence showed that reducing water content leads to encouraging the physiological role of iron compared to full irrigation supply. Regarding the dry weight, the significance was obtained from the interaction effect between water supply and iron levels, where the highest and the lowest value was obtained from the combination W_1Fe_2 and W_3Fe_1 reached 6.23 and 4.90 g plant^{-1} respectively, increasing the soil water tension from W_1 to W_3 reduced the dry weight matter because the iron in the combination of W_3Fe_1 was insufficient to reduce the negative effect of water stress. The results of the statistical analysis listed in Table 3. showed a significant reduction in the total chlorophyll content reached 25.41 and 24.72 mg g^{-1} in W_2 and W_3 respectively, compared to W_1 that achieved 32.01 mg g^{-1}. However, the results showed significant differences when spraying the iron, where the highest value of chlorophyll content was obtained from the treatment Fe_2 reached 28.41 mg g^{-1} compare to Fe_0 that gave 26.00 mg g^{-1}.

Similarly, the interaction effect was significant where the highest value was achieved from W_1Fe_2 reached 35.17 mg g^{-1} while the lowest value of chlorophyll content was 23.07 mg g^{-1} resulted from W_3Fe_1, the decrease in the total chlorophyll content was probably due to the water stress that led to a decrease in the number and size of chloroplasts thereby a reduction in the compounds needed to build chlorophyll such as water, nutrients and carbohydrates, consequently reduces the total chlorophyll content production (Berkowitz 1998).

Table 3. Plant height, leaves number, dry weight and total chlorophyll of onion receiving different irrigation water and iron concentration levels

Averages	Plant height, cm	Number of leaves plant^{-1}	Dry weight, g	Total chlorophyll, mg g^{-1}
W_1	29.18	5.38	9.66	32.01*
W_2	30.09*	4.21*	5.69*	25.41
W_3	26.37	4.71*	5.63*	24. 72
$LSD_{0.05}$	1.742	0.622	0.742	1.956
Fe_0	27.06	4.88	6.06	26.00
Fe_1	27.87	4.73	7.18*	27.73
Fe_2	30.70*	4.69	7.74	28.41*
$LSD_{0.05}$	1.742	N.S.	0.742	1.956

W_1, W_2 and W_3 represent the water supply (100, 75 and 50% respectively); Fe_0, Fe_1 and Fe_2 represent the iron concentration (0, 100 and 200 mg L^{-1} respectively).

Leaves iron content, yield traits and water use efficiency

The results listed in Table 4 showed that the W_3 treatment gave the lowest rate of leaves iron content reached 161.2 mg kg^{-1} compared to W_1 and W_2 with 175.9 mg kg^{-1} and 168.1 mg kg^{-1} respectively, this can be attributed to the lack of water in the leaves and stem that associated with a decrease in water irrigation depth and relative humidity (Heidari *et al.* 2011). While the foliar application of iron had achieved the highest concentration of iron reached 183.5 mg kg^{-1} obtained from the treatment Fe_2 compare to the control treatment which gave 149.9 mg kg^{-1}, with an increasing rate of 22.4%. Since the foliar nutrients absorbed easily by the leaf cuticle and enter the cells, facilitating easy and rapid utilization of nutrients by the crop (Latha, Nadanassababady, 2003). Consequently, increasing its content in the plant tissue.

In terms of interaction, the combination of W_1Fe_2 gave the highest average of iron content reached 194.8 mg kg^{-1} with an increasing rate of 32.52% compare to W_2Fe_0. In addition to Table 5 shows the significant effect of water stress on the average bulb weight, where the water deficiency caused a clear reduction in the bulb weight. In this regard, the treatment W_3 gave the lowest weight of bulb up to 5.57 g compare to 12.46 g resulted from the treatment W_1 with a reduction rate of 55.30%. At the same time, the spraying iron with the concentration of 100 mg L^{-1} had achieved the highest weight of bulb up to 11.70 g with an increasing rate of 43.03 and 33.42% compared to the control treatment and Fe_2, wherein gave 8.18 and 7.79 g, respectively. While the significant increase was obtained from the interaction effect, where the combination W_1Fe_1 gave the highest weight of bulb reached 16.20 g compare to W_3Fe_3 with 5.03 g (Table 4).

Table 4. Interaction of iron foliar spraying and water supply on yield components and water use efficiency some vegetative growth traits of onion

Water supply	Iron levels	Leaves iron content, mg L^{-1}	Bulb weight, g†	Total yield, Mg ha^{-1}	WUE, %
W_1	Fe_0	152.40	9.43	2.552	49.00
	Fe_1	180.40*	16.20*	4.332	84.20*
	Fe_2	194.80*	11.73	3.129	61.00*
W_2	Fe_0	150.40	8.57	2.284	59.40
	Fe_1	170.60*	13.77*	3.671*	84.70*
	Fe_2	183.30*	6.60	1.76	44.70
W_3	Fe_0	147.00	6.53	1.742	67.90*
	Fe_1	164.20*	5.13	1.369	53.40
	Fe_2	172.50*	5.03	1.327	51.70
LSD(0.05)		11.94	2.572	0.685	15.71

†Average weight of the bulbs; WUE – water use efficiency, W_1, W_2 and W_3 represent the water supply (100, 75 and 50% respectively).

The results listed in Table 5 showed that reducing the water supply significantly decreased the bulb yield. Where the yield was decreased from 3.32 to 1.47 Mg ha^{-1} when the water supply was reduced from 100% to 50% of water supply, with a reduction rate of 55.74%. Probably due to water stress that caused an imbalance of nutrients, since the most available elements are present in the soil solution, which negatively affects the metabolic processes of the plant, including photosynthesis that considers the main source of the other physiological processes of the plant (Rout, Sahoo, 2015). On the other hand, the iron application had positively increased the yield, where the treatment

Fe_1 gave the highest yield reached 3.12 Mg ha^{-1} followed by Fe_0 and Fe_2 with 2.18 and 2.07 Mg ha^{-1} respectively. This increase was due to the role of iron in controlling the negative effect of water stress in the aforementioned traits Table 5. Also, iron has a vital role in nucleic acid metabolism.

In terms of interaction, the highest yield was obtained from the combination W_1Fe_1 up to 4.33 Mg ha^{-1}, compare to W_3Fe_2 that gave 1.32 Mg ha^{-1}. Furthermore, the statistical analysis showed an insignificant reduction in the water use efficiency (WUE) when reducing the amount of applied irrigation water, while spraying with iron had a significant effect in increasing the efficiency of water use, where the concentration of 100 mg L^{-1} had achieved the highest water use efficiency with 77.1% compared to 200 mg L^{-1} and control treatment (0 mg L^{-1}) in which gave 52.5 and 58.8% respectively. Regarding the interaction, the combination W_2Fe_1 achieved the highest water use efficiency up to 84.7% compared to W_2Fe_2 which gave 44.7%.

Table 5. Means effect of water supply and iron concentration

Averages	Leaves iron content, mg L^{-1}	Bulb average weight, g	Bulbs total yield, kg ha^{-1}	WUE, %
W_1	175.9*	12.46*	3.321*	64.7
W_2	168.1*	9.64*	2.572*	65.9
W_3	161.2	5.57	1.479	57.7
$LSD_{0.05}$	6.89	1.485	0.396	N.S
Fe_0	149.9	8.18	2.181	58.8
Fe_1	171.7*	11.7*	3.12*	77.1*
Fe_2	183.5*	7.79	2.072	52.5
$LSD_{0.05}$	6.89	1.485	0.396	9.07

W_1, W_2 and W_3 represent the water supply (100, 75 and 50% respectively); Fe_0, Fe_1 and Fe_2 represent the iron concentration (0, 100 and 200 mg L^{-1} respectively).

Conclusions

The results of this study showed that the foliar application of iron with a concentration of 100 mg L^{-1} caused increasing in the traits under study (average of bulb weight, total yield of bulb, WUE) when the plant did not expose to the water stress, this increasing continues with applying the 200 mg L^{-1}, however, when the plant exposed to the water stress, the increasing achieved only when the foliar feeding of iron with the concentration of 100 mg L^{-1} has been used, where the iron has reduced the negative effect of water stress, which positively reflected on all traits under study. While applying the second level of iron (Fe_2), gave adverse results in most traits when exposing the plant to water stress, therefore, iron foliar application with a concentrate of 200 mg L^{-1} is recommended when the onion is grown under sufficient water condition. While the level of 100 mg L^{-1} can be used under water stress conditions. The evidence showed that 25% of the water supply can be saved by applying iron with a concentrate of 100 mg L^{-1} similarly, the interaction (W_1Fe_1) showed increasing in weight bulb, total yield and WUE, these findings support the results obtained from foliar application of iron with a concentration of 100 mg L^{-1}. while when reducing the water content with constant of Fe in the combination of W_2Fe_1 leads to reduce the leaves iron content, weight bulb and total yield. These findings confirm the importance of using iron in reducing the negative effect of water deficiency with acceptable limits, where expose the onion to water stress more than 50% of water supply caused a significant reduction in the yield even though the iron foliar spraying has been used. Regarding the interaction, the combination W_2Fe_1 achieved the highest water use efficiency up to 84.7% compared to W_2Fe_2.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

LG 25%, BB 25%, MA 25%, JA 25% – study conception and design;
LG 50%, MA 50% – acquisition of data;
BB 25%, JA 25%, MA 25%, GH 25% – analysis and interpretation of data;
LG 50%, MS 25%, JA 25% – drafting of the manuscript;
MA 50%, GH 50% – critical revision and approve the final manuscript.

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EXPERIMENTAL RESEARCH INTO OPERATION OF POTATO HARVESTER WITH ROTARY TOOL

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ABSTRACT. The experimental research was carried out on a specially designed laboratory-and-field test unit with the use of a hydraulic vertical rotor drive and strain-gauge equipment mounted on a tractor as well as the set of interchangeable coupling pieces for setting the machine's operating duty. Research into the process of breaking two adjacent potato row beds with the vanes of a vertical rotor has been undertaken. A design and process schematic model has been proposed for the operation of the potato harvester. Experimental research into the geometrical parameters of the potato row has been carried out to select the design parameters of potato harvesters. Based on the results obtained during the experimental investigations, the rational ranges have been established for the work process of the rotary potato harvester, the methods of engineering clod breaking tools have been developed. Following the completion of the full factorial experiment, regression functions have been generated. Their analysis has proved that the following factors have the greatest impact on the optimisation parameters: the rotor diameter and the clearance between the rotor and the spherical discs. The following parameters have been optimised based on the response surface analysis: soil separation ratio S = 93.5%, tuber damage rate P_b = 0.97%, total power consumption by unit operation N_a = 18.27 kW, at the following pre-set values of the factors: n_p = 77 min^{-1}, V_m = 2.2 m·s^{-1}, d_p = 0.825 m, l_2 = 0.3 m. The maximum discrepancy between the results of the theoretical and experimental investigations for determining the design and process parameters of the potato harvester does not exceed 15%. The completed economic testing has proved the advantages of the experimental potato harvester as compared to the existing ones. That said, the separation ratio of the pilot machine is equal to S = 91.4%, which is 23% higher than in the reference case, while its tuber damage rate is equal to 1.14%, which is 5.0% better than in the reference case. The recommendations for the selection of the rational operation duty of the rotary potato harvester as well as the methods for the engineering analysis of the design and process parameters of clod breaking tools have been developed.

Introduction

Harvesting is one of the most complicated and energy-intensive process operations in the potato production structure (Wang *et al.* 2017; Xin, Liang, 2017). The quality, with which this operation is performed, has a significant impact on the labour intensity of the following operations and the storage life of the obtained product (Wei *et al.*, 2019a; Gulati, Singh, 2019). As distinct from many other crops, harvesting the ripe potatoes requires lifting soil slices of great volumes and then separating the tubers from the soil, achieving the cleanness rate in the combined unit hopper of at least 80% and the damage rate not exceeding 3% (Hevko *et al.*, 2016; Wei *et al.*, 2017; Bulgakov *et al.*, 2018).

When potatoes are cultivated in heavy loam soils, which are prone to compacting during tillage, by the time of harvesting, when the potato harvesters lift tuber-bearing soil slices, a great amount of clods is formed and these clods cannot be removed by the separating tools of the combined unit (Petrov, 2004, Kheiry *et al.*, 2018, Pshechenkov *et al.*, 2018; Wei *et al.*, 2019b).

In that case, the soil content in the potato heap arriving at the hopper exceeds 20%, which does not meet the agronomical standards. Because of the above-said, the industrially produced potato harvesters are capable of operating only on sandy, sandy loam and medium grain-size composition soils with optimal moisture contents. When operating on heavy loam soils, the potato heap cleanness rate does not exceed 55–74%, while the tuber damage rate varies within the range of 18–25% (Lü *et al.*, 2015; Bulgakov *et al.*, 2018; Ruzhylo *et al.* 2020).

That complicates the process of potato heap classification and makes it necessary for the farm businesses to use manual labour extensively in the harvesting operations, which significantly increases the production cost of the final output. All efforts before potato harvesting have to be focused on loosening the tuber-bearing soil slice structure, removing hard clods from it and establishing the conditions that provide for essentially reducing the arrival of the soil impurities sized commensurate with potato tubers to the potato harvester separating tools. Meeting these conditions will facilitate raising the efficiency of the use of combined units on heavy soils and reducing the potato tuber loss and damage rates (Bulgakov *et al.*, 2019).

After analysing the known designs of clod breaking tools as well as the results of the research into their operation (Misener, McLeod, 1989; Ruysschaert *et al.* 2006; Bishop *et al.* 2012; Ichiki *et al.* 2013; Lü *et al.*, 2017), it becomes obvious that it is necessary to explore the agronomical characteristics of the operation of such tools. Among the reviewed potato harvesters, the greatest attention is to be paid to rotary-type tools (Petrov, 2004). The analysis of the operation of finger and vane beaters has proved that it is a most promising direction to design the clod breaking devices, in which the tools produce an increased impact on the tuber-bearing soil slice at the initial stage of the work process, at the same time taking into account the tuber damage rate (Brook, 1993; Blahovec, Židova, 2004; Gao *et al.*, 2011; Feng *et al.*, 2017; Bulgakov *et al.*, 2018; Issa *et al.*, 2020).

The experimental research into the operation of the rotary potato harvester has to find out the impact that different factors (shape and type of the rotary tool, machine's translational motion velocity, rotation frequency of the rotor, type and properties of the soil) have on the performance indices of the machine, such as the soil separation ratio, the tuber damage rate, the tuber-bearing heap distribution pattern on the raddle chain (Feller *et al.*, 1987).

The relevancy of carrying out the experimental research is proved by the absence in the available publications of any data on the investigation of the main parameters and operation duties of rotary tools as well as the data that would specify the efficiency of their operation in the vertical position in the inter-row spacing between two adjacent potato rows.

Taking into account the above-mentioned circumstances, the following targets were included in the experimental research programme:

– to explore the relations and patterns observed in the process of breaking clods in the potato row by the vanes of the vertical rotor and justify the positioning of spherical discs relative to the rotor as well as the other design and kinematic parameters of the machine's clod breaking tools;
– to determine experimentally the effect of the machine translation velocity, rotor rotation frequency, rotor diameter, spherical disc positioning, soil type and properties on the potato harvester's performance;
– to determine the tractive resistance of the experimental unit and the total power consumed in the work process;
– to test the operating efficiency of the vertical rotor on the soil contaminated with stones and plant debris;
– to carry out the comparative analysis of the economic and energy efficiency in the operation of the experimental unit and the commercial potato harvester KST-1.4.

The following tasks had to be performed to fulfil the above programme of experimental investigations:

– developing the techniques for carrying out individual stages of research;
– selecting the needed standard equipment;
– engineering and manufacturing the laboratory-and-field test unit;
– producing the necessary quantity of accessory parts for the performance of experiments;
– designing and manufacturing the equipment for the vertical rotor hydraulic drive installation as well as the calibration instruments;
– carrying out the calibration of measuring tools;

– organising the performance of the investigations following the experiment planning procedure and the processing of research results.

The research aimed to study the influence of the rational design, process and energetic parameters for clod crushing tools in the rotary-type potato harvester to improve its separating capacity.

Materials and methods

The method of experimental investigations was developed regarding the GOST 28713-90 and the RD 10.8.5-89 "Testing of agricultural machinery". To carry out the experimental investigations, it was necessary to arrange the monitoring of the work process performance utilizing measurements. That said, the performance of the overall research was divided into two phases: exploratory tests and main experiments.

The exploratory tests were carried out after the following design:

– determining the factors that have an effect on the process of soil separation and clod breaking by the vertical rotor in conjunction with the spherical discs and the raddle chain, i.e. selection of main factors;
– determining the effect the main factors have on the performance of the work process of breaking clods in the potato row bed;
– partially checking the testing method and conditions;
– checking the devices concerning the testing conditions;
– determining the data needed to calculate the number of experiments.

The exploratory tests do not provide for fully discovering the relations in the performance of the work process. They can be applied during the development of the research method, during the research or after it.

To carry out the exploratory and main tests, it was necessary to produce a pilot unit with an assortment of accessory parts, a set of measuring devices, strain-gauge equipment (Bulgakov *et al.*, 2021).

With provision for the above programme of experimental investigations, to explore the performance of the rotary tool operating in combination with the spherical discs and raddle chain, the experimental unit is shown in Fig. 1 and described further was designed and produced.

Frame 1 is used for mounting the components and mechanisms and comprises two parts: main and auxiliary. The main part of the frame carries the following components: depth rollers, vertical rotor, spherical discs and hydraulic drive. The auxiliary part of the frame carries a solid share, raddle chain, carrying wheels, which could be mounted on the main part of the frame. The depth rollers 2 mounted in the forepart of the unit are simultaneously the feeling and carrying members, which allow adjusting the depth of processing.

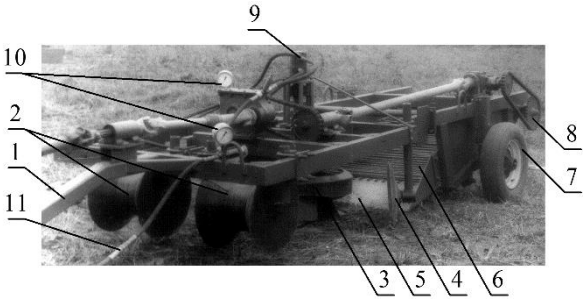

Figure 1. Field experiment unit for research into the operation of new potato harvester (the unit comprises the following components: frame 1, depth rollers 2, vertical rotor 3, spherical discs 4, solid share 5, raddle chain 6, carrying wheels 7, jolting device 8, vertical rotor hydraulic drive 9, pressure gauge 10, connection hoses 11)

The spherical discs 4 positioned at the angle of attack relative to the machine's line of travel overturn the two potato row beds broken by the vertical rotor towards the inter-row spacing centreline, thus forming the windrow to be picked up by the solid share 5.

The raddle chain 6 operates together with the jolting device 8 providing for the dispersion of the broken tuber-bearing soil slice and the separation of the tubers from the soil, the latter being then thrown off onto the field surface. It is to be noted that the travel rate of the conveyor belt and the amplitude of its jolting are equal to those of the conventional potato harvester raddle chains (Lovkis, 1991; Misener, McMillan, 1996). The carrying wheels 7 are used for adjusting the running depth of the unit's tools as well as for its transportation.

The vertical rotor 3 mounted on the centreline of the machine is used for breaking the two adjacent potato row beds by shifting them away from the inter-row spacing centreline, in order to disintegrate the soil clods. It is shown in more detail in Fig. 2.

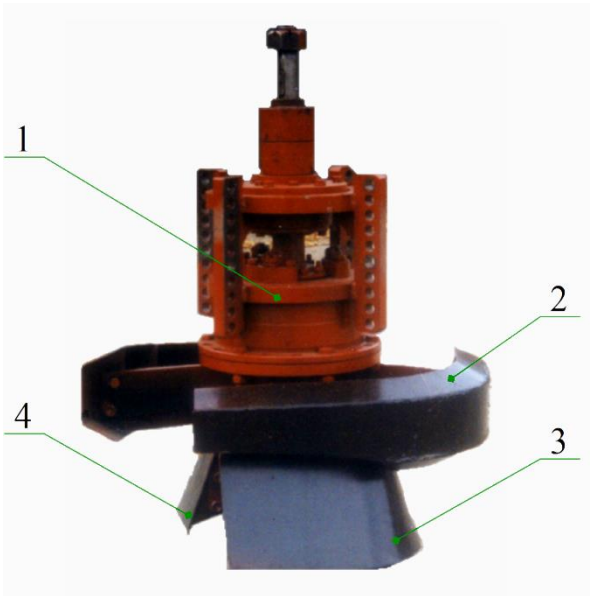

Figure 2. Vertical rotor: 1 – planetary reduction gear, 2 – upper beater, 3 – lower beater, 4 – disk blade

The design of the vertical rotor is as follows. The top-mounted planetary reduction gear 1 changes the senses of rotation of the upper 2 and lower 3 beaters that rotate at different angular velocities with a reduction ratio of 2.39. The upper beater 2 and the lower beater 3 each feature two curved vanes. The vanes of the lower beater have a cone-shaped surface with the offset radius facilitating the smooth penetration and disintegration of the lower layer of the potato row. The vanes of the upper beater have a cylinder-like surface with the offset radius and change the direction of motion of the tuber-bearing soil slice broken by the lower beater. The disk blade 4 of the vertical rotor provides for the uniform penetration and smooth motion of the vertical rotor.

The tools in the pilot unit can be driven by the tractor's power take-off (PTO) shaft, so by its hydraulic system. In particular, an MGP-160 hydraulic motor has been installed for driving the vertical rotor.

The power transmission comprises a reduction gear, two transmission gears, three transmission shafts, a universal-joint drive, a V-belt drive and a chain gear.

After selecting the main factors, in the second phase of research, the experiments had to be carried out. For that purpose, it was necessary to prepare the design of a full factorial experiment 2^n.

The measurement results were recorded in the test log, then processed with the use of the mathematical statistics methods (Brandt, 2014).

As is known, it is necessary to change during the experiments the machine's operating duty as well as the geometric parameters of its tools. Therefore, the rotor rotation frequency was changed with the use of a flow metering valve, the machine translation velocity – by shifting the tractor's gears, the rotor diameter – with the use of the assortment of accessory connection plates for the vanes of the lower and upper beaters, as shown in Fig. 3 and 4.

Figure 3. Assortment of accessory connection plates for vertical rotor

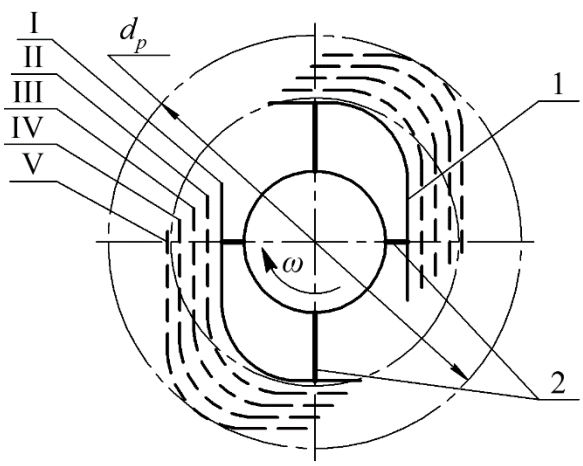

Figure 4. Schematic model of changing rotor diameter with the use of connection plates: 1 – rotor vane; 2 – connection plate

The spherical discs were mounted on the frame of the experimental unit and positioned in the longitudinal and transverse directions with the use of special brackets fastened with clamps (Fig. 5).

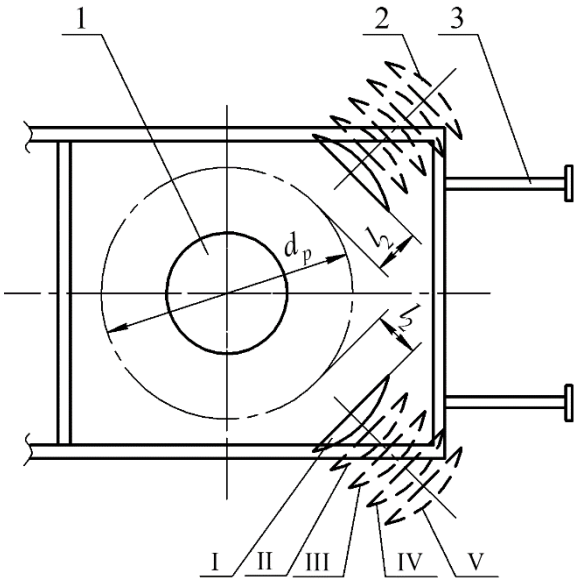

Figure 5. Schematic model of positioning spherical discs on experimental unit's frame: 1 – vertical rotor; 2 – spherical disc; 3 – frame

To determine the main relations between the performance indices and provide for the objective analysis of the experimental data, it was necessary to define the conditions for carrying out the research (Fig. 6).

Using the equipment installed in the tractor's cabin, the above-listed parameters, except for the tractive resistance, were synchronously recorded on the oscillograph tape. The traction resistance of the experimental unit was measured by the strain-gauge installation mounted in the rear part of the tractor's cabin.

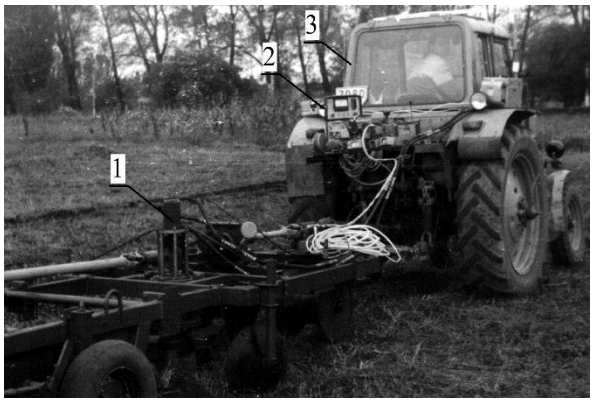

Figure 6. Experimental unit with strain-gauge equipment during measurement of energy characteristics: 1 – experimental unit; 2 – strain-gauge equipment; 3 – carrying tractor MTZ-80

The experimental investigations were carried out taking into account the following analytic relations.

The hydraulic moment on the vertical rotor is equal to:

$$M_p = q_M \Delta P \, \eta_{g.g.} \frac{1}{2\pi}, \quad (1)$$

where q_M – constant of the hydraulic motor (cm^3 rev^{-1}); $\Delta P = P_n - P_{zl}$ – pressure drop on the hydraulic motor (MPa);
$\eta_{g.g.}$ – mechanical efficiency of the hydraulic motor.

Angular velocity of the rotor is equal to:

$$\omega = \frac{\pi \, n_p}{30}, \quad (2)$$

where n_p – rotation frequency of the rotor (according to the oscillogram)

$$n = \frac{60 \, K_0}{\Delta t \, n_0}, \quad (3)$$

where K_0 – number of revolution marks on the oscillogram;
Δt – time-division value on the oscillogram;
n_0 – number of time marks on the oscillogram.

The power rating of the rotor drive is equal to:

$$N_P = M_i \, \omega_i, \quad (4)$$

Total power consumption by the experimental unit operation is equal to:

$$N_a = R_a V_m + N_P + N_{PTO}, \quad (5)$$

where R_a – tractive resistance of the unit (kN); N_{PTO} – power consumption by the tractor's power take-off shaft (kW).

Results

Design and operating parameters

Following the programme and methods of experimental investigations, to obtain reliable and full information about the process under consideration, the main experiments were carried out following the method of full factorial orthogonal central-composition planning.

After carrying out the theoretical analysis and exploratory screening tests, the following parameters had been identified as those having the greatest effect on the performance of the work process: machine translation speed V_m, rotor rotation frequency n_p, rotor diameter d_p, the distance between the spherical discs and the rotor's diameter l_2. Thus, the main experiments had to be carried out as four-factor full factorial experiments 2^4.

The soil separation ratio S and the tuber damage rate P_b were taken as the optimisation parameters that represented the quality of operation of the experimental unit. Simultaneously with the assessment of the machine's operation quality, the energy characteristics were estimated. For that purpose, the unit's tractive resistance R_a and the rotation moment on the rotor shaft M_p were measured with the use of the strain-gauge installation mounted on the tractor, then the total power consumption by the unit operation N_a was determined taking into account the power output at the tractor's PTO, which was equal to 6.25 kW (Bulgakov *et al.*, 2020). The numbers of measurements needed to ensure the target reliability of experiments were determined from the table by Brandt (2014). The number of experiment replications was equal to five.

After processing the experimental data to determine the effect of the above-mentioned factors on the optimisation parameters, the following three regression equations were obtained in the encoded form:

For the soil separation ratio S:

$$y_1 = 91.39 + 1.13x_1 + 2.06x_4 + 0.281x_1x_2 + 0.219x_2x_4 - 0.344x_3x_4 - 1.003x_1^2 - 1.288x_2^2 - 0.553x_3^2 - 0.445x_4^2 \quad (6)$$

For the tuber damage rate P_b:

$$y_2 = 2.159 - 0.557x_1 - 0.593x_2 + 0.245x_3 + 0.584x_4 - 0.258x_2x_4 + 0.108x_1^2 + 0.138x_3^2 \quad (7)$$

For the total power consumption by the unit operation N_a:

$$y_3 = 17.24 + 1.934x_2 - 0.813x_3 + 0.33x_4 + 0.625x_1x_3 + 0.813x_1x_4 + 0.275x_3x_4 - 1.72x_2^2 - 1.02x_3^2 \quad (8)$$

After a transition from the encoded form of the regression equations to the natural one, the following equations were obtained for each of the optimisation parameters, respectively:

$$S = 26.418 + 108.179d_p + 0.135n_p + 4.496d_pV_m + 9.713V_m + 0.018V_mn_p + 0.092l_2n_p + 7.866l_2 - \\ -64.171d_p^2 - 4.912V_p^2 - 24.577l_2^2 - 0.00071n_p^2 \quad (9)$$

$$P_b = 8.308 - 15.861d_p + 0.362V_m - 2.047l_2 + 0.054n_p - 0.021V_mn_p + 6.912d_p^2 + 6.133l_2^2 \quad (10)$$

$$N_a = 18.511 + 24.508V_m - 11.22l - 0.223n_p + 33.333d_pl_2 - 29.511d_p + 0.26d_pn_p + 0.073l_2n_p - 6.88V_m^2 - 45.333l_2^2 \quad (11)$$

where $$x_1 = \frac{d_p - 0.825}{0.125}; \quad x_2 = \frac{V_m - 1.5}{0.5}; \quad x_3 = \frac{l_2 - 0.3}{0.15}; \quad x_4 = \frac{n_p - 75}{25}. \quad (12)$$

Testing the above relations by Fisher's ratio allowed us to state that the model adequacy hypothesis was not rejected at a significance level of 0.05.

The obtained equations were analysed with the use of the path-of-steepest-ascent method applied on the PC in the MathCAD environment. For that purpose, two of the factors were fixed at pre-set values, while the other two were variable. The response surfaces for different cases and their analysis with fixed values of the factors of soil separation ratio S and tuber damage rate P_b are presented in Fig. 7–10.

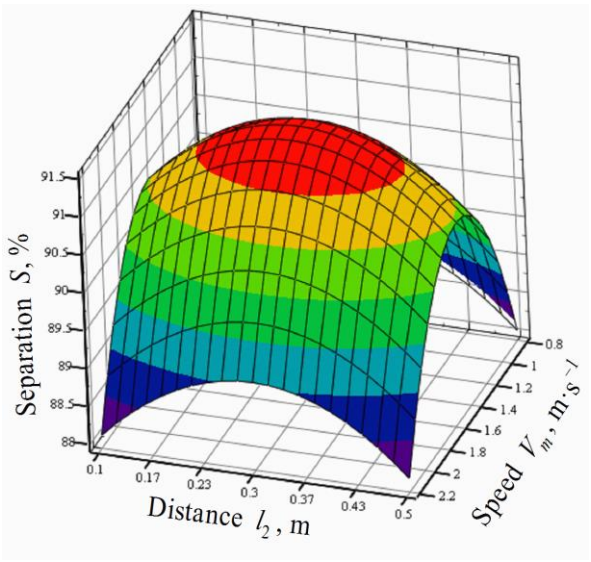

Figure 7. Relation between soil separation ratio S, on the one hand, and machine translation speed V_m and distance between rotor diameter and spherical discs l_2, on the other hand (at d = 0.825 m, n_p = 75 min^{-1})

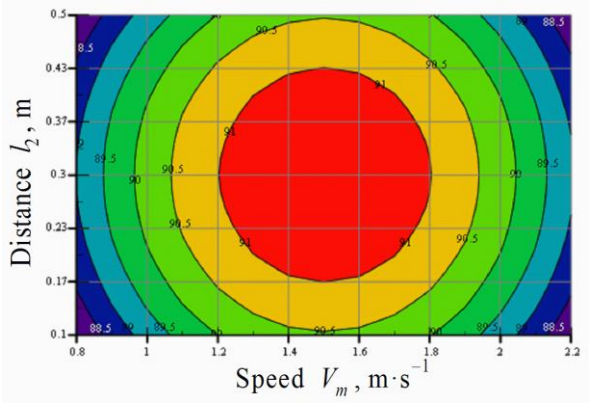

Figure 8. Analysis of response surface for soil separation ratio S

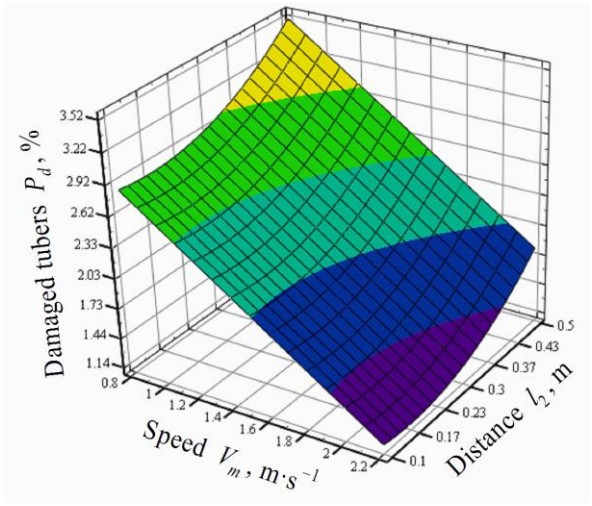

Figure 9. Relation between tuber damage rate P_b, on the one hand, and machine translation speed V_m and distance between rotor diameter and spherical discs l_2, on the other hand

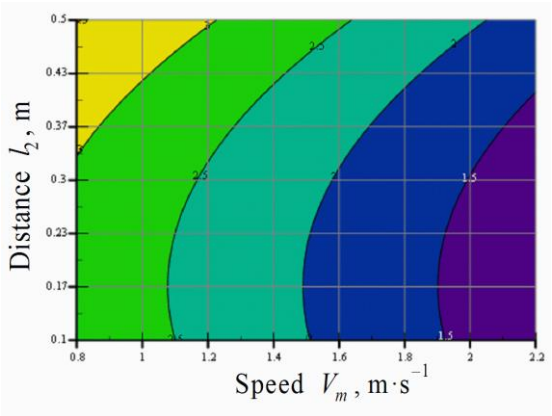

Figure 10. Analysis of response surface for tuber damage rate P_b

Energetic parameters

Following the method of research with the use of strain-gauge equipment, the following data were recorded: oil pressure in the pressure P_n and return P_z lines of the hydraulic rotor drive, rotor rotation frequency n_p as well as the tractive resistance of the experimental unit R_a. By the analytic relations described in the experiment procedure, the following parameters were determined: the rotor shaft rotation moment M_p, the power consumption by rotor drive N_p as well as the power consumption by the unit operation N_a (Fig. 11, 12).

Basing on the analysis of response surfaces, the optimum value has been established for the total power consumption in the operation of the two-row tractor-hitched potato harvester developed by the authors. It is equal to N_a = 18.27 kW at the following pre-set values of the other design and kinematic parameters: n_p = 77 min^{-1}, V_m = 2.2 m·s^{-1}, d_p = 0.825 m, l_2 = 0.3 m. That said, the maximum discrepancy between the results of the theoretical and experimental investigations does not exceed 15%..

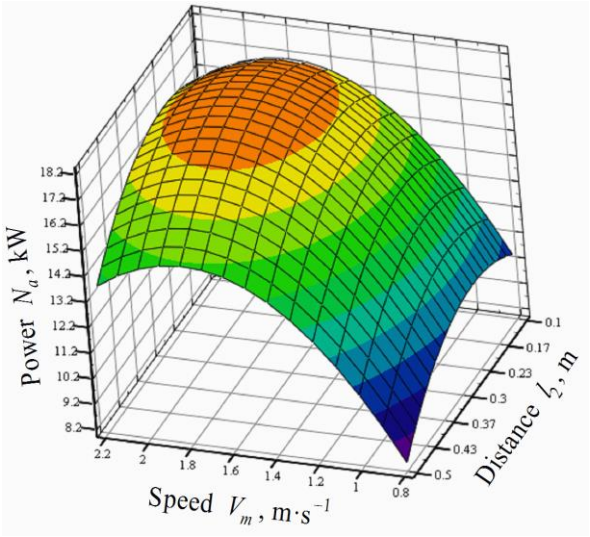

Figure 11. Relation between total power consumption for unit operation N_a, on the one hand, and machine translation speed V_m and distance between rotor diameter and spherical discs l_2, on the other hand.

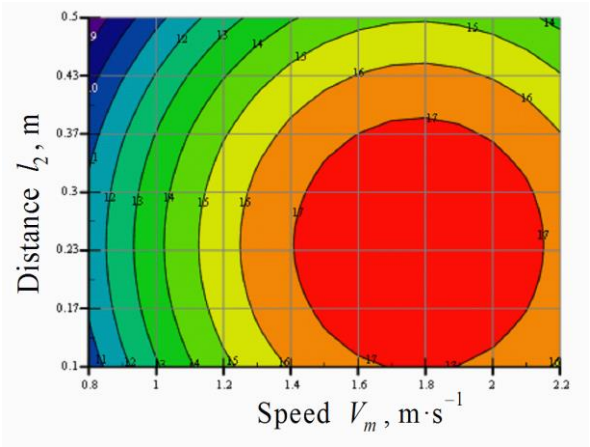

Figure 12. Analysis of response surface for total power consumption by unit operation N_a

Conclusions

1. In the completed exploratory tests, the factors that have the greatest effect on the performance of the potato harvester have been established: machine translation speed V_m, rotor rotation frequency n_p, rotor diameter d_p, the distance between the spherical discs and the rotor diameter l_2.

2. The working capacity of the machine has been established at different operating duties and the revealed deficiencies have been eliminated.

3. The main experiments have been carried out following the design of the full factorial experiment 2^4. As a result, the rational design and process parameters of the potato harvester have been established.

4. The regression equations have been generated for the following three optimisation parameters: soil separation ratio S, tuber damage rate P_b, total power consumption by the unit operation N_a.

5. The response surfaces have been analysed with the use of the path-of-steepest-ascent method.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

Author contributions

VB, YI – study conception and design;
VB, JO – drafting of the manuscript;
VB, VB, VM, JO, YI – analysis and interpretation of data;
YI, ZR, AZ, VV – acquisition of data.
JO – critical revision and approval of the final manuscript.

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BODY CONDITION EFFECTS ON DRY MATTER INTAKE AND METABOLIC STATUS DURING THE TRANSITION PERIOD IN HOLSTEIN DAIRY COWS

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ABSTRACT. The objective was to evaluate dry matter intake, metabolite concentrations and milk production of cows with different dry period body condition score (BCS). In addition, to support these results with previously reported insulin resistance and adipose tissue mRNA data on the same cows. Multiparous Estonian Holstein cows (n = 42) were assigned to three experimental groups on the basis of BCS 28 days before expected calving (d –28) as follows: BCS ≤ 3.0 (2.25–3.00; thin (T), n = 14); BCS = 3.25–3.5 (optimal (O), n = 14); BCS ≥ 3.75 (3.75–4.50; over-conditioned (OC), n = 14). Blood samples were taken between d –21 and d 42 in relation to calving, milk production data were collected throughout lactation. The OC cows' adaptation to the demands of lactation was the worst based on the comparison of dynamics of blood parameters between BCS groups. They had the most unbalanced metabolism and used more stored lipids compared to T and O cows. Fatty acids concentrations in the first week of lactation, related to insulin resistance status in the dry period and DMI in the first days of lactation, describe most of the variation ($R^2 = 0.55$) in BCS loss during the first 42 days of lactation.

Introduction

The transition period is metabolically the most demanding period throughout the productive period, which is illustrated by high disease incidence and culling rates (Ingvartsen, Moyes, 2013). In order to have trouble-free transitions, the cow needs to successfully adapt to lactation challenges (Gross, Bruckmaier, 2019) and homeorchetic changes during pregnancy play a vital role in the ability to adapt (Bauman, Currie 1980). The main goals for herd managers during the dry-period in supporting good adaption are to avoid overfeeding, overconditioning, and declining dry matter intake (DMI) before parturition (Drackley, Cardoso, 2014).

It has been shown that overfeeding either during the far-off or close-up periods results in higher lipid mobilization postpartum (Dann *et al.*, 2006, Mann *et al.*, 2016). However, the negative effect of overfeeding seems to be more pronounced with diets high in starch and its residues as studies with grass-based feeding have not given similar results (Agenäs *et al.*, 2003, Salin *et al.*, 2018). Long term overfeeding, irrespective of diet, will lead to increased fat reserves as the excess of dietary energy is mainly stored in the adipose tissue. Body condition score (BCS) is a good proxy for the amount of fat reserves, and over-conditioned cows face a greater risk of high lipid mobilization leading to high risk for health problems, while under-conditioned cows produce less milk and have lower fertility (Roche *et al.*, 2009). The range of optimum body condition is still debated and may depend on a genotype as cows with higher genetic merit or milk production are prone to a higher rate of lipolysis compared to lesser peers (Khan *et al.*, 2013, Karis *et al.*, 2020). Adequate intake during the transition period indicates cows' adaption to lactation and leads to reduced negative energy balance (NEB). Both overfeeding and overconditioning are associated with lower DMI postpartum (Drackley, Cardoso, 2014).

At the beginning of lactation cows' endocrine status favours lipid mobilization mainly through adrenergic signalling and the blunted effect of its main antagonist insulin on adipocytes will further induce lipolysis. This

situation of insulin resistance (IR) is deemed to develop during the dry period (De Koster, Opsomer, 2013). We have previously shown that the development of IR, changes in the expression of mRNAs and proteins related to glucose and lipid metabolism are dependent on the body condition in the dry period (Jaakson *et al.*, 2018, Karis *et al.*, 2020). In this study, we set out to test the hypothesis that the factors through which the amount of fat reserves before calving affects cows' adaption to lactation are interrelated. Our objective was to evaluate DMI around calving, concentrations of metabolites related to a potential overload of metabolism, and milk production of cows with different dry period BCS.

Material and methods

Experimental design

The European Council Directive regarding the protection of animals and the Estonian Animal Protection Act have been complied with in this experiment. The study was approved by the Committee for Conducting Animal Experiments at the Estonian Ministry of Rural Affairs. The study was carried out over two consecutive years (2013–2015) on the experimental farm of the Estonian University of Life Sciences with a herd size of 120 cows and a mean annual milk yield of 9 200 kg cow^{-1}.

Cows BCS was assessed fortnightly according to the method described by Edmonson *et al.* (1989) starting from dry-off approximately 60 days before expected calving. Cows whose BCS 28 days before expected calving (d –28) was the same as at dry-off were eligible for the study. In total, 46 Estonian Holstein cows were assigned to three experimental groups on the basis of BCS on d –28 as follows: BCS ≤ 3.0 (2.25–3.00, thin (T)); BCS 3.25–3.5 (optimal (O)); BCS ≥ 3.75 (3.75–4.50, over-conditioned (OC)). Due to culling in the first two weeks of lactation, 4 cows were excluded from the study. The remaining 42 cows were equally distributed between three experimental groups, 14 each. The average parity of the cows after calving during the trial period was the following: T – 2.6 ± 0.9 (parity 2 [n = 9], 3 [n = 3], 4 [n = 1], 5 [n = 1]), O – 3.2 ± 1.2 (parity 2 [n = 5], 3 [n = 4], 4 [n = 3], 5 [n = 1], 6 [n = 1]), OC – 3.7 ± 1.0 (parity 2 [n = 1], 3 [n = 6], 4 [n = 4], 5 [n = 2], 6 [n = 1]). Cows were enrolled in accordance with blocked design with each block consisting of three cows, one from each experimental group. Fortnightly BC scoring was continued until d 42 postpartum.

On d –28 cows were removed from the dry cow barn to tie-stall housing and from the seventh milking, cows were moved to a free-stall barn with a milking parlour.

The cows were milked twice daily at 05:00 and 15:00. Milking parlour hardware (DeLaval, Tumba, Sweden) recorded milk yields. Morning and evening milk samples were collected either on Sundays and Thursdays (from March 2013 to April 2014) or on Sundays, Thursdays and Tuesdays (from May 2014 to December 2015) with in-line milk meters (DeLaval, MM27BC, Sweden). Milk samples were stabilized with bronopol (Broad Spectrum Microtabs, D&F Control Systems Inc., Norwood, MA) and were analysed for fat and protein with an automatic infrared milk analyser (System FT+, Foss Electric, Hillerød, Denmark) in the Milk Analysis Laboratory of Estonian Livestock Performance Recording Ltd. ECM-yields were calculated according to Sjaunja *et al.* (1990). A fourth-order polynomial was fitted to milk, ECM, milk fat and milk protein production values. The area under the curve was calculated for each of the variables as the definite integral of the fitted polynomial and used as an estimate for total production over 42 and 305 DIM.

Disease events during the trial period are reported elsewhere (Jaakson *et al.*, 2018), these disease events were not taken into account in the current study. In addition, three cows from group T and three cows from group OC were culled before the end of lactation. The culling reasons were the following: two incidences of feet disease and four incidences of udder disease.

We have previously reported data on glucose tolerance test carried out on d –21 and d 21, data on mRNA and protein abundance in subcutaneous adipose tissue taken on d –21 and d 21 of the same cows (Jaakson *et al.*, 2018, Karis *et al.*, 2020). Blood samples were taken approximately one hour before the start of the glucose tolerance test.

Feeding, DMI drop, BCS loss

Diets, ingredients and chemical composition are presented in table 1. Cows were fed TMR *ad libitum* twice a day, at 05:30 and 14:30. Diets were calculated according to Estonian feeding recommendations: metabolizable energy (ME) according to Oll and Tölp (1995), metabolizable protein (MP) as described by Kärt *et al.* (2002). Between d –28 to d 2 cows were fed individually and orts were collected and weighed twice daily before the fresh feed was offered. Daily intake was calculated as the difference between the weight of feed offered and the weight of orts. To calculate the DMI drop average DMI between d –14 to d –8 were calculated for each cow, which was thereafter subtracted from DMI on d –1.

Third-order polynomial was fitted to BCS data, and BCS loss was calculated as the difference between the model's value on d 42 minus the value on d 1.

Blood sampling and laboratory analyses

Blood samples were taken on d –21 ± 2.3, d –14 ± 2.1, d –7 ± 2.6, d 7 ± 0.9, d 14 ± 0.8, d 21 ± 1.0, d 28 ± 1.1 and d 42 ± 2.1 at around 10:00 from the coccygeal vein into vacuum tubes containing Li-heparin (VACUETTE®, Greiner Bio-One International GmbH, Kremsmünster, Austria). Samples were centrifuged (5 000 × g, 15 min, +4°C) and stored at –80 °C. Clinical chemistry analyser (ERBA XL300, ERBA Diagnostics Mannheim GmbH, Mannheim, Germany) was used to measure the concentration of plasma fatty acids (also known as non-esterified fatty acids (NEFA)) (cat. no. FA 115; Randox Laboratories Ltd., Crumlin, United Kingdom), β-hydroxybutyrate (BHB) (cat. no. RB 1007; Randox Laboratories Ltd.),

total antioxidant status (TAS) (cat. no. NX 2332; Randox Laboratories Ltd.), aspartate aminotransferase (AST) (product code XSYS016, ERBA Diagnostics Mannheim GmbH, Germany), glucose (product code XSYS012, ERBA Diagnostics Mannheim GmbH), albumin (product code XSYS001 ERBA Diagnostics Mannheim GmbH), blood urea nitrogen (BUN) (product code XSYS020 ERBA Diagnostics Mannheim GmbH), uric acid (product code BLT00062 ERBA Diagnostics Mannheim GmbH). All of the inter- and intra-assay CVs were below 8.3 and 6.1%, respectively.

Insulin was analysed from the same eight samples by bovine-optimized sandwich ELISA (cat no 10-1201-01; Mercodia AB, Uppsala, Sweden), with a detection limit of 0.025 ng mL^{-1}, on a microplate reader (SunriseTM, Tecan Group Ltd., Switzerland); results were calculated using cubic spline regression (Magellan™ data analysis software; Tecan Group Ltd., Switzerland). The inter-assay coefficients of variation for plasma insulin concentrations of 0.2 ng mL^{-1} and 1.6 ng mL^{-1} were 4.6 and 6.6%, respectively and the intra-assay coefficients of variation were 4.2 and 4.1%, respectively.

Statistical analysis

Statistical analyses were performed with software R (version 3.5.0, R Foundation of Statistical Computing, Vienna, Austria). The DMI data from d –28 to d –15 and from d –14 to d –8 were averaged for each cow. To estimate the effect of the BCS group and time on the dependent variables a mixed linear model was fitted, considering the fixed effects of the BCS group, time, their interaction and the confounding factors of parity and relative breeding value for milk production, and random effects of animal and block. Pre- and post-partum blood sample data were modelled separately with the exception of DMI between d –7 to d 2 in relation to calving. For variables that were measured once or pooled, the fixed effect of time and random effect of the animal was removed from the model. If the modelling resulted in a singular fit due to the effect of a block being close to zero it was removed from the model. Modelling was performed with the function "lmer" in the package "lme4" and the least square means (LSM, *alias* marginal or model-based means) were estimated with the function "emmeans". The pairwise comparison of LSM was performed with the function "contrast". P-values were adjusted for multiple testing with the Tukey method. The significance of the effect of factors considered in the model was estimated with the type 2 Wald Chi-Square Test with the function "Anova". Two analyses were performed for variables that were not normally distributed (normality tested with the Kolmogorov-Smirnov test): at first, the models were fitted and the LSM were estimated on an arithmetic scale, and subsequently, the P-values were estimated fitting the same models on logarithm-transformed values. Linear regression lines and coefficients of determination were calculated to investigate the relationships between fatty acids on d 7 and DMI on d 2, between fatty acids on d 7 and BCS loss. Statistical significance was declared at $P < 0.05$.

Table 1. Diets ingredients and chemical composition (Mean ± SD) of total mixed rations diets (published in Jaakson *et al.*, 2018 and Karis *et al.*, 2020)

Item	Diets				
	Far-off Until d –15	Close-up d –14 to –1	Lactation 1 d 1 to 6	Lactation 2 d 7 to 14	Lactation 3 from d 15
Ingredient, g kg^{-1}					
Grass silage	955 ± 81	599 ± 71	604 ± 72	460 ± 49	384 ± 37
Hay	33.6 ± 81	28.3 ± 69	28.6 ± 70	20.3 ± 49	15.6 ± 37
Barley meal	–	301 ± 10	303 ± 10	309 ± 0.1	296 ± 0.2
Cornmeal	–	–	–	64.3 ± 0.0	120 ± 0.1
Heat-treated rapeseed cake	–	47.1 ± 0.6	47.5 ± 0.6	129 ± 0.0	168 ± 0.1
Mineral-vitamin feed	11.7 ± 0.11	7.82 ± 0.21	10.5 ± 0.32	8.58 ± 0.02	7.99 ± 0.02
Anionic mineral feed[3]	–	10.4 ± 0.2	–	–	–
Limestone	–	6.26 ± 0.2	–	4.29 ± 0.0	4.41 ± 0.7
Sodium chloride	–	–	5.78 ± 0.1	4.72 ± 0.0	4.40 ± 0.0
DM of diet	348 ± 78	435 ± 64	433 ± 64	483 ± 53	511 ± 45
Chemical composition					
CP, g kg^{-1} of DM	131 ± 10.5	144 ± 7.2	145 ± 7.3	161 ± 5.6	169 ± 4.8
MP, g kg^{-1} of DM	72.8 ± 3.3	86.6 ± 2.1	87.3 ± 2.1	97.9 ± 1.6	104 ± 1.3
ME, MJ kg^{-1}	8.70 ± 0.3	10.1 ± 0.2	10.2 ± 0.2	10.9 ± 0.1	11.3 ± 0.1
NDF, g kg^{-1} of DM	534 ± 51	455 ± 32	458 ± 32	410 ± 25	380 ± 22
ADF, g kg^{-1} of DM	387 ± 47	278 ± 29	280 ± 29	242 ± 22	220 ± 18
Ca, g kg^{-1} of DM	11.4 ± 1.9	10.3 ± 1.3	9.33 ± 1.3	9.54 ± 1.0	8.97 ± 0.8
P, g kg^{-1} of DM	3.42 ± 0.4	4.06 ± 0.2	4.03 ± 0.2	4.48 ± 0.2	4.77 ± 0.2

[1] Composition (as-fed basis): 170 g kg^{-1} of Ca; 50 g kg^{-1} of P; 30 g kg^{-1} of Na; 140 g kg^{-1} of Mg; 30 g kg^{-1} of S; 1000 mg kg^{-1} of Cu; 4500 mg kg^{-1} of Zn; 4000 mg kg^{-1} of Mn; 40 mg kg^{-1} of Se; 50 mg of Co; 200 mg of I; 800 000 IU kg^{-1} of vitamin A; 190 000 IU kg^{-1} of vitamin D; and 8000 IU kg^{-1} of vitamin E.

[2] Composition (as-fed basis): 150 g kg^{-1} of Ca; 35 g kg^{-1} of P; 75 g kg^{-1} of Na; 90 g kg^{-1} of Mg; 1 g kg^{-1} of S; 4000 mg kg^{-1} of Cu; 6667 mg kg^{-1} of Zn; 6452 mg kg^{-1} of Mn; 94 mg kg^{-1} of Se; 109 mg of Co; 650 000 IU kg^{-1} of vitamin A; 150 000 IU kg^{-1} of vitamin D; and 4000 IU kg^{-1} of vitamin E.

[3] Composition (as-fed basis): 9 g kg^{-1} of Ca; 1 g kg^{-1} of P; 5 g kg^{-1} of Na; 100 g kg^{-1} of Mg; 1000 mg kg^{-1} of Cu; 5000 mg kg^{-1} of Zn; 2000 mg kg^{-1} of Mn; 27 mg kg^{-1} of Se; 40 mg of Co; 100 mg of I; 1 000 000 IU kg^{-1} of vitamin A; 60 000 IU kg^{-1} of vitamin D; and 10 000 mg kg^{-1} of vitamin E, 100 000 mcg kg^{-1} of biotin.

Results

Group differences in milk yield, DMI and BCS loss

Milk yield for the first 42 DIM, 305-day milk and ECM production did not differ between the groups, but ECM production up to d 42 was greater in group OC compared to group T ($P < 0.05$; Table 2). OC cows had a higher milk fat percentage in the first 42 days of lactation compared to O ($P < 0.05$) and T ($P < 0.01$) cows.

DMI from d –14 to d –8 was lower in OC cows compared to T cows ($P < 0.05$; Table 2). In addition, OC cows DMI were lower than those of T cows on d –3 and d –1 ($P < 0.05$; Fig. 1). On d 1 and 2 postpartum DMI was the greatest in T cows ($P < 0.01$). A DMI drop was significant for all groups (T – $P < 0.05$; O and OC – $P < 0.01$), but it did not differ between groups.

The BCS on d –28 was different between the groups ($P < 0.01$) and the loss until 42 DIM was the greatest in OC cows ($P < 0.01$).

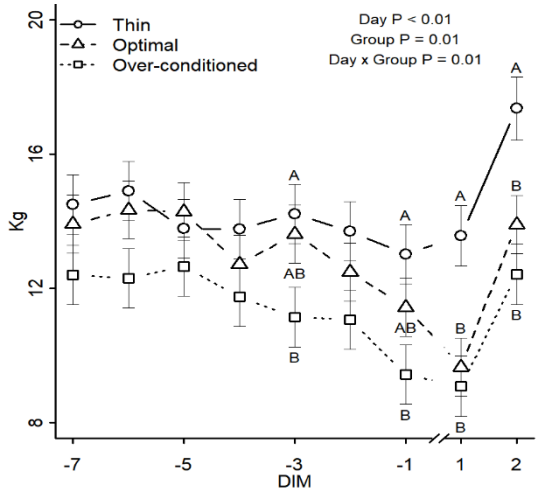

Figure 1. Dry matter intake around calving in multiparous Holstein cows grouped according to BCS on d –28 as follows: ≤ 3.0 – Thin; 3.25–3.5 – Optimal; ≥ 3.75 – Over-conditioned (n = 14 each). Values are expressed as LSM ± SEM. Letters "A" and "B" indicate a difference ($P \leq 0.05$)

Group differences in blood metabolites

Throughout the study period OC cows had a higher concentration of fatty acids, differing from T and O cows on d –28 and d –14, and from T cows on d –7, d 7 and d 21 ($P < 0.05$; Fig. 2). The BHB concentration was lowest on d –14 for OC cows and higher than in T cows on d 14 ($P < 0.05$). On d –21 the glucose concentration in T cows was lower than in OC cows ($P < 0.05$; Fig. 3). From pre- to postpartum glucose and insulin followed the same dynamics. No differences were recorded in the concentrations of insulin between the BCS groups. The activity of AST was highest in OC cows on d 7 ($P < 0.05$), on d 14 ($P < 0.01$), and higher than in T cows on d 21 ($P < 0.05$; Fig. 4).

Albumin concentration was higher prepartum in OC cows compared to T cows ($P < 0.01$), in addition, it was higher than O cows on d –14 ($P < 0.01$; Fig. 5). No differences were recorded postpartum. No differences were also found in TAS throughout the experimental period. Uric acid concentration differed only on d –21, at which point OC cows had greater concentrations compared to T cows (Fig. 6). The BUN concentration did not differ between groups prepartum, but in the postpartum period, T cows had the highest concentration on d 21 (compared to O, $P < 0.05$; compared to OC, ($P < 0.01$)) and higher than OC cows on d 28 ($P < 0.05$).

Associations between DMI, BCS loss and blood fatty acids

In the calculation of regression lines and coefficients of determination, the BCS groups were not differentiated. There was a negative correlation between fatty acids on d 7 and DMI on d 2 and a positive correlation between fatty acids on d 7 and BCS loss during the first 42 DIM (Fig. 7). DMI on d 2 described 37% of fatty acids variance on d 7 and fatty acids on d 7 described 55% of BCS loss variance ($P < 0.01$).

Table 2. Milk yield and composition, DMI, and body condition score (BCS) characteristics. Values are expressed as LSM. Letters "a", "b" and "c" indicate a difference ($P \leq 0.05$)

Characteristic	Group			SEM	P-value
	Thin	Optimal	Over-conditioned		
Milk					
up to d 42, kg d^{-1}	36.0	36.7	38.0	54.4	0.573
up to d 305, kg	10 079	10 639	10 824	463	0.495
ECM[1] up to d 42, kg d^{-1}	37.1^{a}	38.6^{ab}	42.7^{b}	56.2	0.019
ECM up to d 305, kg	9 901	10 484	10 909	384	0.133
Fat up to d 42, %	4.25^{a}	4.38^{a}	4.99^{b}	0.15	0.000
Fat up to d 305, %	3.89	3.87	4.02	0.14	0.717
Protein up to d 42, %	3.32	3.36	3.33	0.06	0.816
Protein up to d 305, %	3.22	3.31	3.34	0.06	0.308
DMI, kg					
d –28 to d –15	14.2	13.8	12.2	0.72	0.126
d –14 to d –8	15.1^{a}	14.8^{ab}	12.7^{b}	0.68	0.017
drop[2]	1.77	3.52	3.46	0.85	0.235
BCS					
on d –28	2.86^{a}	3.33^{b}	3.89^{c}	0.06	0.000
loss[3]	0.45^{a}	0.64^{a}	1.07^{b}	0.08	0.000

[1] Energy corrected milk, calculated according to Sjaunja *et al.*, 1990.
[2] The subtract between the average DMI on d –14 to d –8 and d –1.
[3] Total BCS loss on the first 42 days in milk.

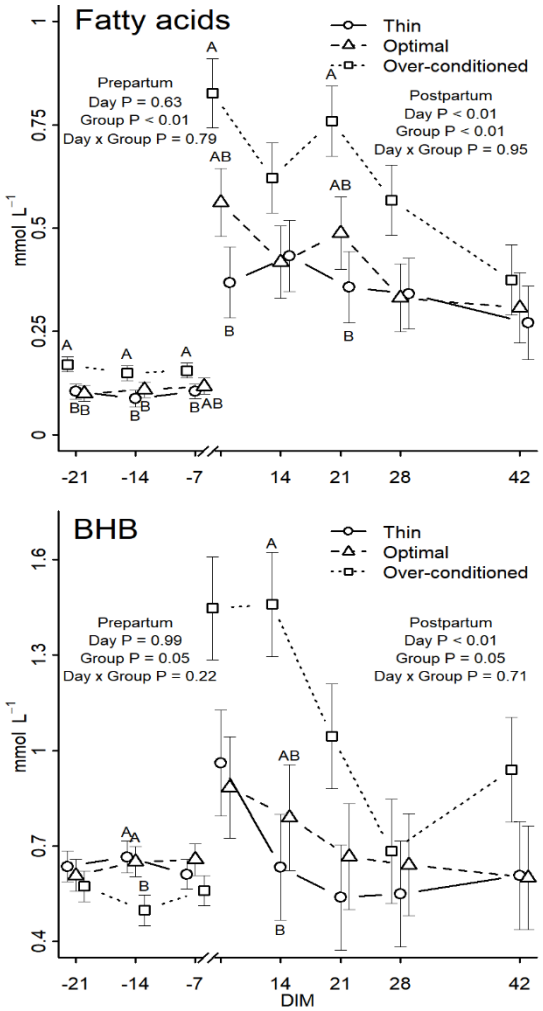

Figure 2. Concentrations of fatty acids and β-hydroxybutyrate (BHB) during the experimental period in multiparous Holstein cows grouped according to BCS on d –28 as follows: ≤ 3.0 – Thin; 3.25–3.5 – Optimal; ≥ 3.75 – Over-conditioned (n = 14 each). Values are expressed as LSM ± SEM. Letters "A" and "B" indicate a difference (P ≤ 0.05)

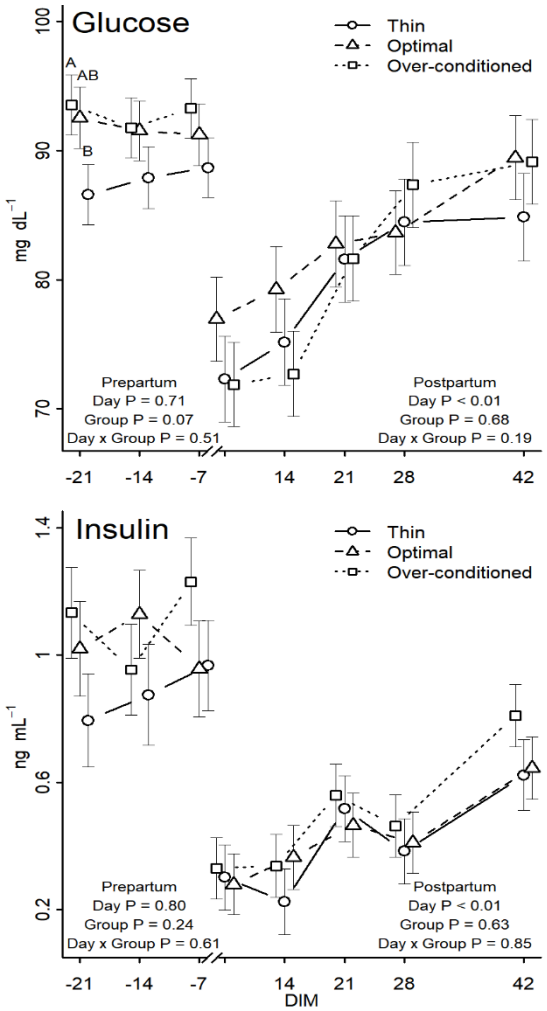

Figure 3. Concentrations of glucose and insulin during the experimental period in multiparous Holstein cows grouped according to BCS on d –28 as follows: ≤ 3.0 – Thin; 3.25–3.5 – Optimal; ≥ 3.75 – Over-conditioned (n = 14 each). Values are expressed as LSM ± SEM. Letters "A" and "B" indicate a difference (P ≤ 0.05)

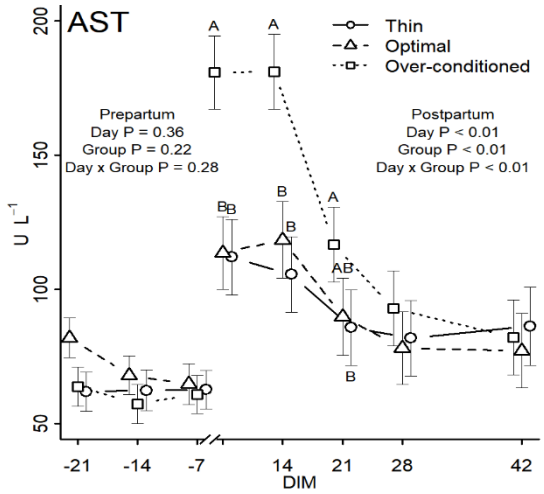

Figure 4. The activity of aspartame aminotransferase (AST) in plasma during the experimental period in multiparous Holstein cows grouped according to BCS on d –28 as follows: ≤ 3.0 – Thin; 3.25–3.5 – Optimal; ≥ 3.75 – Over-conditioned (n = 14 each). Values are expressed as LSM ± SEM. Letters "A" and "B" indicate a difference (P ≤ 0.05)

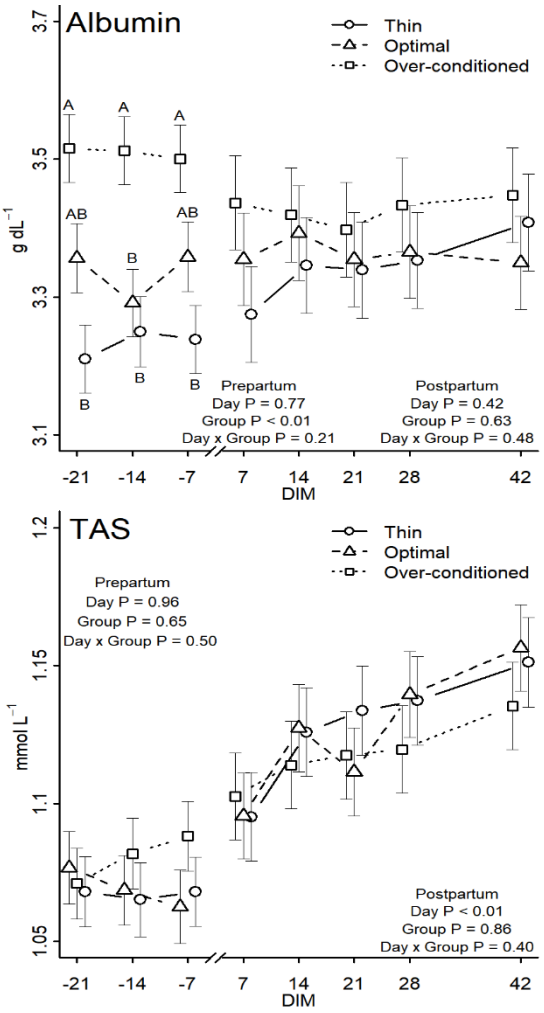

Figure 5. The concentration of albumin and total antioxidant status (TAS) during the experimental period in multiparous Holstein cows grouped according to BCS on d –28 as follows: ≤ 3.0 – Thin; 3.25–3.5 – Optimal; ≥ 3.75 – Over-conditioned (n = 14 each). Values are expressed as LSM ± SEM. Letters "A" and "B" indicate a difference (P ≤ 0.05)

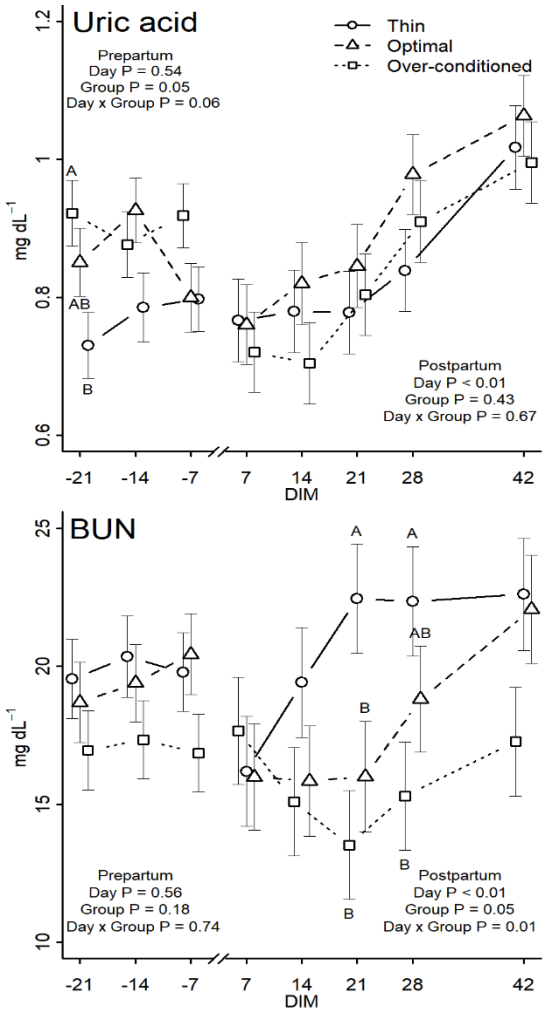

Figure 6. The concentrations of uric acid and blood urea nitrogen (BUN) during the experimental period in multiparous Holstein cows grouped according to BCS on d –28 as follows: ≤ 3.0 – Thin; 3.25–3.5 – Optimal; ≥ 3.75 – Over-conditioned (n = 14 each). Values are expressed as LSM ± SEM. Letters "A" and "B" indicate a difference (P ≤ 0.05)

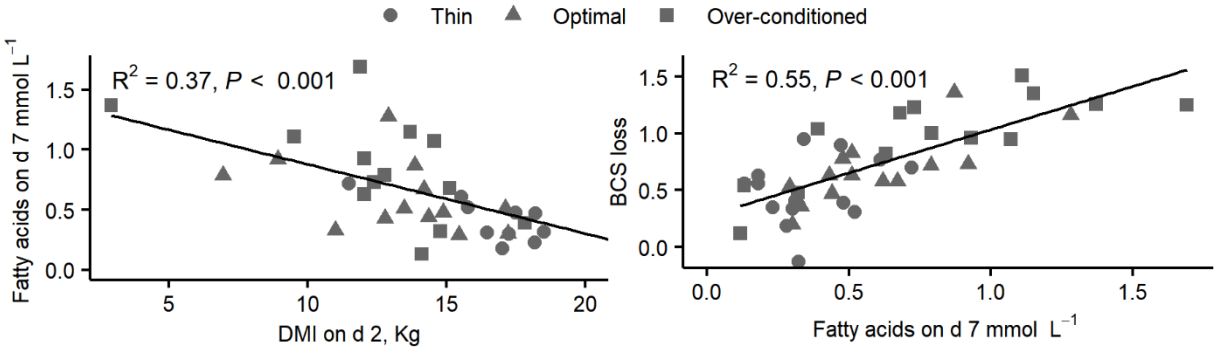

Figure 7. Regression line and coefficient of determination (R^2) of dry matter intake (DMI) on d 2 postpartum and body condition score (BCS) loss during first 42 days in milk with plasma fatty acids concentration on day 7. Points represent the values of individual multiparous Holstein cows

Discussion

This paper is a companion paper to that of Jaakson *et al.* (2018) and Karis *et al.* (2020) and proposes an integration of those findings with longitudinal dynamics in DMI, BCS, blood metabolite concentrations and milk composition of the same cows.

A limitation of our study is the uneven distribution of parity between the experimental groups. To compensate, we added the effect of parity to each mixed linear model as a confounding factor. In most cases, the effect

of parity was small and insignificant; the only exception is DMI between d –28 to d –15.

The magnitude of change in most of the concentrations of the measured metabolites illustrates the drastic shift in cows' metabolism in order to adapt to lactation.

The effect of BCS on DMI, milk and blood biomarkers

Greater glucose concentrations on d –21 and fatty acids concentrations throughout the prepartum period in OC cows compared to T cows might be a reflection of IR, as we have previously reported that OC cows had greater glucose and insulin AUC (Jaakson *et al.*, 2018) as well as longer fatty acids latency (Karis *et al.*, 2020) after glucose infusion on d –21. The differences in glucose concentrations between groups disappeared after d –21 probably because the DMI of OC cows was lower between d –14 to d –8 compared to T cows, and diet starch concentrations were increased two weeks before calving.

We observed differences in uric acid and albumin concentrations prepartum, but the physiological causes and meaning remain uncertain. Albumin serves as a marker of liver activity, inflammation, protein degradation or undernutrition (Agneäs *et al.*, 2003; Bertoni, Trevisi, 2013), but neither of those seems plausible explanations for these cows prepartum. Roche *et al.* (2013) also found a tendency for lower albumin in thin cows prepartum. The difference in uric acid, a marker for microbial protein degradation in the intestine that correlates with DMI (Tas, Susenbeth, 2007), is counter-intuitive as its concentration was lower for T cows even though the DMI was higher on d –28 to d –15 (though, no significance was observed).

It has become common knowledge that a dairy cow's DMI gradually decreases up to 30% over the last three weeks of gestation (Ingvartsen, Andersen, 2000). However, our findings do not support this notion as we observed a DMI drop only in the final days of gestation, and this seemed to be dependent on BCS as we saw a slight DMI drop in T, but a noticeable drop in O and OC cows in the final days of gestation. In agreement with our results, Agenäs *et al.* (2003) and Salin *et al.* (2018) did not report a depressed DMI during close-up in cows overfed with grass silage. High starch intake seems to be one of the possible reasons for DMI depression prepartum (Grummer *et al.*, 2004, Roche *et al.*, 2013). The reason could be the fact that glucose, availability of which is increased, is more tightly regulated and has more of an effect on the endocrine system (Bradford, Allen, 2007). We hypothesize that grass-based diets in the dry period protect cows from a gradual DMI drop. This is also consistent with the hepatic oxidation theory for DMI as acetate, the more dominant volatile FA in grass silage based diets is not extracted from the blood by the liver (Allen, 2020).

The DMI drop for O and OC cows resulted in a difference between T cows in the first two days of lactation. Regarding the T and O groups, this difference was not reflected in the concentrations of blood metabolites as we only recorded differences on BUN concentrations on d 21 between the groups postpartum. Our DMI data end at d 2, thus there is a 5-day discrepancy between the first postpartum blood samples. We hypothesize that the DMI of O cows must have caught up with that of the T cows. This is supported by the facts that we previously reported no difference in the total energy balance during the first 21 DIM (Jaakson *et al.*, 2018) for the same cows nor a difference in body condition loss between groups T and O.

Even though OC cows' fatty acids and BHB concentrations postpartum differed only from the T cows, we conclude that OC cows had the highest lipolysis up to the third week of lactation. We argue that the excess of fatty acids released from the adipose tissue of OC cows was divided between compartments within the organism (*e.g.* liver, udder) and synthesized into other metabolites (*e.g.* BHB), thereby lowering fatty acids concentration in plasma. This is supported by the very high BHB concentrations on d 7 and d 14 in OC cows and the highest total ECM production during the first 42 DIM, which is driven mainly by a high-fat content in milk. In the first week of lactation, the uptake of fatty acids from the blood by the mammary gland accounts for approximately 40% of total milk fatty acids (Bell, 1995). Probably the OC cows' high milk fat percentage, particularly in the first week of lactation, is caused by high lipolysis and fatty acids concentrations in the blood. This is supported by the highest C18:1 *cis*-9 fatty acid concentration in the milk ($P < 0.01$, unpublished data) and the greatest BCS loss in OC cows during six weeks of lactation.

The high activity of AST in OC cows agrees with other metabolites and milk traits data indicating again high lipolysis in OC cows that put them at risk of metabolic and infectious disease (Ingvartsen, Moyes, 2013). Over-conditioned cows are known to be at risk for fat infiltration to liver cell and AST activity is one of its indicators (Bobe *et al.*, 2004). In agreement with his, low urea is also associated with increased TAG in the liver (Jorritsma *et al.*, 2001) and lower expression of some mRNA encoding of enzymes involved in ureagenesis in the liver for cows at risk of high lipid mobilization (Graber *et al.*, 2010). Pires *et al.* (2013) reported evidence indicating higher labile protein mobilization in thin cows, presumably to compensate for the lack of energy from adipose tissue, thus it is plausible that the highest BUN for T cows on d 21 may arise from the differences in protein degradation in addition to liver function.

Increasing TAS postpartum shows that dairy cows are challenged with high rates of oxidation, which is caused by ongoing metabolic stress and NEB. This leads to increased synthesis of reactive oxygen species that may overwhelm the antioxidant capacity and lead to oxidative stress and inflammation response (Sordillo, Raphael, 2013). Even though high lipid mobilization (Sordillo, Raphael, 2013) and high BCS (Bernabucci *et al.*, 2005) are associated with systemic oxidative stress and inflammation, we observed no

differences in TAS or albumin between the BCS groups postpartum. There are more specific biomarkers for inflammation and oxidative stress, for example, tumour necrosis factor α and glutathione peroxidase, than used in this study and tissue-specific alterations might occur (*e.g.* adipose tissue inflammation) (Contreras *et al.*, 2018), therefore a definitive decision on this cannot be made.

Association between DMI, fatty acids and BCS loss

DMI on d 2 describes 37 per cent of the variation of fatty acids on d 7, which in turn seems to be the major determinant for the overall BC loss over the first 6 weeks of lactation. Thus, the success of the transition period is determined in early lactation and relies on the adaption of cows metabolism to a new physiological state during the dry period. In agreement, according to the hepatic oxidation theory, increased plasma fatty acid concentration, and thus intensified oxidation in the liver, is the limiting factor for DMI during early postpartum (Allen, Piantoni, 2013) and improving cows DMI intake pre- and postpartum is seen as the main goal to ensure a trouble-free transition period (Drackley, Cardoso, 2014). De Koster *et al.* (2019) clustered cows based on glucose, fatty acids, BHB and insulin-like growth factor 1 concentrations in blood at the beginning of lactation and reported that the main differences between metabolically balanced and unbalanced cows are DMI and BCS loss postpartum. Our data show that in addition to unfavourable metabolite concentrations OC cows had lower DMI compared to T cows and greater BCS loss than T and O cows and therefore can be classified as metabolically unbalanced. Although, DMI between OC and O cows did not differ on d 1 and 2 postpartum, OC cows still mobilize more body lipids, especially in the first weeks of lactation, suggesting that there are more factors determining the balance of metabolism or, in other words, OC cows experience higher lipolysis regardless of DMI. As only two days of postpartum DIM data was available in our study, and it might not reflect the actual feed intake during early lactation, our interpretation must be taken with caution. The underlying reason for higher lipolysis might be IR, as we have shown its interaction with body condition, and that cows with greater insulin AUC prepartum have decreased lipogenesis potential (lower *LPL* and *DGAT2* mRNA abundance) and greater fatty acids concentration on d 7 (Karis *et al.*, 2020). With good management practices the BCS of dry off cows can be optimized, but the reason for IR prepartum needs to be further studied, including the role of a genetic component in its development.

Conclusions

Overconditioning causes minor differences in glucose and fatty acids concentrations during the last three weeks of prepartum. During the first six postpartum weeks, the OC cows' adaptation to the demands of lactation was the worst, they had the most unbalanced metabolism and used more stored lipids compared to T and O cows. This also reflected in higher milk fat percentage and higher ECM production in OC cows during the first six lactation weeks. Fatty acids concentrations in the first week of lactation, related to IR status in the dry period and DMI in the first days of lactation, describe most of the variation in BCS loss during the first six weeks postpartum. Thus, metabolic processes during the dry period and in the first week of lactation are important determinants for metabolic health in the first weeks of lactation.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

HJ, KL, MH, AW, MO – study conception and design;
PK, HJ, KL, MH, AW – acquisition of data;
PK, HJ, KL – analysis and interpretation of data;
PK – drafting of the manuscript;
PK, HJ, KL, MR, MH, AW, MO – critical revision and approval of the final manuscript.

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FACTORS OF INCREASING ALFALFA YIELD CAPACITY UNDER CONDITIONS OF THE FOREST-STEPPE

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ABSTRACT. It was found that southern ecotype alfalfa (*Medicago sativa*) of variety 'Angelica' adapted to the soil and climatic conditions of the Forest-Steppe and was not inferior in yielding capacity to variety 'Rosana' which provided maximum indices in the phase of the beginning of budding at sowing rates of 6.0 million pcs ha^{-1} and ordinary row sowing method with row spacing 12.5 cm. It was discovered that with the increase of alfalfa sowing rate from 4.0 to 8.0 million ha^{-1} the dry matter content of varieties increased by 0.11–0.20% for sowing with row spacing of 25.0 cm. compared to row spacing of 12.5 cm. (22.62–22.83%). The average crude protein content in variety 'Rosana' was 20.68–21.37 and 'Angelica' 20.67–21.07%. Narrowing of row spacing contributed to an increase in crude protein content by 0.55–0.58%. The highest content NDF and ADF were observed in the second year of alfalfa grass life, respectively 30.72–34.91 and 23.02–24.60%. During the third year of alfalfa grass usage, the indices decreased to 27.09–33.03 and 19.53–24.18%, respectively. Thus, during three years of life at different geographical origins, alfalfa in the phase of budding provided a stable dry matter output of 27.45–27.81 and crude protein output of 5.86–5.87 t ha^{-1} for sowing with row spacing of 12.5 cm. and sowing rate of 6.0 million pcs ha^{-1}.

Introduction

One of the priority development directions for the agro-industrial complex of Ukraine is the provision of husbandry with high-protein fodders at unstable moisture supply and high-temperature regime (Kvitko *et al.*, 2014; Petrychenko, Hetman, 2017). Therefore, the creation of long-term agro-phytocenoses requires innovative approaches, especially in the selection of alfalfa varieties, which, with the maximum realization of genetic potential can form stable yields under extreme hydrothermal conditions. Today, alfalfa (*Medicago sativa L.*) due to its biological characteristics is the most drought-resistant crop (Holoborodko, Pohinaiko, 2018) and an unsurpassed component of agro-phytocenoses of cheap plant protein for the preparation of various fodder types (Petrychenko *et al.*, 2020).

In fodder production intensification for the production of high-quality fodder primarily is used new generation alfalfa varieties (Syniuha) which can produce on soils of high acidity (Hetman, Tsyhansky, 2014) resistant to salt stress ('Nadezhda') (Tyshchenko *et al.*, 2013) and have high nitrogen-fixing ability ('Angelica', 'Zoriana', 'Veselka' and others) (Vozhegova, Tyshchenko, 2017).

The method of sowing alfalfa with row spacing of 15 cm and sowing rate of 8–10 million ha^{-1} for uncovered growing provides an increase in crude protein output by 27.4–36.0 and 30.4% – 12 million ha^{-1} compared to cover sowing under spring barley for grain has been scientifically substantiated (Petrychenko, Kvitko, 2010).

Scientists from around the world have established the dependence of crop production processes on the width of row spacing and soil-climatic conditions of growing. Thus, in the United States on sub-sandy soils of 'Sesil' the yield of alfalfa green mass increased with the narrow-row sowing method compared to the broad-row sowing method (Madhav *et al.*, 2020[a]; Karbivska *et al.*, 2020; Tonkha *et al.*, 2021) and crude protein content

remained unchanged with increasing row spacing (Stringer *et al.*, 1996). On clay soils in Bandeirantes, Pararana state (Brazil), no significant influence of row spacing (15, 20, 30, 40 cm) on plant height and alfalfa dry matter output was observed (Bellettini *et al.*, 1997; Karbivska *et al.,* 2019; Karpenko *et al.,* 2019), and under conditions of southern Serbia (Geren *et al.*, 2003) with the increase of row spacing were improved water balance and the number of generative organs (Madhav *et al.*, 2020[b]).

Based on literature analysis was not established a unanimous opinion concerning the width of alfalfa row spacing when growing for green fodder. Under conditions of climate change, this issue has become the subject and object of in-depth study of the life cycle of alfalfa new varieties in the Forest-Steppe of Ukraine.

The research aims to study alfalfa (*Medicago sativa* L.) yielding capacity depending on variety, seeding rates and row spacing.

Materials and methods

The field study was conducted during 2017–2019 in the experimental field of the department of field fodder crops, hayfields and pastures at the Institute of fodders and agriculture of Podillia NAAS of Ukraine.

The soil of the experimental field is grey forest, medium loamy by mechanical composition. The arable layer of soil (0–30 cm) contained 2.06% of humus (according to Tiurin), alkaline hydrolyzed nitrogen – 7.7 mg per 100 g of soil (according to Cornfield), movable phosphorus and metabolic potassium, respectively 14.2 and 8.0 mg per 100 g of soil (according to Chirikov), $pH_{sal.}$ – 5.9. Soil analysis was conducted in the Vinnytsia branch of SE Derzhgruntohorona at the Institute of soil protection of Ukraine.

The weather conditions in May-September 2017 were arid. The air temperature was 18.2 °C and the hydrothermal coefficient 0.68. In 2018–2019, air temperature during this period fell to 16.7–17.8 °C and the hydrothermal coefficient (HTC) increased by 1.21–1.25 (Fig. 1).

The technology of alfalfa growing was generally accepted for the Forest-Steppe of Ukraine. Predecessor – spring rye for grain. In the experiment was sown alfalfa varieties 'Rosana' (Forest-Steppe) and 'Angelica' (southern Steppe) which are included in the State Register of Plant Varieties Suitable for Growing in Ukraine. Superphosphate and potassium-magnesium were used as mineral fertilizers and lime was added according to hydrolytic acidity. Sowing was carried out in the third decade of April 2017 by the uncovered method. Alfalfa was sown by row method with row spacing of 12.5 and 25.0 cm at sowing rates of 4, 6, 8 million pcs ha^{-1} of similar seeds.

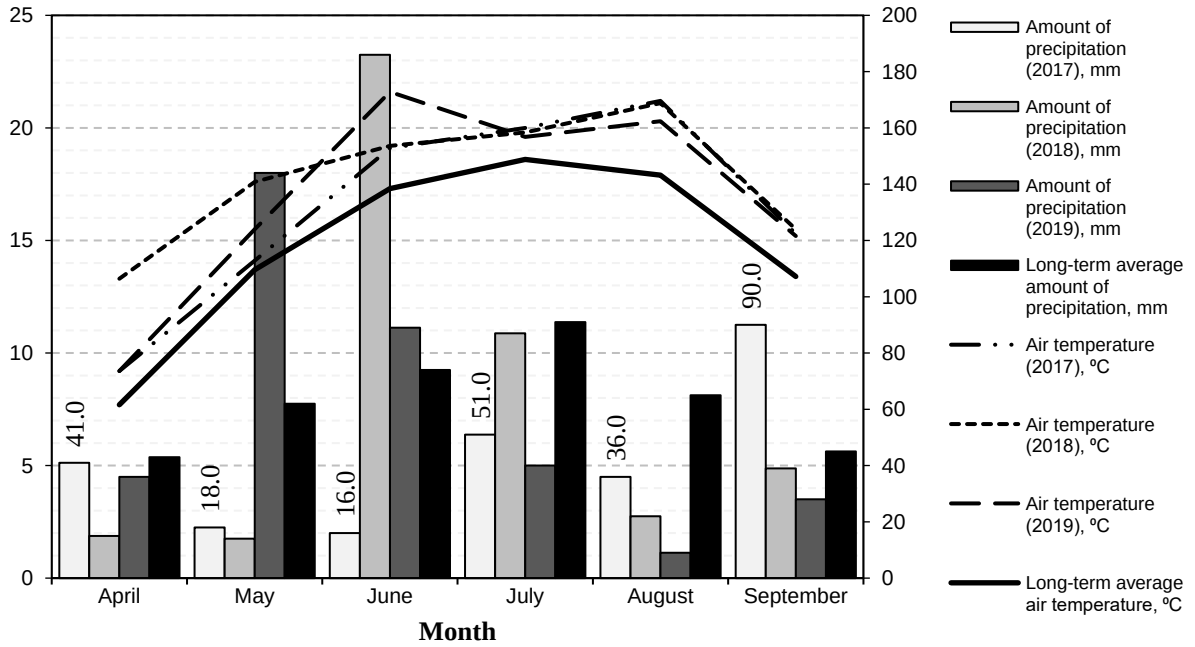

Figure 1. Hydrothermal conditions during the years 2017–2019

In the year of sowing, alfalfa was sprayed with herbicide Bazagran (a.m. bentazol, 3.0 l ha^{-1}) + Achiba (a.m. Hizalofol-P-ethyl, 50 g l^{-1}, Bayer) in a norm of 2.0 l ha^{-1} at alfalfa plant height of 12–14 cm. During the 2nd and 3rd years of life, alfalfa grasses were mowed at the beginning of the budding phase.

The research was carried out by the method of field experiments. The chemical composition of alfalfa dry matter was determined in a certified laboratory of the Institute of fodders and agriculture of Podillia NAAS.

Statistical and correlation-regression analysis of research results was conducted on a personal computer using modern packages of applied programs for mathematical processing MS Excel, Statistica 6.0 and appropriate methods for conducting field experiments (Dospekhov, 1985; Ushkarenko *et al.*, 2009).

Resuts and discussion

With the uncovered method of cultivation, alfalfa crops were infested with weeds, which took the largest share of crops in terms of botanical composition. The cropped grass was mainly represented by such weed species as *Raphanus raphanistrum, Chenopodium album, Thlaspi arvense, Setaria pumila* and others, which germinated at low average daily air temperature and inhibited the growth processes of the main crop. After spraying alfalfa crops in the year of sowing weeding decreased by 85–90%. Due to arid conditions of May and June, as well as uneven distribution of precipitation in July and August, alfalfa plants formed a bush and reached the stemming phase. Already in the first decade of September, it was in the phase of budding-beginning of flowering with a plant height of 29–33 cm, yield capacity of 8.39–8.88 and 2.17–2.26 t ha^{-1} of green mass and dry matter respectively.

During the second and the next years of life, to obtain high-quality plant material (green mass, hay) mowing of alfalfa grass was carried out at the beginning of the budding phase as harvesting in the flowering phase although provides a higher yield of green mass, but of low quality. It is worth noting that regrowth and formation of subsequent hay harvest depended on the timing of mowing and weather conditions.

After the restoration of vegetation in the second year of life alfalfa reached the beginning of the budding phase in 40 days. The grass was formed mainly due to the usage of productive moisture of the autumn-winter period but not from precipitations of April-May (29 mm). In the third year of life, the phase of budding began 5 days later after the restoration of vegetation in spring. It is explained by excessive moisture in May (144 mm, long-term norm of 63 mm). Therefore, there was a reduction in the time of green mass formation to 30–40 days, compared to 2018 – 37–42 days.

Optimization of row spacing from 25.0 to 12.5 cm. provided the highest yield capacity of alfalfa green mass. The studied varieties differed slightly in terms of green mass yield and realization of biological potential. The southern variety of alfalfa 'Angelica' adapted to the conditions of forest-steppe cultivation and in the third year of life provided the largest yield of green mass of 66.15 t ha^{-1} with sowing rates of 8.0 million ha^{-1} and row spacing of 12.5 cm. Variety 'Rosana' distinguished by the stability of green mass yield formation at the level of 58.33 t ha^{-1} (2018) and 61.13 t ha^{-1} (2019). With row spacing of 25.0 cm, the green mass yield was 53.66–56.05 t ha^{-1} for variety 'Rosana' and 51.10–60.20 t ha^{-1} for variety 'Angelica'.

The distribution of biomass between hay harvests differed over the years and amounted to 21.7–27.2% of total yield. The largest percentage difference was observed between the third and the fourth hay harvests – 5.5% and the smallest between the first – the third 1.3–2.3% in the second year of life. Opposite conditions were created for the formation of alfalfa green mass for the third year of life where the percentage of the first hay harvest was the highest – 31.6 and 14.7% for the fourth hay harvest.

The influence of factor "precipitation" increased the duration of the stalking phase in 2019, and therefore the beginning of plant budding was observed after 45 days. High yields of green mass in the first hay harvest were obtained by increasing the height of plants by 7.7–8.1 cm. compared to 2018 which was 69.2–71.9 ± 3.5 cm. Hence, we can state that alfalfa can form stable yields of green mass in compliance with mowing dates.

It is established that there is a strong positive correlation R = 0.731–0.742 between green mass yield formation and hydrothermal conditions. Correlation between obtained data are described by the following multiple regression equations:

$$y = 20.2316 + 0.0299\ x_1 - 0.5143\ x_2;\ R = 0.731 \quad (1)$$

where: y – green mass yield, t ha^{-1}; x_1 – precipitation, mm, x_2 – average daily air temperature, °C.

$$y = 14.7522 + 0.0205\ x_1 - 0.2222\ x_2;\ R = 0.742 \quad (2)$$

where: y – green mass yield, t ha^{-1}; x_1 – precipitation, mm, x_2 – length of daylight, hours. min.

In characterizing the nutritional value of alfalfa fodder, the dry matter content plays an important role, the indices of which were determined by hydrothermal conditions, seeding rates and row spacing. Obtained data are confirmed by scientists from different countries. When passing the stages of organogenesis, the dry matter content in plants increases and proportion of leaves decreases (Aksoy, Nursoy, 2010; Ayhan *et al.*, 2004) and the percentage of fibre increases compared to early stages of growth and development (Chatepa, 2012; Geren *et al.*, 2003; Homolka *et al.*, 2008; Yu *et al.*, 2003; Karpenko *et al.*, 2020).

During crop vegetation with the increase of daylight duration (14:23–16:19) and optimal hydrothermal conditions, dry matter content in alfalfa green mass increased from the first to the second hay harvests from 23.72–23.76 to 26.74–26.91% respectively. And with reduction of daylight duration and temperature lowering, in the third hay harvest dry matter decreased to 21.34–21.44 % and in the fourth – to 21.77–22.01%.

Under conditions of excessive moisture, dry matter content in the green mass of the first hay harvest of alfalfa was the lowest 18.52–19.27%, in the second hay harvest it gradually increased to 20.28–20.58%. With air temperature rising and uneven distribution of precipitation in the third hay harvest, the indices were already 21.62–22.06% and the largest ones were received in the fourth hay harvest – 27.32–27.52%.

It can be concluded that dry matter content was determined by hydrothermal conditions and the factors studied. With the increase of seeding rate from 4.0 to 8.0 million ha^{-1}, it decreased from 23.02 to 22.52% and increased with increasing row spacing from 22.62–22.83 to 22.82–22.94%, regardless of variety. Alfalfa

variety 'Rosana' dominated in dry matter content by 0.16% (22.88%) over 'Angelica' variety – 22.72%.

It was found that twice the narrowing of row spacing contributed to dry matter yield increase by 0.63–2.04 t ha^{-1} at alfalfa seeding rates of 6.0–8.0 million ha^{-1}. At the same time, yielding capacity indices, regardless of variety, distinguished by stability, which for 'Rosana' variety amounted to 12.65–12.97 t ha^{-1}, and in sum for three years – 27.45 t ha^{-1} (Table 1).

Table 1. Accumulation of dry matter in the green mass of alfalfa (*Medicago sativa* L.) depending on sowing rates and row spacing, t ha^{-1}

Row spacing, cm (factor C)	Seeding rate, million ha (factor B)	Variety 'Rosana' (factor A)				Variety 'Angelica'			
		Vegetation years			Sum for 3 years	Vegetation years			Sum for 3 years
		2017	2018	2019		2017	2018	2019	
12.5	4.0	1.96	12.05	12.21	26.22	1.81	11.50	13.12	26.43
	6.0	1.83	12.65	12.97	27.45	1.58	12.42	13.81	27.81
	8.0	2.26	12.06	12.89	27.21	2.17	11.64	13.79	27.60
25.0	4.0	1.62	12.44	12.59	26.65	1.17	11.84	13.18	26.19
	6.0	1.68	12.49	12.65	26.82	1.27	11.72	13.14	26.13
	8.0	1.21	12.46	11.72	25.39	1.00	11.90	12.66	25.56
LSD_{05}	2017: A – 0.04; B – 0.05; C – 0.07; 2018: A – 0.11; B – 0.10; C – 0.12; 2019 A – 0.09; B – 0.09; C – 0.11.								

Southern ecotype of alfalfa 'Angelica' provided maximum dry matter output of 13.81 t ha^{-1} at the third year of life at a seeding rate of 6.0 million $^{-1}$ha and row spacing of 12.5 cm or was 6.5% higher than 'Rosana' and 11.2% compared to the second year of life. During three years of grass usage, the output of dry matter was 27.81 t ha^{-1} and there was a tendency to reduction of dry matter output in other variants.

It is known that fodder quality is one of the yielding capacity indices of cattle where alfalfa is the main component in the diet of animals in the form of green mass, hay or haylage.

Content of crude protein, neutral and acid-detergent fibre was determined in the dry matter of alfalfa green mass collected at the beginning of the budding phase. With the reduction of daylight length (period of the 3–4th hay harvest formation) was established regularity of increase in crude protein content from 21.11 to 23.47%. In the first and second hay harvests, there was a decrease in the percentage of crude protein 19.79–20.31 and 17.83–20.14% respectively, which is explained by increased dry matter content in the green mass. Regardless of the width of row spacing, the content of crude protein in variety 'Rosana' increased by 0.38–0.53 and in variety 'Angelica' by 0.63–0.79% – (Fig. 2, 3).

The southern ecotype of alfalfa variety Anzhelika provided the highest indicators during the third year of vegetation (13.81 t ha^{-1}) under the seeding rates of 6.0 million seeds ha^{-1} and row spacing of 12.5 cm. Over three years of mowing the dry matter yield were 27.81 t ha^{-1}. Under other seeding rates and changes in-row the spacing, there was observed a tendency towards the decrease in the dry matter yield.

Agricultural and ecological conditions and the elements of cultivation technology influenced the crude protein content, neutral- and acid-detergent fibre content over the years of vegetation of alfalfa of different dormancy classes. During the second year of vegetation with a reduction in the daylight duration and optimal moisture supply of plants at the time of formation of the third and fourth harvests, the crude protein content was the highest, which was 22.10–23.47 and 21.11–22.40%, respectively. In the first and second harvests, the percentage of crude protein in the dry matter of alfalfa decreased significantly and amounted to 20.18–20.25 and 18.08–18.32% (Fig. 2).

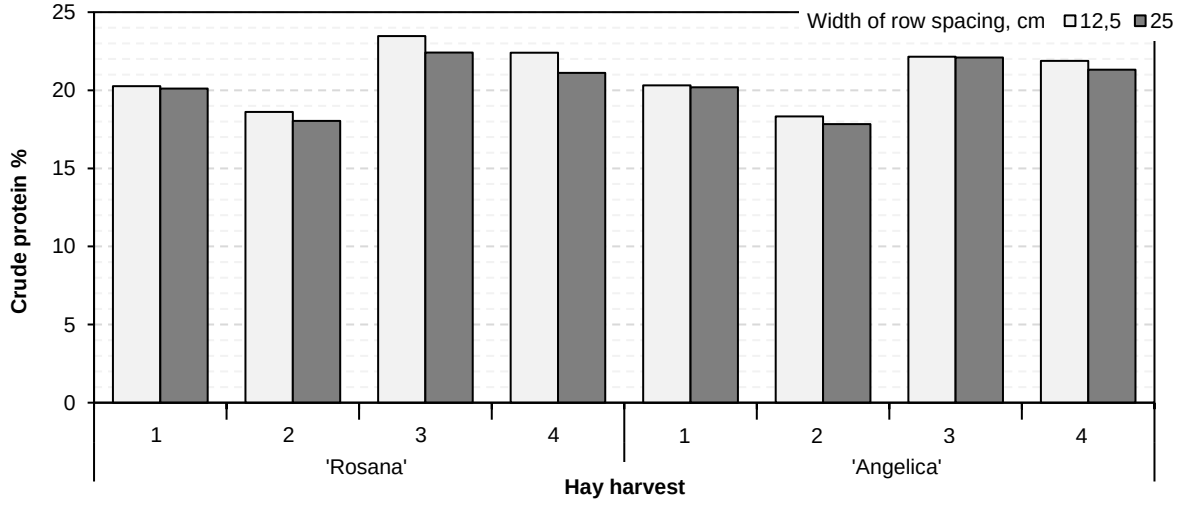

Figure 2. The content of crude protein in alfalfa dry matter of varieties 'Rosana' and 'Angelica' depending on hay harvest and row spacing width for the second year of life, %

In our opinion, due to dense grass formation of the crop, evaporation of moisture decreased with narrowing of row spacing, and conditions for nitrogen transformation in plants improved. Therefore, the content of crude protein in alfalfa of varieties 'Rosana' and 'Angelica' differed little between them and averaged 20.68–21.37 and 20.67–21.07% respectively. In particular, narrowing of row spacing increased crude protein content by 0.55–0.58% and the factor "variety" provided a difference between ecotypes of 0.15% in favour of the 'Rosana' variety.

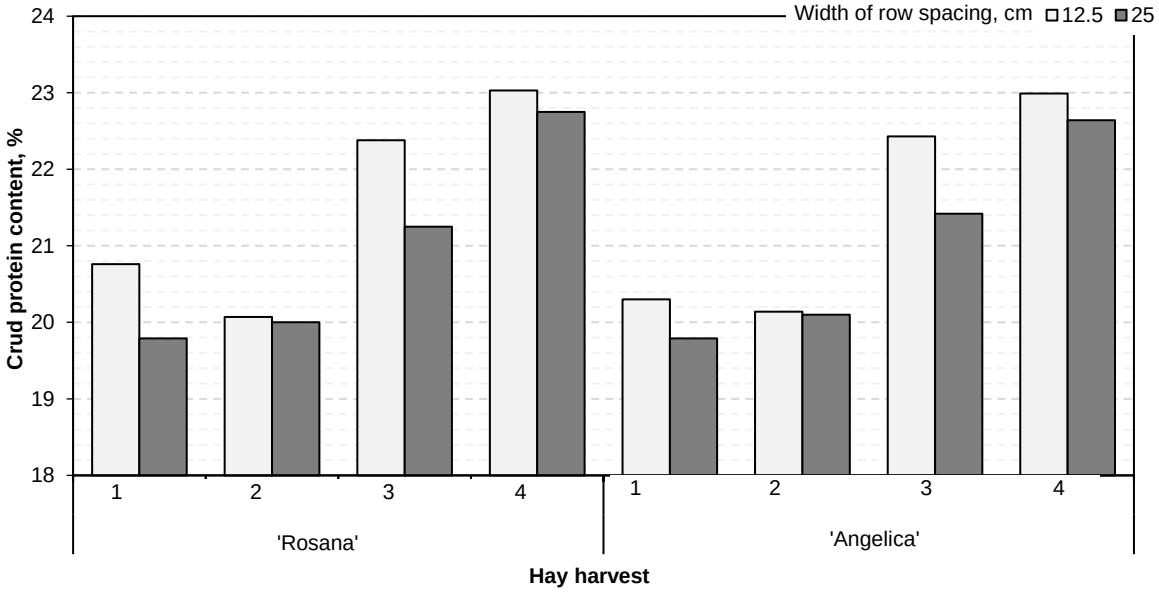

Figure 3. The content of crude protein in alfalfa dry matter of varieties 'Rosana' and 'Angelica' depending on hay harvest and row spacing width for the third year of life, %

The highest output of crude protein (5.86–5.87 t ha^{-1}) the varieties provided at sowing rates of 6.0 million ha^{-1} and sowing with row spacing of 12.5 cm. For three-year usage of alfalfa grass at the beginning of budding phase with an increase in sowing rate from 6.0 to 8.0 million ha^{-1} and twice increase of row spacing width was registered the tendency to reduction of crude protein output in both varieties from 5.40–5.55 to 5.37–5.39 t ha^{-1} (Fig. 4).

According to the practice and research of scientists in the system of evaluation of fodder carbohydrate nutrition in the diets of ruminants, it is advisable to control the content of neutral-detergent fibre (NDF) and acid detergent fibre (ADF). The level of NDF in fodder is related to dry matter consumption and ADF is related to digestibility. The authors believe that for highly productive cows (40 kg day^{-1} of milk) it is recommended to optimize rations with a content of NDF not more than 32%, and for cows with lower-yielding capacity (20 kg day^{-1} of milk), so as not to minimize fodder consumption – not higher than 44% (Ruban *et al.*, 2018).

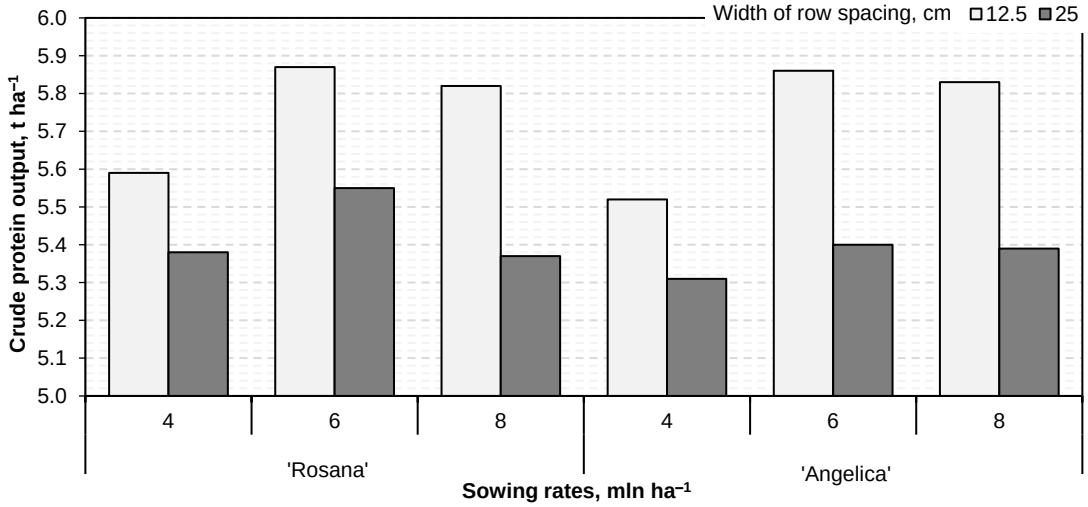

Figure 4. The output of alfalfa crude protein for varieties 'Rosana' and 'Angelica', depending on sowing rates and row spacing width for three years of life, t ha^{-1}

Scientists from province Gansu (Nan *et al.*, 2019) and Huang-Huai-Hai, Shandong Province (China) (Lü *et al.*, 2019) found a tendency to decrease the content of neutral and acid-detergent fibre when increasing seeding rate and narrowing row spacing from 40 to 15 cm. They received the highest content of raw protein 20.06% at a sowing rate of alfalfa 16 kg ha^{-1}.

According to data of scientists (Nan *et al.*, 2019), the lowest content of neutral detergent fibre (31.74%) and acid detergent fibre (25.64%) was at alfalfa seeding rates of 24 kg ha^{-1} with row spacing of 10 and 15 cm, others proved the dependence of indices on phases of alfalfa growth and development (Mysenko *et al.*, 2019).

Our data are confirmed by the results of foreign and domestic scientists and for variety 'Rosana' they are within limits from 29.11 to 33.97% NDF and 22.21–24.64% ADF, depending on the sequence of mowing. Alfalfa variety 'Angelica', from the first to the fourth hay harvest, provided indices at the level of 29.01–31.47% of NDF and 21.32–23.01% of ADF. The highest percentage of NDF and ADF was observed on alfalfa grass of the second year of life and was within limits of 30.72–34.91 and 23.02–24.60% respectively. For the third year of alfalfa grass usage, the content of NDF and ADF in dry matter decreased to 27.09–33.03 and 19.53–24.18% respectively (Fig. 5).

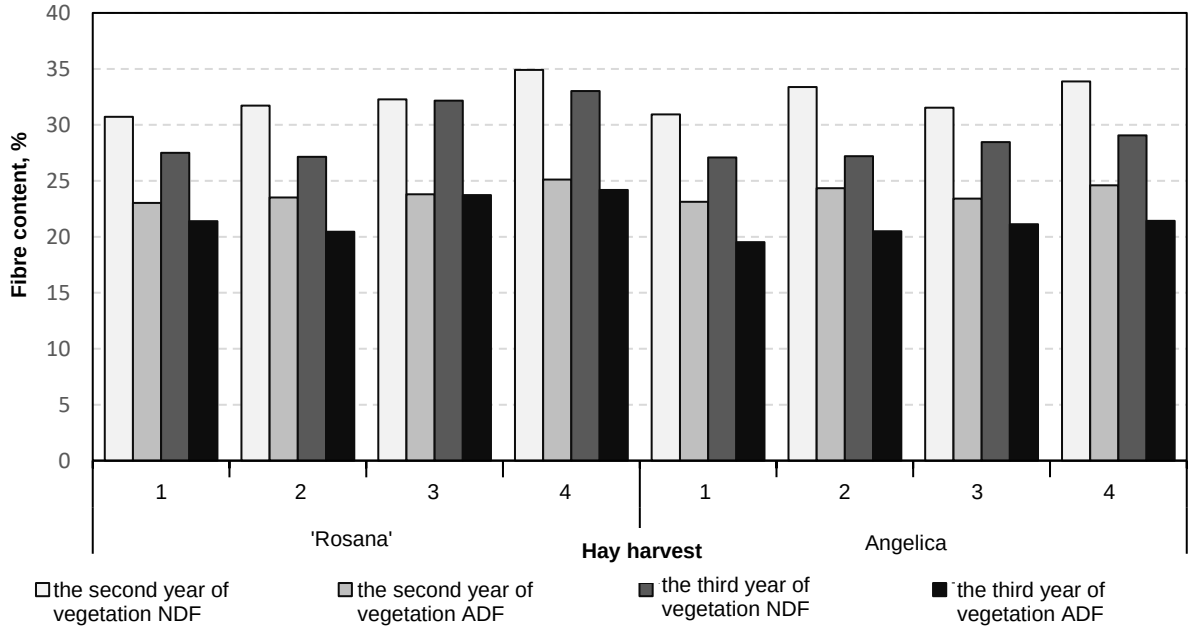

Figure 5. Content of neutral and acid detergent fibre in dry matter of alfalfa green mass depending on variety and hay harvest, %

Conclusion

Alfalfa (*Medicago sativa* L.) of southern ecotype, variety 'Angelica' adapted to the soil and climatic conditions of the Forest-Steppe and was not inferior to variety 'Rosana' in terms of yielding capacity. The content of alfalfa crude protein in the phase beginning of budding averaged in varieties 'Angelica' and 'Rosana' 21.07–21.37% for sowing with row spacing of 12.5 cm and 20.67–20.68% – 25.0 cm. Narrowing of row spacing contributed to the increase of crude protein content by 0.55–0.58%. The content of neutral and acid-detergent fibre during two years of life averaged 31.18 and 23.15% in variety 'Rosana' and 30.19 and 22.26% in variety 'Angelica' at a sowing rate of 6.0 million pcs ha^{-1} and sowing with row spacing of 12.5 cm. In terms of fodder yielding capacity, alfalfa varieties 'Angelica' and 'Rosana' were equivalent and provided dry matter output of 27.45–27.81 and crude protein output of 5.86–5.87 t ha^{-1}.

Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this paper.

Author contributions

MK – study conception and design, drafting of the manuscript;
GD – an author of the idea, guided the research;
AB – analysis and interpretation of data and is the corresponding author;
NG, LB – acquisition of data, drafting of the manuscript;
VM – performed the literature data analysis and discussion of the results;
SS, VO – critical revision and approval of the final manuscript.
All authors read and approved the final manuscript.

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RÄHKMULDADE SEISUND JA LEVIK EESTI PÕLLUMAJANDUS-NING METSAMAASTIKES

STATUS AND DISTRIBUTION OF RYHKY SOILS IN ESTONIAN AGRICULTURAL AND FOREST LANDSCAPES

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ABSTRACT. In the overview nomenclature, properties, distribution, productivity and usage of *ryhky soils*, as the Year 2021 Soil of Estonia, are treated. As synonyms of the name *ryhky soils*, also the names *pebble rendzina* and *gravelly soils* have been used in Estonian Soil Classification (ESC). In overview, the main attention is paid to dry, fresh and moist rich in coarse calcareous material mild-humuous (mull-type) soils. After WRB these soils may be characterized by prefix qualifiers as *calcaric* or *eutric*, *skeletic* or *hyperskeletic* and *rendzic* or *mollic CAMBISOLS, LITHOSOLS* or *REGOSOLS*, whereas all of them have *endogleyic* versions as well. The wet and eroded *ryhky soils* have been excluded from the overview as their properties depend not so on the coarse calcareous earth content as on feeding their soil water or water erosion. The area of *ryhky soils* forms 6.3% from whole Estonian soil cover and 11.1% from the arable land. The main criteria of *ryhky soil* species' determination are calcareousness, content and shape of coarse soil fragments, and water regime of soil cover. By ESC six soil species have been determined, from which three ones have *endogleyic* character. The fine earth texture of *ryhky soils* is mainly loam. From the coarse fractions, the indicative role belongs to the small stones (ryhk, pebble and shingle). The morphology, humus status and suitability of *ryhky soils* for management are treated on the level of soil species independence of land use (arable, forest or grassland).

Sissejuhatus

Eesti aasta 2021 mullaks on valitud rähkmuld. Rähkmuldade perekonda iseloomustab suur liikide omaduste amplituud ja erimite mitmekesisus. Järgnevas ülevaates tutvustatakse suhteliselt lähedaste agronoomiliste ja metsakasvatuslike omadustega normaalse arenguga põuakartlikke, parasniiskeid ja niiskeid koreserikkaid karbonaatseid muldasid. Siinjuures ei käsitleta alaliselt liigniiskeid (märgi) rähkseid gleimuldi ega vee-erosioonist tugevasti mõjutatud erodeeritud rähkmuldi, kuna nende olemus ei sõltu niivõrd korese sisaldusest kui liigniiskusest või vee-erosioonist.

Rähkmuldade tunnused ja nomenklatuur

Rähkmullad on rohkesti karbonaatset korest sisaldavad (ehk koreselised) humaatse (ehk pehmehuumusliku või mull-tüüpi) huumusega mullad. Rähkmuldade üheks olulisemaks tunnuseks on karbonaatse mullapeenese ja korese sügavus maapinnalt, mida testitakse 10% soolhappega kihisemise järgi ning mis on kõigis rähkmuldades kõrgemal kui 30 cm. Eestis kasutatava mulla korese ja lõimiste käsitlemise Katšinski süsteemi järgi on mulla korese ja peenese osakeste läbimõõdu vaheliseks piiriks 1 mm (Astover jt, 2013).

Rähkmullaliikidele nime andvaks tunnuseks on karbonaatsete peenkivide (Ø 1−10 cm) kuju ja sisaldus. Rähkmuldade kores pärineb valdavalt massiivsest lubja- ja/või dolomiidirikkast kivimist. Sõltuvalt nende murenemisele (rabenemisele) järgnevatest geoloogilistest protsessidest (kulutus, transport) eristatakse kolme erineva kujuga peenkivide liiki: rähk, veeris ja klibu. Rähk on teravaservaliste murdepindadega, veeris suuremal või vähemal määral ümardunud rähk ning klibu ümardunud ja lapikuks lihvitud peenkivid. Vastavalt koresele eristatakse rähk-, veeris- ja klibumuldasid. Niiskusoludest lähtuvalt on käesolevas töös

vaatluse all põuakartlikud (kuivad), parasniisked (värsked) ja niisked (rõsked) rähkmullad.

Eesti muldade klassifikatsiooni (EMK) järgi käesoleva ülevaate jaoks valitud rähkmuldade liiginimetused, mullastiku kaartidel kasutatavad koodid ja tüüpprofiilid on esitatud tabelis 1. Rähkmuldade profiilis on huumus-(A)horisondi järgne või metsades O–A (kus O on metsakõdu) järgne sisseuhte-(B-, Bw-)horisont nõrgalt välja kujunenud või puudub. Väljauhte horisondid (leetjad ja leede-) aga hoopiski puuduvad. Tüüpiliste profiilide kõrval leidub paljuski üksteisest suuremal või vähemal määral erinevaid profiile. Nii võivad profiilis esineda üleminekuhforisondid AB ja AC, millistest esimene näitab nõrka sisseuhet huumushorisondi alumisse ossa, teine aga hoopiski sisseuhte puudumist kuna A-horisont läheb sujuvalt üle lähtekivimiks ehk C-horisondiks. Hästi väljakujunenud B-horisonti esineb vaid tüsedamatel, enamarenenud muldadel. Rähkmuldade hulgas on ka mullad, millistes lähtekivimile järgneb lausaldane paas ehk (R-horisont), mis lasub 30 cm-st sügavamal ning erandina ka koresevabad karbonaatsed liivmullad. Eriilmelisi rähkmuldi näeb joonistelt 1–5.

Tabel 1. Rähkmuldade liiginimed, koodid ja tüüpprofiilid
Table 1. *Species names, codes and typical profiles of ryhky (pebble) soils*

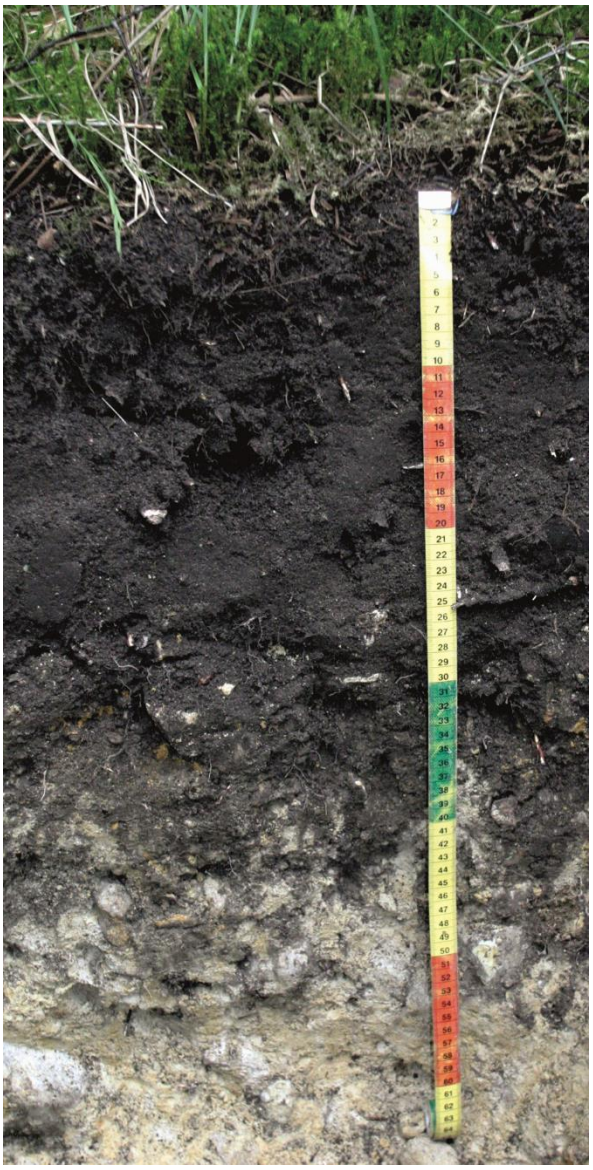
Kood *Code*	Mulla liik *Soil species*	Tüüpprofiilid *Typical profiles*	Osatähtsus[1] *Role*, %
K	Rähk-(veeris)mullad *Ryhky (pebble) soils*	A-AB-BC[1]-C, A-AB-Bw[2]-C	61
Kg	Gleistunud rähk-(veeris)mullad *Gleyed ryhky (pebble) soils*	A-AB-BC-Cg[3], A-Bw-BC-Cg	17
Kr	Koreserikkad rähk-(veeris-)mullad *Coarse rich ryhky (pebble) soils*	A-AB-BC-C	14
Krg	Gleistunud koreserikkad rähk-(veeris-)mullad *Gleyed coarse rich ryhky (pebble) soils*	A-ABC-Cg	5
Kk	Klibumullad *Shingle soils*	A-(B)C-C	2
Kkg	Gleistunud klibumullad *Gleyed shingle soils*	A-(BC)-Cg	<1

[1] Osatähtsuse % on hinnanguline / *Expert estimation*

Joonis 1. Rähkmuld. Mullateaduse õppetooli fotokogu
Figure 1. *Ryhky soil*

Joonis 2. Koreserikas rähkmuld. Mullateaduse õppetooli fotokogu
Figure 2. *Coarse rich ryhky soil*

Joonis 3. Rähkmuld peenel rähal. Mullateaduse õppetooli fotokogu
Figure 3. *Ryhky soil on fine ryhk*



Joonis 4. Gleistunud rähkmuld. Mullateaduse õppetooli fotokogu
Figure 4. *Gleyed ryhky soil*

Joonis 5. Klibumuld. Mullateaduse õppetooli fotokogu
Figure 5. *Shingle soil*

Need rähkmullad, mis on kevaditi ja sügiseti lühiaegselt liigniisked, mida näitab roostetäppide või –pesakeste ja sinakashallide või roostevärvi gleilaikude esinemine alusmulla horisontides (Cg, Bg), on gleistunud ehk niisked rähkmullad (Kg, Krg, Kkg). Kui haritavate rähkmuldade huumushorisont kihiseb juba mulla pinnalt, siis metsa- ja rohumaade rähkmuldade kihisemise piir on sügavamal, kuid diagnostilisele tunnusele vastavalt 0−30 cm sügavusel.

Looduslike koreserikaste rähk(veeris)- ja klibumuldade (Kr, Kk, Krg, Kkg) muldkate on tunduvalt õhem võrreldes enamarenenud muldadega (K, Kg; tabel 2). Õhukeste huumushorisontide (12−20 cm) esinemine looduslikel aladel näitab, et tegemist on noorte muldadega. Haritaval maal on huumushorisondid seoses maaharimisega muudetud ühtlasemaks ja tüsedamaks (24−30 cm).

Tabel 2. Rähkmuldade huumushorisondi ja muldkatte tüsedused
Table 2. *Thicknesses of ryhky soils' humus horizon and soil cover*

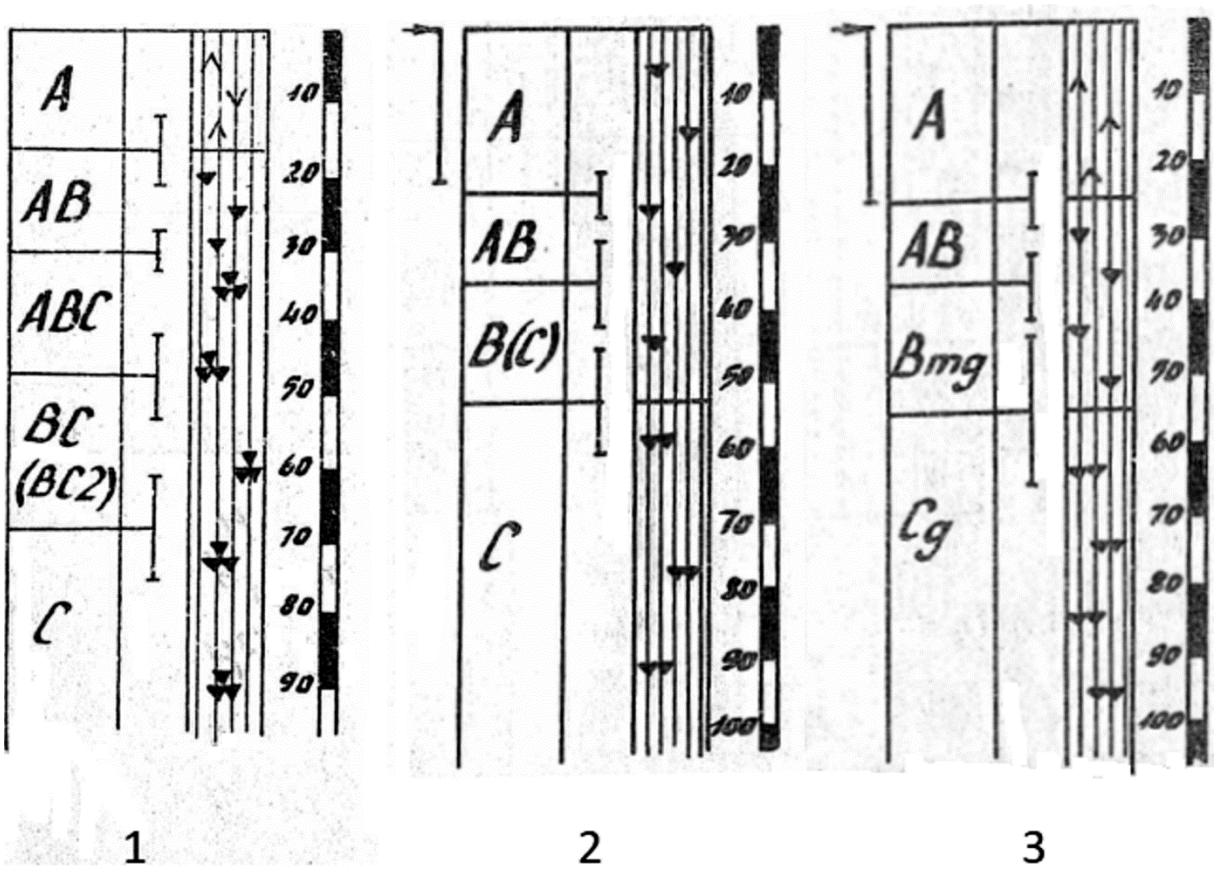
Mulla kood *Soil code*	Maakasutus *Landuse*	A-horisont *A horizon*, cm	Muldkate *Soil cover*, cm
K Kg	Põld / *Arable land*	23−31	40−65
	Mets / *Forest land*	19−27	40−60
Kr Krg	Põld / *Arable land*	17−25	30−40
	Mets / *Forest land*	13−21	25−35
Kk Kkg	Rohumaa / *Grassland*	12−20	25−30

Eestis domineerivad valkjashallist rähkmoreenist moodustunud (nn tüüpilised) rähkmullad. Veerismuldade lähtekivimiks on veeriselised ja kruusased mandrijää sulamisvete oosistike või voorestike setted. Üsna tavaline on räha ja veerise koosesinemine muldkattes ja lähtekivimis. Klibumuldade lähtematerjaliks on rannavallide klibu. Mulla erinevate horisontide rähksus (peenkivisus) määratakse välitöödel nende sisalduse mahu ja maapinnal või kaeve seinal oleva korese katteväärtuse järgi (Astover jt, 2013).

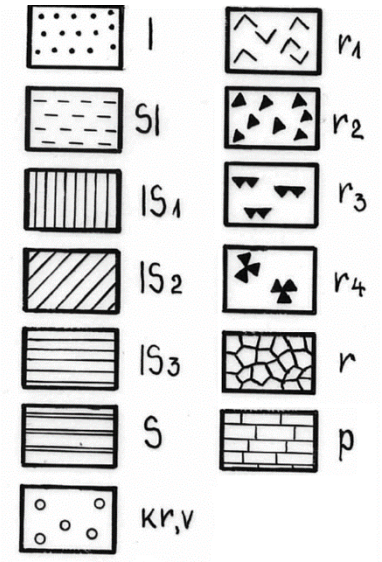
Peale peenkivide esineb rähkmuldades tavaliselt ka suuremat kivifraktsiooni (väikekivid Ø 10−20 cm ja suurkivid Ø >20 cm) ning peent korest (osised Ø 1–10 mm ehk kruusa ja mügi) (EPP, 1983). Olenevalt rähkmuldade lähtematerjalist võib nendes esineda karbonaatse korese kõrval ka suuremal või vähemal määral raudkivimilist (tardkivimitest pärinevat) korest, suuri kive, kruusa (kr) ja jämedateralist liiva (jl). Kui peenkivide esinemise määr tehakse kindlaks väliuurimiste käigus, siis peene korese sisaldus määratakse laboris proovide sõelumise teel. Kivide sisalduses on suured erinevused huumushorisondi ja mulla lähtekivimi vahel. Klibumuldades (Kk Kkg) on mullapeenese sisaldus väga väike või praktiliselt puudub, kuid samas ei ole neis ka suuremaid kive.

Rähkmuldade peenese põhilõimiste vähenev järjekord on: liivsavi (ls), saviliiv (sl), liiv (l) ja savi (s) ehk protsentides vastavalt 80 > 15 > 4 > 1. Lisaks põhilõimistele esineb täiendsõnadega täpsustatud lõimiseid nagu tolmjas (tsl, tls), peenliivakas (pl) ja kruusakas (kr). Detailsel lõimise käsitlemisel eristataks kerged (ls_1), keskmised (ls_2) ja rasked (ls_3) liivsavid. Mullakaartidel ja tabelites on peenese lõimised esitatud koos koresega valemi kujul, kus erinevate lõimisekihtide happesused on näidatud tingmärkide abil: ("=" näitab, et pH on 4,6–5,0; "–" et pH on 5,1–5,5; ilma tingmärgita on nõrgalt happelised (pH 5,6–6,5) või neutraalse pH-ga (6,6–7,2) kihid ning "+" tähendab, et muld on karbonaatne (pH ≥7,3).

Eesti domineerivate rähkmullaerimite koondprofiile, mis on koostatud suure hulga üksikprofiilide alusel, tuleks sisuliselt võtta kui vastavate erimite Eesti keskmisi mudelprofiile (joonis 6 ja 6A).



Joonis 6. Rähkse liivsavimulla mudelprofiil metsas (1 – K) ja põllul (2 – K) ning gleistunud rähkse liivsavimulla mudelprofiil põllul (3 – Kg)
Figure 6. *Ryhky loamy soil model profile in forest (1 – K) and in field (2 – K), and gleyed ryhky loamy soil model profile in the field (3 – Kg)*



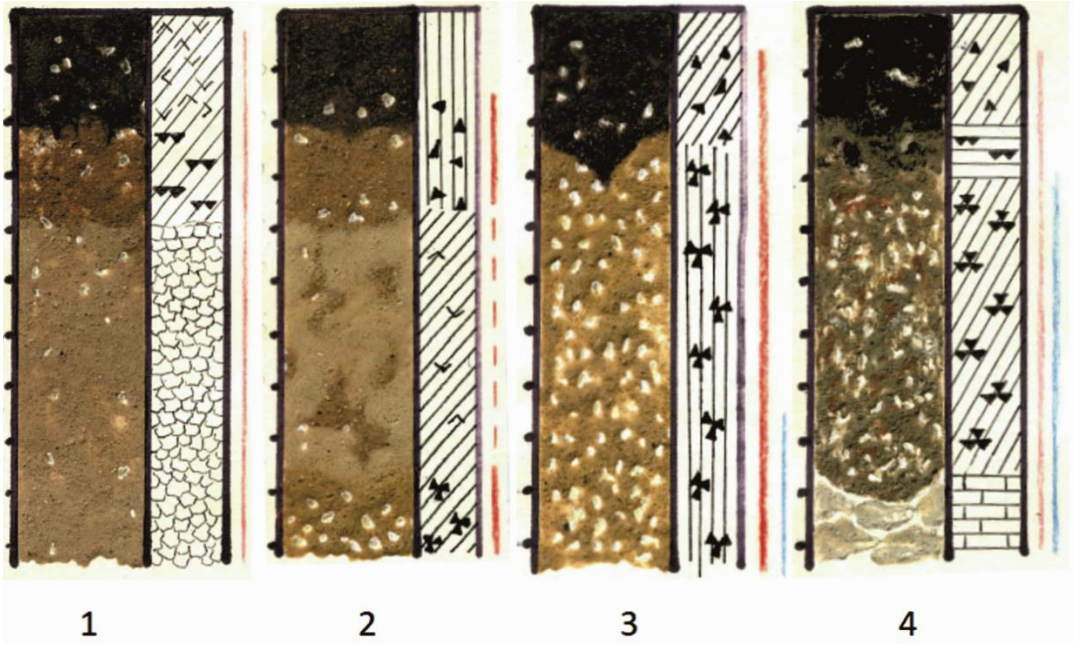
Joonis 6A. Tingmärgid mudel-, liim- ja joonisprofiilide kohta. Mullapeenes: l – liiv, sl – saviliiv, ls_1 – kerge liivsavi, ls_2 – keskmine liivsavi, ls_3 – raske liivsavi, s – savi, kr, v – kruus, veeris; koreselisus: r_1 – nõrgalt, r_2 – keskmiselt, r_3 – tugevasti või r_4 – väga tugevasti rähkne (või veeriseline); r – rähk, p – paas
Figure 6A. *Signs to model, glue and drawing profiles. Fine earth: l – sand, sl – loamy sand, ls_1 – light loam, ls_2 – medium loam, ls_3 – heavy loam, s – clay, kr, v – gravel, pebble; content of coarse fractions: r_1 – lightly, r_2 – average, r_3 – strongly or r_4 – very strongly ryhky (or pebbly); r – ryhk, p – limestone*

Mudelprofiilide kohta on andmebaasides olemas kõigi horisontide mulla olemust ja taimekasvatuslikku potentsiaali kajastavad karakteristikud ja nende variatsioon-statistilised analüüsid (EPP, 1983). Üldreeglina sisaldavad kõigi rähkmuldade huumuskatted (pealismullad) oluliselt vähem korest võrreldes alusmulla ja eriti lähtekivimiga (tabel 3). Põllumaade K ja Kg muldade künnikihid on valdavalt väga nõrgalt või nõrgalt rähksed (veeriselised), kuid nende alumiste horisontide koresesisaldus on valdavalt tugev või ka keskmine. Samas võib esineda ka mitmesuguseid teisi variante. Koreserikaste rähk(veeris-)muldade (Kr, Krg) huumushorisondid on valdavalt keskmiselt koreselised, kuid alumised kihid kas tugevasti või väga tugevasti koreselised või esineb lausaldane rähk, veeris või klibu. Välipäevikutes, aruannetes või publikatsioonides võivad rähkmullad olla fikseeritud kas liimprofiilide või joonisprofiilide kujul (joonis 7 ja 8).

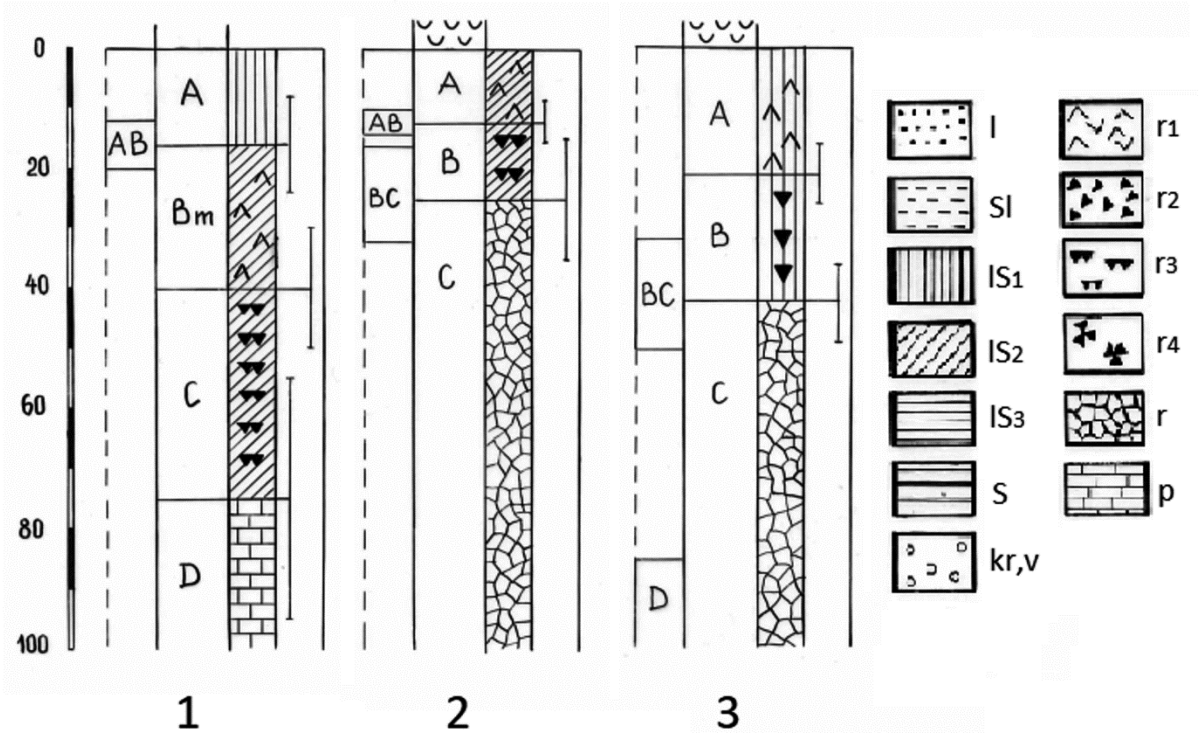
Tabel 3. Peenkivid (Ø 1–10 cm), peen kores (Ø 1–10 mm) ja kivid (Ø >10 cm) rähkmuldades
Table 3. *Small stones (Ø 1–10 cm), coarse earth (Ø 1–10 mm) and large-size stones (Ø >10 cm) in ryhky soils*

Mullad *Soils*	Peenkivisuse valem[1)] *Formula of small stones*	Peene korese osatähtsuse % *Percentage of coarse earth*	Kivisus *Stoniness*, $m^3\,ha^{-1}$
K Kg	$r(v)_{1\text{-}2}$ / $r_{3\text{-}2}$ ($v_{3\text{-}2}$)	5−7 / 24−36	5−20, II aste / *degree*
Kr Krg	$r(v)_3$ / $r_{4\text{-}5}$ ($v_{4\text{-}5}$) või r(v)	22−24 / 39−65	20−50, III aste / *degree*
Kk Kkg	$kb_{3\text{-}4}$ / kb_5 või kb	10−20 / <5[3)]	<5, I aste / *degree*

1) Peenkivisus (% mahust) huumuskattes/alusmullas: 1 – 2–10; 2 – 10–20; 3 – 20–30; 4 – 30–50; 5 – 50–70 ning >70 on lausaldane r, v, või kb; 2) kruusa ja mügi % mullapeenese massist (huumuskate/alusmuld), 3) osakeste Ø 1–10 mm protsent klibu (Ø 1–10 cm) mahust.
1) Stoniness (volume %) of small stones in humus cover / subsoil: 1 – 2–10; 2 – 10–20; 3 – 20–30; 4 – 30–50; 5 – 50–70 and >70 ryhk (r), pebble (v) or shingle (kb); 2) Percentage of gravel from fine earth weight (humus cover/subsoil); 3) Particles Ø 1–10 mm percentage from shingles (Ø 1–10 cm) volume.



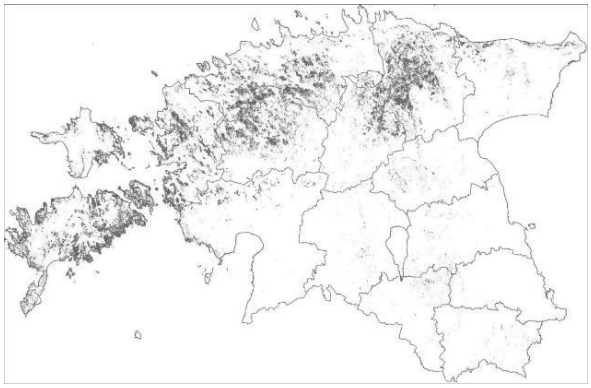
Joonis 7. Rähkmuldade liimprofiilide kogum.
Mullaerimid: 1 – Rähkmuld, $r_1ls_2/r_3ls_2/r$; 2 – Rähkmuld, $ls_1/r_2ls_1/r_1ls_2/r_4ls_2$; 3 – Koreserikas rähkmuld, r_2ls_2/r_4ls; 4 – Gleistunud koreserikas rähkmuld, $r_2ls_2/r_3ls_3/r_4ls_2/p$; mullapeenese ja korese koodidele vastavaid nimetusi vt. jooniselt 6A
Figure 7. *Ryhky soils' profiles prepared with glue.*
Soil varieties: 1 – Ryhky soil, $r_1ls_2/r_3ls_2/r$; 2 – Ryhky soil, $ls_1/r_2ls_1/r_1ls_2/r_4ls_2$; 3 – Coarse rich ryhky soil, r_2ls_2/r_4ls; 4 – Gleyed coarse rich ryhky soil, $r_2ls_2/r_3ls_3/r_4ls_2/p$; for the names of fine earth and coarse fractions after their codes see Figure 6A



Joonis 8. Rähkmuldade joonisprofiilide kogum.
Mullad: 1 – Rähkne põllumuld (K), 2 – Koreserikas metsa rähkmuld (Kr) ja 3 – Rähkne metsamuld (K); mullapeenese ja korese koodidele vastavaid nimetusi vt jooniselt 6A
Figure 8. *Collection of drawings about ryhky soils' profiles.*
Soils: 1 – Ryhky arable soil (K), 2 – Coarse rich ryhky forest soil (Kr) and 3 – Ryhky forest soil (K); for the names of fine earth and coarse fractions after their codes see Figure 6A

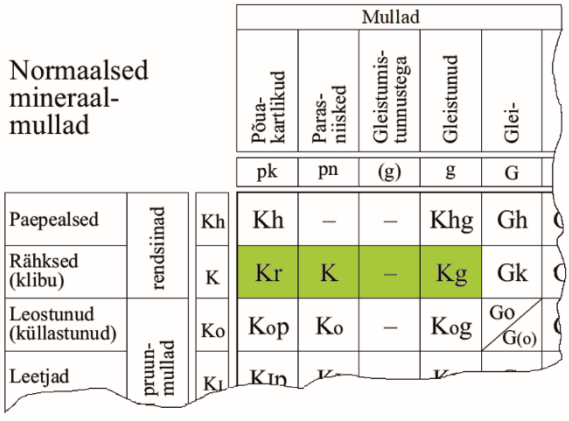
Rähkmuldade levik Eestis

Rähkmuldade peamised levikualad on Põhja- ja Loode-Eesti ning saared. Nad on valdavateks muldadeks Harju, Lääne ja Saare maakonnas (joonis 9). Rähkmuldadega kaasnevateks (kooslusi moodustavateks) mullaliikideks on reljeefi kõrgematel osadel parasniisked leostunud (Ko) ja paepealsed Kh) mullad (joonis 10). Reljeefi madalamatel osadel võivad rähkmuldadele kaasneda gleistunud leostunud (Kog) ning rähksed (Gk) ja leostunud (Go) gleimullad. Rähkmuldade ülekaaluga muldkattele on iseloomulik mullastiku suur kirjusus nii lõimise, niiskusrežiimi kui ka produktiivsuse suhtes (joonis 11).

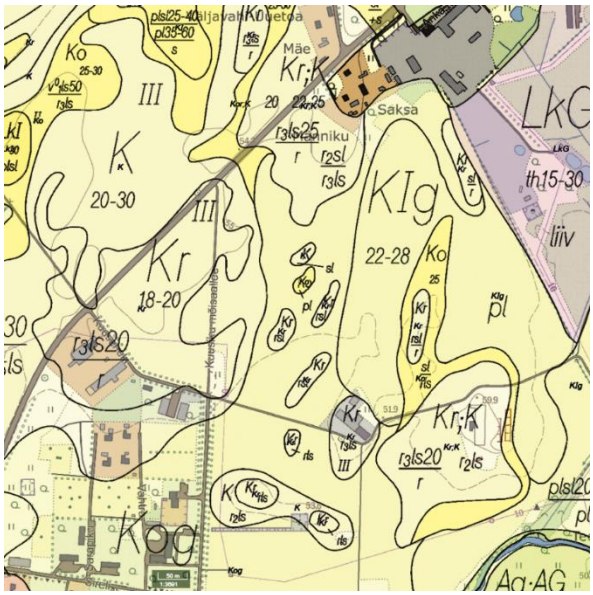


Joonis 9. Rähkmuldade levik Eestis
Figure 9. *Distribution of ryhky soils in Estonia*

Eesti maapinnast on rähkmuldadega kaetud *ca* 6,3%. Kõigist rähkmuldadest on veidi alla 2/3 põllumaad, veidi alla 1/3 metsamaad ning vaid *ca* 4% looduslikke rohumaid. Kui kogu kaardistatud pindalast moodustavad põllumaade rähkmullad tähelepanuväärselt suure osa (*ca* 11,3%), siis metsa – ja rohumaade puhul on see protsent kordades väiksem (vastavalt 3,2 ja 2,5%).



Joonis 10. Rähkmuldade hüdrokateena alates kuivadest kuni niiskete muldadeni ja nendega kaasnevad mullaliigid.
Kateena mullaliigid: Kr – kuivad rähkmullad sh Kk – klibumullad; K – parasniisked rähkmullad; Kg – niisked rähkmullad sh niisked koreserikkad (Krg) ja klibumullad (Kkg)
Figure 10. *Hydrocatena of ryhky soils beginning from dry to moist soils and associated to them soil species.*
Soil species of the catena: Kr – dry ryhky soils among them Kk – shingle soils; K – normal moisture (fresh) ryhky soils; Kg – moist ryhky soils, among them moist coarse rich ryhky soils (Krg) and shingle soils (Kkg)



Joonis 11. Väljavõte Eesti 1:10 000 mullastiku kaardilt (Maa-amet, 2001; Maa-uuringud, 2009)
Figure 11. *Excerpt from the 1:10,000 digital soil map of Estonia*

Rähkmuldade huumuskatte omadused

Haritavate rähkmuldade huumuskate koosneb A-horisondist ja poolest üleminekukihist järgnevasse horisonti. Looduslikel aladel lisandub huumuskattesse mineraalse mulla väline orgaanilise aine kiht ehk metsa- või rohumaade kõdu. Huumuskatte ülesehitus ja aineline koostis peegeldab hästi huumusseisundit, mis on sisuliselt orgaanilise aine koosseisus olevate ainete (sh süsinik) ringe ja energia voog. Huumusseisundit hinnatakse (a) huumuskatte ülesehituse (horisondid ja nende tüsedused), (b) mulla orgaanilise aine (MOA) sisalduse (kontsentratsioon ja varu), (c) MOA paiknemise ja talitlemise järgi ning (d) MOA C:N suhte järgi. Mulla huumusseisundit ja talitlemist iseloomustavad veelgi paremini dünaamilised näitajad, milleks on igaaastane MOA sisend mulda, selle lagunemise-muundumise kulg seoses mullaelustiku tegevusega, stabiilse huumuse akumuleerumise määr mulda ning süsiniku väljund mullast seoses heterotroofse hingamise või väljauhtumisega. Huumusseisundi erisuste peamisteks teguriteks on mulla lõimis ja niiskusolud.

Rähkmuldade neutraalse huumuse koosseisus on ülekaalus kaltsiumiga seotud huumushapped. Heaks kompleksekseks huumusseisundi indikaatoriks on huumuskatte tüüp (ehk huumusvorm), mille klassifikatsioonid on olemas eraldi looduslike ja haritavate muldade kohta. Rähkmuldade mullaelustiku tegevus on koondunud pindmisse (5−7 cm) kihti, milles on head bakteriaalse lagunemise tingimused eriti just niisketel soojadel kevad- ja sügisperioodidel. Kuivadel suvedel on bioloogiline tegevus miinimumis. Tüsedamates rähkmuldades on mullaelustiku tegevusest haaratud peale A-horisondi ka struktuursed metamorfsed sisseuhte- (Bw) ja ülemineku-(AB, BC) horisondid, mis sisaldavad rohkesti taimejuuri. Elustiku aktiivsus neis karbonaatsetes horisontides sõltub õhustatusest ning optimaalsete niiskustingimuste olemasolust.

Looduslike gleistunud rähkmuldade rikkaliku koostisega rohurinde juurestik soodustab kamardumist ja igaaastase värske orgaanilise aine sattumist mulla pindmisse kihti. Rähkmuldadel kujunenud taimkatte varis on tuha- (eriti kaltsiumi-) ja lämmastikurikas, mis soodustab varise laguproduktide küllastumist. Gleistunud muldades on kevadeti ja sügiseti lagunemise-muundumise protsess liigniiskuse tõttu mõnevõrra pidurdunud ning MOA akumuleerub veidi toorhuumuslikumal kujul.

Põlluks haritud rähkmullad on pehmehuumusliku humaatse ehk mull-tüüpi huumusega mullad. K muldade huumusesisaldus varieerub piirides 23–45 g kg^{-1}, Kg muldades aga 35–55 g kg^{-1}. Põllumuldade A-horisondi huumuse kontsentratsioonid on mõnevõrra väiksemad võrreldes looduslikega. Põllu rähkmuldade (K, Kg) huumuse C:N suhe (10–11) on kitsam metsamuldade omast, mis viitab huumuse paremale kvaliteedile. Lämmastiku sisaldustes põllu- ja metsamuldade vahel olulist erinevust ei ole (mõlemal on see piirides 2,0–2,5 g kg^{-1}). Metsas asuvate rähkmuldade iseärasuseks võrreldes põlluks ülesharitud rähkmuldadega on mineraalse mulla välise kõdu-(O-)horisondi esinemine A-horisondi peal. Rähkmulla kõduhorisont on seoses mullaelustiku aktiivse bioloogilise tegevusega, mulla lubjarikkusega ning varise mullaga segunemisele õhuke ja kihistumata. Purujast pooleldi-lagunenud varisest koosnev metsakõdu võib siin täielikult laguneda ühe suve jooksul, mistõttu suve keskel võib metsakõdu hoopiski puududa.

Tüsedamatele parasniisketele K muldadele on metsades moodustunud värske kaltsi- või metsa-mull huumuskate. Nende huumusprofiilid koosnevad 1–2 cm tüsedusest O-horisondist, mis lasub huumusrikka (50–75 g kg^{-1}) A-horisondi peal. Vähem rähka sisaldavates A-horisontides on ka huumusesisaldus madalam. Seoses liikuvate fulvohapete osatähtsuse suurenemisega taolistes muldades on neil moodustunud metsa-mull tüüpi huumuskate (tabel 4). Kr ja Kk metsamullad on tavaliselt K-dest huumusrikkad (60–100 g kg^{-1}), mille põhjuseks on nii mullapeenese kaltsiumirikkus kui ka ajutine läbikuivamine, mille tagajärjel pooleldi või hästilagunenud MOA kondenseerub ja polümeriseerub. Samas on nende huumusvaru väike.

Kg, Krg ja Kkg huumushorisont on nõrgalt toorhuumuslik. Nende muldade huumusesisaldus (80–110 g kg^{-1}) on suurem parasniisketest muldadest, seoses mulla mineraalosaga seostumata huumuse suurema osatähtsusega. Kuigi Kg muldade kõduhorisont on natuke tüsedam kui parasniisketel muldadel, paikneb valdav osa orgaanilisest ainest ikkagi huumushorisondis. Rähksete metsamuldade huumuse C:N suhe (15–16) on laiem võrreldes põllumuldadega. Kuigi huumuskatete omadustes on tuntavad erinevused värskete (parasniiskete) ja niiskete rähkmuldade vahel, on suuremad erinevused tingitud ikkagi maakasutusest (mets, põld, rohumaa). Haritavate rähkmuldade huumushorisondi pH_{KCl} on piirides 6,5–7,3. Metsades ja rohumaadel, kus kihisemine soolhappe lahusega esineb huumushorisondi alumises osas, võib huumushorisondi pindmises osas pH_{KCl} langeda kuni 5,5-ni.

Tabel 4. Rähkmuldade huumuskatted põllul ja metsas ning nende A-horisondi pH_{KCl}
Table 4. *Humus covers on field and forest, and their A horizon's* pH_{KCl}

Huumuskate[1)] *Humus cover*	Kõlvik *Land use*	Muld *Soil*	pH_{KCl}
Kuiv pehmehuumuslik vähese huumusvaruga / *Dry mild-humuous with scars humus stock*	Põld / *Field*	Kr Kk	6,9−7,3
Värske pehmehuumuslik rähkne / *Fresh mild-humuous ryhky*	Põld / *Field*	K	6,8–7,2
Niiske pehmehuumuslik rähkne / *Moist mild-humuous ryhky*	Põld / *Field*	Kg	6,8–7,2
Kuiv mull / *Dry mull*	Mets / *Forest*	Kk Kr	6,3−6,9
Värske kaltsi-mull / *Fresh calci-mull*	Mets / *Forest*	Kr K	6,1−6,5
Värske metsa-mull / *Fresh forest-mull*	Mets / *Forest*	K	5,5−5,8
Niiske kaltsi-mull / *Moist calci-mull*	Mets / *Forest*	Krg Kkg	5,6−6,0
Niiske metsa-mull / *Moist forest-mull*	Mets / *Forest*	Kg	5,5−5,9

1) Põllud – lühiiseloomustus, metsad – klassifikatsiooni (Astover jt, 2013) alusel määratud tüüp.
1) For field short characterization, for forests determined by local classification humus cover type.

Tompjas või teralis-tompjas struktuursus on hästi väljakujunenud liivsavilõimisega looduslikel rähkmuldadel, nõrgemini saviliivadel ja eriti liivadel. Intensiivselt haritavatel põldudel on mõningane osa agregaatidest purustatud. Tingituna heast struktuursusest ja tihenenud mullakihtide puudumisest on rähkmullad hea loodusliku drenaažiga. Veeläbilaskvus on parim koreserikastel ja väikseim sügavatel koresevaesematel rähkmuldadel. Rähkmullad on kevadeti kiiresti tahenevad ja soojenevad, mida soodustab rähkmuldade huumuserikkusest tingitud tume värvus. Rähkmullad on hästi õhustatud ning nendes domineerivad hapendustingimused.

Rähkmuldade A-horisondi peenese eripind, mis sõltub mulla lõimisest ja huumusesisaldusest, on liivsavimuldadel valdavalt 40–90 $m^2\ g^{-1}$ ja saviliivmuldadel – 25–65 $m^2\ g^{-1}$. Looduslike rähkmuldade huumushorisondi hüdrolüütiline happesus on <1,5 cmol kg^{-1} ning alusmullas nullilähedane (<0,5 cmol kg^{-1}). Neeldunud aluste sisaldus muutub hüdrolüütilisele happesusele vastupidises suunas. Keskmisena on rähkmuldade huumushorisondis neeldunud aluseid kuni 35–40 cmol kg^{-1}. Sellega kooskõlas oleva mullapeenese neelamismahutavus on väiksem alusmulla huumusvaestes horisontides. Rähksete muldade huumushorisondi küllastusaste on kõrgem haritavates muldades (>95%) võrreldes looduslikus olekus olevate muldadega (>90%). Küllastusaste suureneb sügavuse suunas, ulatudes 100%-ni karbonaadirikastes horisontides.

Rähkmuldade alusmullast ja lähtematerjalist

Rähkmuldade alusmullas suuri füüsikaliste ja keemiliste omaduste erinevusi sõltuvalt kasutusviisist (põld, mets, rohumaa) eriti ei ole. Alusmulla ülesehituse erinevused on tingitud peamiselt mullaliikide koreselisusest (korese liik ja sisalduse määr) ja alusmulla tüsedusest, vähemal määral ka mulla niiskusoludest ja lõimisest. Tüsedamate rähkmuldade (K, Kg) B-horisondi heast struktuursusest sõltuv lasuvustihedus on B-horisondis *ca* 0,1–0,2 Mg m^{-3} võrra suurem kui A-horisondis. Samas on B-horisondi tasakaalustunud lasuvustihedus ikkagi suurem põllumuldadel võrreldes metsamuldadega. Suurem üldpoorsus teeb metsarähkmullad vett hästi läbilaskvateks ehk nad on hea loodusliku drenaažiga.

Väliveemahutavus on suurim koresevaestel raskema lõimisega muldadel. Tingituna õhukeste koreserikaste muldade peenese ja huumuse vähesest massist on need rähkmullad (Kr, Kk) põuakartlikud. Produktiivsust limiteeriv aktiivvee mahutavus koreserikaste muldade 1 m kihis on alla 80–100 mm. Suurema tüseduse ja väiksema rähasisaldusega muldades on see ühemeetrise mullakihi kohta üle 160–180 mm. Sügavamate gleistunud rähkmuldade aktiivveemahutavus on võrreldav parasniisketega, kuid tunduvalt kõrgemal asuva põhjavee tõttu need mullad (Kg, Krg) suviti põua all ei kannata. Veerežiimi muudab ebastabiilsemaks muldkatte all lasuv paas. Mida lähemal maapinnale paas on, seda põuakartlikum muld on. Enamarenenud rähkmuldades esinev savistunud Bw-horisont loob taimede kasvuks soodsa vee- ja õhurežiimi ning samas ei ole häiritud oluliselt ka vee läbilaskvus.

Rähkmuldade levinumateks lõimisteks on rähksed liivsavid ja saviliivad ning veeriselised kruusad ja liivad. Liivsavidest on ülekaalus kerged liivsavid. Vähem leidub keskmist liivsavi, kuna rasket liivsavi ja savi esineb väga piiratud ulatuses. Rähkmuldade jämedad liivad on enamasti fluvioglatsiaalse päritoluga. Peenliiva ning jämeda tolmu osatähtsus on rähkmuldades väike. Mullapeenese osatähtsus alusmullas (B, BC) ja lähtekivimis on kordselt väiksem võrreldes huumuskattega. Veerismuldadele on iseloomulik peale suure veerise, kruusa ja liiva osatähtsuse ka nende kihilisus ja sorteeritus.

Alusmulla, kui ülemineku ala, koresesisaldus on kooskõlas nii huumuskatte kui lähtekivimi korese sisaldusega. Kivirikastes moreenide ja fluvioglatsiaalsete setete koresesisaldus võib ulatuda kuni 60–70 mahuprotsendini, kuid klibuvallides kuni 80–95%-ni. Üldreeglina on huumuskatted 1–2 astme võrra väiksema koresesisaldusega võrreldes lähtekivimi ja/või alusmullaga. Samas võib domineerivate koresesisalduste kõrval (tabel 3) leida palju alternatiivseid korese ja peenese osakaaluga mullaerimite kombinatsioone (joonised 6–8).

Rähkmuldade produktiivsus ja selle seos huumusseisundiga

Mulla produktiivsust hinnatakse temal kasvava (taim-muld süsteemi moodustava) taimkatte aastafütoproduktiivsuse (AFP) järgi (Kõlli, 1987). AFP on antud kuiva fütomassi pindtihedusena ajaühiku kohta (tonni $ha^{-1}\ a^{-1}$). Võrreldavuse huvides on metsamuldade pro-

duktiivsus määratud kiire kasvufaasi läbinud eelvalminud kuni raieküpsete puistutega metsaökosüsteemides. AFP hõlmab puhta primaarse produktsooni, millest on maha arvatud aasta jooksul irdunud varis ja kulud sekundaarse (loomse) biomassi moodustumisele.

Madalaima AFP-ga (5–6 t ha^{-1} a^{-1}) on kuivadel õhukese huumushorisondiga koreserikastel rähk-(veeris-) ja klibumuldadel (Kr, Kk) ning ebastabiilse veerežiimiga gleistunud rähkmuldadel (Krg, Kkg) kasvavad hõredad leesika- ja kastikuloo männikud. Sügavamate ja väiksema koresesisaldusega parasniiskete liivsavimuldade (K) sinilille kuusikute (vähemal määral ka männikute ja kaasikute) AFP ulatub 10–12 tonnini hektari kohta. Sügavate gleistunud rähksete (Kg) muldade salumetsad on veelgi kõrgema potentsiaalse viljakusega (AFP 10–13 t ha^{-1} a^{-1}). Puistuteks on siin laialehelisi puuliike sisaldavad naadi kaasikud ja kuusikud.

Rähkmuldade liigirikka alusmetsa tihedus sõltub puurinde tihedusest, kuid on valdavalt hõre kuni keskmise ehk varieeruva tihedusega, maapealse AFP-ga piirides 0,1–0,4 t ha^{-1} a^{-1}. Rohurinne on rähkmuldadel liigirikas, kusjuures liikide levikumuster sõltub peale huumuskatte tüüpide ka maapinna valgustatusest. Rohurinde AFP varieerub sellest tingitult piirides alates 0,4 kuni 0,9 tonni ha^{-1} a^{-1}. Rähkmuldade samblarinne on enamjaolt liigivaene, hõre ja katkendlik AFP-ga 0,1–0,4 t ha^{-1} a^{-1}. Puistute AFP erinevus väljendub ka puistute boniteedis, mis ulatub väheviljakast V-st boniteediklassist kuni kõrge produktiivsusega Ia-ni. Muldade rea Kr(Kk)–K–Kg(Krg) keskmised boniteedid on seega vastavalt IV-V–I-II–I-Ia.

Võrreldavuse huvides hinnatakse ka agroökosüsteemide AFP ühe kindla kultuuri (meie odra) järgi. Odra AFP kalkuleeriti odra erinevate osade (lehed, kõrred, pähikud, terad, juured) fütomassi pindtiheduse dünaamika alusel. Teatavasti saabub erinevate fütomassi osiste maksimum erinevatel aegadel, samas toimub kasvuperioodi jooksul varise irdumine ja kaasneb umbrohtude fütomassi moodustumine. Odra AFP on reeglina teatud määral suurem odraga moodustunus agroökosüsteemi maksimaalsest massist. Meie uurimuste järgi on odra AFP K mullal 6–11 t ha^{-1} a^{-1} sh umbrohud 0,1–0,6 t ha^{-1} a^{-1}.

Haritavate rähkmuldade hindepunktid erinevad metsamuldadega sarnase seaduspärasuse järgi. Kergema lõimisega koreseliste rähkmuldade boniteet on ligikaudu 25 hindepunkti, liivsavilõimisega sügavamatel rähkmuldadel aga *ca* 50. Kuivendatud liivsavilõimisega Kg muldade boniteet on keskmiselt 40–43 hindepunkti. Suured koresesisalduse kõikumised võivad muuta rähkmuldade viljakuse varieeruvaks ja põllud ebaühtlaseks (joonis 12).

Joonis 12. Maastik rähkmuldadel. Valli Loide foto
Figure 12. *Landscape on ryhky soils*

Enamlevinumateks rähkmuldadega looduslikeks rohumaadeks on loo- ja künka-arud ning lausk-arurohumaad. Taimkate on neil liigirikas, kuid põuakartlikel muldadel (Kr, Kk) on paljud taimeliigid kääbusjad. Looduslike rohumaade produktiivsust näitab kuiva heina (niiskust *ca* 14%) saagikus. Rähkmuldade reas Kr(Kk)–K–Kg(Krg) ulatub kuiva heina saagikus 0,5 t ha^{-1} kuni 3 t ha^{-1}. Võttes arvesse rohumaaökosüsteemide kõigi maapealsete osade aastase juurdekasvu võib järeldada, et kateena Kr(Kk)–K–Kg(Krg) ulatuses suureneb AFP alates 1,1 kuni 2,5 t ha^{-1} a^{-1}, mis aga moodustab vaid ligikaudu poole rohumaaökosüsteemi kogu AFP-st.

Rähkmuldade produktiivsus sõltub huumuskatte huumuse-, lämmastiku- ja teiste oluliste toiteelementide sisaldusest, millised vähenevad alates kuivadest koreserikastest kuni niiskete rähavaeste muldadeni. Liigirikka ja lubjalembese taimestikuga rähkmuldade AFP-st limiteerivaks teguriks on koreserikaste õhukeste muldade puhul väike aktiivvee mahutavus. Vaid sügavad rähkmullad koresevaesel lähtekivimil suudavad valdaval osal aastatest taimi normaalselt veega varustada. Parasniisketel rähkmuldadel asub põhjavesi sügaval ja ei ole taimedele kättesaadav. Taimede poolt kasutatava mullavee hulk on suurim rohkema mullapeenese- ja vähema koresesisaldusega gleistunud rähkmuldades, millistele on kujunenud kõrge produktiivsusega looduslikud rohumaad.

Rähkmuldadest on suurima huumusvaruga (>150 t ha^{-1}) tüseda huumushorisondiga liivsavi- ja saviliivmoreenil kujunenud mullad. Huumusvarudes suuri erinevusi seoses maakasutusega ei ole. Arvestatav huumusvaru on rähkmuldadel ka AB- ja Bw-horisontides ja juurekäikudes, olles näiteks liivsavidel keskmiselt 20–35 Mg ha^{-1}. Kg huumusvaru ulatub 110–140 tonnini hektari kohta. Metsakõdu tuhavaba orgaanilise aine mass on K muldadel piirides 9–13 t ha^{-1}, Kg metsakõdul aga valdavalt 14–18 t ha^{-1}. Haritavate rähkmuldade (peamiselt K ja Kg) huumuskatte tüsedused ning mulla orgaanilise süsiniku sisaldused, mis olenevad mullapeenese lõimise ja niiskusolude kõrval ka kasutatud agrotehnoloogiast, on suuresti ühtlustunud ning nende MOA varud varieeruvad suhteliselt vähesel määral (60–80 t ha^{-1}).

Rähkmuldade EMK ja WRB nimetuste korrelatsioon

Eesti muldasid tuleks määratleda ja nende omadusi teada ennekõike ikkagi lokaalse s.o EMK järgi. Muldade nimetusi võib ju ka tõlkida inglise keelde, kuid need tavaliselt ei ole kuigi informatiivsed ilma vastavat klassifikatsiooni kui töövahendit tundmata. Seega on hoopiski otstarbekam need konverteerida rahvusvaheliselt laialtkasutatavasse süsteemi. Antud juhul on selleks World Reference Base for Soil Resources (WRB; IUSS 2015).

Rähkmuldade profiilide ülesehituse ja omaduste järgi on tegemist vähearenenud (noorte) muldadega, milliseid kajastab WRB süsteemi järgi kõige adekvaatsemalt referentsmuld *Cambisols*. Koreserikaste vähearenenud rähkmuldade vasteks WRB järgi on *Regosols* ja *Lithosols*. WRB järgi kasutatakse detailse mullanimetuse andmiseks referentsmullale lisatud kvalifikaatorite ehk täiendsõnade süsteemi (tabel 5). Selgituseks olgu öeldud, et taoline mullanimede konventeerimine ei saa olla kunagi üks-ühele, sest suuremal või vähemal määral erinevad omaduste jaotuse põhimõtted. Sarnaselt WRB süsteemiga võiks ka eestikeelsetes kirjutistes anda mullaerimi nimetuse mahukamalt ja komplekssemalt, lisades sellesse võimalikult palju vastavat erimit iseloomustavat terminitel põhinevat informatsiooni.

Tabel 5. EMK ja WRB mullanimetuste korrelatsioon
Table 5. *Correlation between soil names of EMK (Estonian Soil Classification) and WRB*

Grupp[1)] *Group*	Kvalifikaatorid ja referentsmullad *Qualifiers and Reference soils*	K[2)]	Kg	Kr	Krg	Kk	Kkg
1	*calcaric*	+	+	+	+	+	+
	eutric	+	+	+	+	+	+
	skeletic	+	+	–	–	–	–
	hyperskeletic	–	–	+	+	+	+
	rendzic	–	–	+	+	+	+
	mollic	+	+	–	–	–	–
	endogleyic	–	+	–	+	–	+
	protic	–	–	–	–	+	+
2	*CAMBISOLS*	+	+	–	–	–	–
	LEPTOSOLS	–	–	+	+	+	+
	REGOSOLS	–	–	–	–	+	+
3	*arenic*	–	–	+	–	+	+
	loamic	+	+	+	+	–	–
	aric	+	+	–	–	–	–

1) Grupp: 1 – täiendsõna eesliitena, 2 – referentsmulla grupp, 3 – täiendsõna järelliitena; 2) Mullanimetusi vt tabelist 1.
1) Group: 1 – prefix qualifiers, 2 – Soil Reference Groups, 3 – suffix qualifiers; 2) Soil names by code see Table 1.

Rähkmuldade kasutamissobivus

Kuna muldade parim kaitse on nende õige kasutamine, siis oleks vaja teada nende erinevate liikide kasutussobivust. Tingituna kaltsiumi- ja huumuserikkusest on rähkmuldade A-horisondi struktuursus hästi väljakujunenud ja nad on harimisele vastupidavad ehk harimiskindlad mullad. Rähkmuldade haritavust (põlluna kasutamisel) mõjutab korese hulk ja kuju. Rohkem, võrreldes veerise ja klibuga, takistab harimist rähk oma teravaservalisuse tõttu. Raskesti haritavateks muldadeks on õhukesed rähk- (veeris-, klibu-) mullad (Kr, Krg, Kk, Kkg) oma väga õhukese huumuskatte ja koreserikka alusmulla tõttu. Õigem oleks taolised mullad jätta looduslikku olekusse või võtta ehituste alla. Rähkmuldade põuakartlikkuse tõttu tuleb mullaniiskuse maksimaalse ärakasutamise huvides teha kevadine mullaharimine ja külv esimesel võimalusel (joonised 13–14).

Rähksed, veeriselised või klibused mullad on kohasemad tugeva ja sügavale ulatuva juurestikuga kultuuridele (lutsern, mesikas) või vee ökonoomsetele kasutajatele (oder, rukis). Sügavamad rähksed (K) mullad suudavad kultuure veega paremini varustada. Teraviljadest on siin odral eelised kaera ja rukki ees. Põldhein (ristik, timut) on nendel muldadel suhteliselt lühikese

kestvusega kuna juba teise aasta põldheina saagid on madalamad esimese aasta saakidest. Kuivendamata Kg mullad, mis on hästi sobivad heintaimede ja metsa kasvatamiseks, vajavad põllumaana kasutamisel kuivendamist. Kuivendatud gleistunud rähksed liivsavimullad on sarnaselt parasniiskete muldadega universaalse kasutussobivusega. Kivised, räha-(veerise-) ja kliburikkad mullad (Kr, Kk) on vähesobivad rühvelkultuuride kasvatamiseks ja kartuli mehhaniseeritud koristamiseks. Tugevasti kivised ja koreserikkad gleistunud karbonaatsed mullad (Krg, Kkg) on piiratud kasutussobivusega. Nende kuivendamine ei ole otstarbekas.

Joonis 13. Rähkne põld. Mullateaduse õppetooli fotokogu
Figure 13. *Ryhky field*

Joonis 14. Põldkatse räharikkal mullal. Valli Loide foto
Figure 14. *Experimental field on ryhky soil*

Status and distribution of ryhky soils in Estonian agricultural and forest landscapes

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Summary

The year 2021 Soils of Estonia are *ryhky soils*, which may be characterized as dry, fresh and moist rich in coarse calcareous material mild-humuous (mull-humus) soils. The main criteria of *ryhky soil* species' identification after Estonian Soil Classification (ESC) are the fine earth calcareousness, content and shape of small stones (Ø 1–10 cm), and water regime of soil cover. The wet and eroded *ryhky soils* have been excluded from the overview as their properties depend not so on the coarse calcareous earth content as on feeding their soil water or water erosion. By ESC six soil species have been distinguished, from which three ones have *endogleyic* character (Table 1). The fine earth texture of *ryhky soils* is mainly loam, but as well are presented with loamy sands, sands and clays. Stoniness by volume per cent of small stones (ryhk, pebble and shingle) in humus cover and subsoil varies to a very large extent from 2–10% to >70% (Table 3). After WRB *ryhky soils* may be characterized as *calcaric* or *eutric, skeletic* or *hyperskeletic* and *rendzic* or *mollic CAMBISOLS, LITHOSOLS* or *REGOSOLS.* The area of *ryhky soils* forms 6.3% from whole Estonian soil cover and 11.1% from the arable land. The morphology, humus status and suitability of *ryhky soils* for management are treated on the level of soil species in dependence of land use (arable, forest or grassland). In connection with this more profoundly are characterized humus cover types of arable and forest soils and their agrochemical properties. *Ryhky soils* productivity, which is characterized by their annual phyto-productivity in ton ha^{-1}, is varied to great extent in all land use conditions (arable, forest and grassland) in dependence of soil types' humus status and content of skeletal material in soil cover. The overview contains 5 Tables, 14 Figures. The list of used literature contains 6 sources.

Huvide konflikt / *Conflict of interest*

Autor kinnitab artikliga seotud huvide konflikti puudumist.
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Autorite panus / *Author contributions*

RK, TT – artikli kontseptsioon ja planeerimine / study conception and design;
RK, TT – andmete kogumine / acquisition of data;
RK – andmete analüüs / analysis of data;
TT – illustreeriva materjali vormistamine / design of figures;
RK – käsikirja mustandi kirjutamine / drafting of manuscript;
RK, TT – lõpliku käsikirja toimetamine ja heaks kiitmine / *critical revision and approve the final manuscript.*

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BOTTLE GOURD (*Lagenaria siceraria* L.) CROP RESPONSE TO DIFFERENT PLANTING DENSITIES UNDER BOTH DRIP AND WIDE-SPACED FURROW IRRIGATION METHODS

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ABSTRACT. Although bottle gourd (*Lagenaria siceraria* L.) is an important vegetable crop in rural communities in the arid Mediterranean region, still no sufficient information regarding its cultivation practices is available. A two-year field experiment (2019 and 2020) was carried out to assess the effects of planting density and irrigation method on bottle gourd yield, following a split-plot experimental design with two planting densities of about 11 111 and 5555 plant ha^{-1}, and two irrigation methods (drip irrigation and wide-spaced furrows as surface irrigation), with three replicates. Significant effects of both factors on bottle gourd fruit characteristics, dry matter, fresh marketable yield, water productivity (WP), and irrigation water use efficiency (IWUE) were found. Seasonal evapotranspiration and irrigation water amounts were considerably reduced by about 20% under drip irrigation as compared with surface irrigation. Moreover, dry matter, fresh marketable yield, WP, and IWUE were doubled. Combining drip irrigation with the lower planting density was the most favourable practice for the bottle gourd crop productivity under the studied context. These findings of high fresh marketable yield and water productivity suggest that bottle gourd crop could be considered as an alternative crop for food security and economic prosperity of rural communities. Adopting drip irrigation can effectively address the water shortage issue and sustain crop production in the arid Mediterranean area.

Introduction

Lagenaria siceraria (Molina) Standl., known as a bottle gourd or the white-flowered gourd, is a member of the Family of *Cucurbitaceae*. Bottle gourd is a very popular vegetable crop in Asia and Africa. Young fruits are commonly consumed by boiling, frying, or stuffing. Shoots, tendrils and leaves are also consumed, while the seeds are used for oil and protein due to their richness of essential amino and fatty acids (Rahman, 2003; Chimonyo, Modi, 2013). The dried hard rind of mature fruits is used as a container, musical instrument, or decorations in some cultures. Moreover, different components of this plant (seeds, tendrils, and young leaves) are used for medical purposes (Ahmad *et al.*, 2011; Milind, Satbir, 2011). Although a lot of information is documented on the medicinal aspects of this plant, its potential as a possible food security crop has been poorly reported (Ahmad *et al.*, 2011; Chimonyo, Modi, 2013; Mabhaudhi *et al.*, 2017). Its high morphological and genetic variability in nature might indicate its wide environmental adaptation (Given, 1987; Koffi *et al.*, 2009). Due to its huge canopy cover, bottle gourd is considered a natural smother of weeds (Koffi *et al.*, 2009). It is often intercropped with other crops and could play a role in live mulching (Ouma, Jeruto, 2010). Bottle gourd is used as rootstock for watermelon and cucumber against low soil temperature and soil-borne diseases. Using bottle gourd as rootstock could also save water and therefore, increase water use efficiency, especially in arid and semi-arid areas (Yetisir *et al.*, 2008; Guler *et al.*, 2013; Yavuz *et al.*, 2020; Aslam *et al.*, 2020). Given such benefits, it is surprising that the bottle gourd crop productivity received the least amount of scientific research attention, as compared with the other members of its family, especially in the arid Mediterranean region.

In Syria, bottle gourd crop is cultivated mostly under irrigated cropping system, due to the lack of rainfall over the production period between April and September (Ragab, Prudhomme, 2002; Turner, 2004).

Surface irrigation method with very low water use efficiency is mostly used. Since water scarcity is a constraint to crop production in this region, efficient irrigation water use is a vital need to sustain crop production for ever-increasing food demands. Higher benefits may be obtained by adopting water-saving irrigation methods such as the drip irrigation method (Goyal, 2014, 2015; Venot *et al.*, 2017). To the best of our knowledge, there are no published field studies conducted on bottle gourd species in Syria under the arid Mediterranean environment. Since bottle gourd is one of the neglected and underutilized species, important scientific outcomes of its cultivation (especially for plant density) and productivity under drip irrigation method are much needed.

This two-year field experiment aimed to evaluate the response of bottle gourd to different planting densities under both drip irrigation and traditional surface (wide-spaced furrows) irrigation methods. The results may contribute to introduce a practical alternative that would sustain crop productivity with efficient water use.

Materials and methods

Field experiments were carried out during the 2019 and 2020 growing seasons at the Agricultural Experiment Station, Deir Al-Hajar, Damascus Countryside in Syria (33°20′ N, 36°26′ E, 600 m above sea level). The arid Mediterranean climate dominates the study region, with annual potential evapotranspiration (ET_0) of more than 2000 mm, as estimated using the FAO Penman-Monteith formula (Allen *et al.*, 1998). The mean annual precipitation based on 20 years' record (2000–2019) is about 120 mm. Table (1) shows some climatic data of the study site, collected during both studied growing seasons.

Table 1. Some climatic data for the study site during both growing seasons 2019 and 2020

Year	Parameter	Apr.	May	Jun.	Jul.	Aug.	Sep.
2019	T_{min}, °C	8.1	15.0	19.0	19.4	20.1	17.6
	T_{max}, °C	22.2	34.2	36.6	37.3	38.0	34.7
	T_{mean}, °C	15.1	24.6	27.8	28.3	29.0	26.1
	RH, %	56.1	61.1	56.9	55.7	56.5	59.9
	ET_0, mm day^{-1}	5.22	8.70	9.26	9.81	9.17	7.19
	Rain. mm	11.6	0.0	0.0	0.0	0.0	0.0
2020	T_{min}, °C	9.7	13.4	16.5	20.0	20.2	20.6
	T_{max}, °C	24.3	31.6	34.7	40.0	37.7	39.5
	T_{mean}, °C	17.0	22.5	25.6	30.0	28.9	30.0
	RH, %	62.8	56.6	55.6	59.0	58.8	67.6
	ET_0, mm day^{-1}	5.82	8.23	9.01	10.47	9.01	9.05
	Rain, mm	6.7	3.0	0.0	0.0	0.0	0.0

T_{min} = minimum temperature, T_{max} = maximum temperature, T_{mean} = average temperature, RH = relative air humidity, ET_0 = reference evapotranspiration.

The top 30 cm of the soil profile had a clay loam texture with sand 27.8%, silt 42.7%, clay 29.5%, bulk density 1.35 g cm^{-3}, organic matter of about 1%, pH of 8.0, EC of 0.6 dS m^{-1}, available P of 22.0 ppm, $NH4^+$ of 13.3 ppm, and NO_3^- of 20.0 ppm. The volumetric soil water content at field capacity was 0.38 cm^3 cm$^{3\,-1}$, and that at the permanent wilting point was 0.18 cm^3 cm$^{3\,-1}$. Irrigation water characteristics were NH_4^+ 1.99 ppm, NO_3^- 1.05 ppm, EC_w 0.46 d Sm^{-1}, and pH of 8.4.

At the beginning of the spring season, the studied field was ploughed to a depth of 0.30 m with a mould-board plough. Experimental units (plots), 12×3 m each, were prepared. Two different irrigation methods were tested: drip and surface irrigation methods. For drip irrigation, lateral driplines of 16 mm diameter with a built-in 40-cm emitter spacing with a discharge of 10 L h^{-1} m^{-1} were used. One lateral dripline per plant row was installed. The lateral dripline spacing was 3.0 m. For the surface irrigation method, wide-spaced furrows (3.0 m apart) adopted by local farmers were used in this study. The depth and width of each furrow were 25 and 75 cm, respectively.

Due to the dimensions of experimental units, it was more practical to make the furrow length equal to the length of the units, therefore, short furrows (12 m) were used. This allowed furrow to be irrigated more efficiently as it is much easier to keep the percolation losses low. A local variety "Baladi" of bottle gourd (*Lagenaria siceraria* L.), extensively planted by farmers in Syria, was used. Bottle gourd seeds were planted on April 25th and May 15th in 2019 and 2020, respectively. Rows were spaced 3.0 m apart. Under both irrigation methods, two different distances between planting holes of 0.6 and 1.2 m were studied. After establishment, plants were hand thinned to two plants per hole. This resulted in 11 111 (Pd1) and 5555 (Pd2) plants per hectare, respectively. Experiments were arranged in a split-plot design involving two irrigation methods (drip and surface) as main plots and two planting densities (Pd1 and Pd2) as sub-plots, with three replicates.

The experimental field was fertilized in early spring before each cropping season, with 100 kg P_2O_5 per hectare as triple superphosphate. However, 200 kg N ha^{-1} as urea was supplied to prevent any nitrogen deficiency. It was applied in two equally split applications: two weeks after thinning and one month later. Thus, all plants received the same quantities of both chemical fertilizers.

Regarding irrigation scheduling, the soil water content (SWC) status in the root zone was observed using the neutron scattering technique. Irrigating plants was initiated immediately after sowing. The active roots depths were set to 0.30 m from sowing until the early flowering, and then to 0.60 m till termination. Bottle gourd was irrigated once a week when the soil water content in the specific soil layer reached 75–80% of the field capacity. In all treatments, plants received 100% of the depleted water amount between two consecutive irrigation events, so that the SWC in the root zone was replenished to the field capacity. Crop evapotranspiration (ETc) was calculated using the water balance relationship as follows (Mubarak, Janat, 2020):

$$ET_c = I + P - D_p - R_o \pm \Delta(SWC), \quad (1)$$

where I = the amount of irrigation water applied (mm); P = the precipitation (mm); D_p = the deep percolation (mm) and R_o = the amount of runoff (mm), $\Delta(SWC)$ = the change in soil water content (mm) in the specified soil profile.

Under controlled water application, R_o was assumed to be zero. Monitoring SWC indicated that D_p was negligible below the depth of 0.60 m. P was also negligible during both cropping seasons (Table 1). Due to the small canopies of young plants and the wide row spacing (3 m), a reduction factor (k_r) was used. The ground cover, GC, which is the fraction of the total surface area covered by the foliage of the plants, was measured every two weeks. The reduction factor was estimated as follows (FAO, 1980):

$$k_r = (GC + 0.1)\ or\ 1, \quad (2)$$

whichever is the smallest.

The volumes of applied amounts of irrigation water were measured by inline flow meters, under both irrigation methods.

For appearance and marketing purposes, the young bottle gourd fruits were harvested at the tender green stage to prevent hard seeds and coarse dry skin, which are not palatable for consumers and do not provoke a good price for farmers. For that, the marketable size fruits were harvested carefully using a knife, three times weekly during morning hours. A 4-m row length from the centre of each plot (experimental units) was selected for plant sampling. The weight (FW), length (FL) and largest diameter (FD) of fresh fruits were measured. Subsamples were air-dried for few days then oven-dried at 70 °C until a constant mass was obtained for determining fruit dry matter yield (DM). Fresh and dry weights were converted into t ha^{-1}. Crop water productivity (WP, kg m$^{3-1}$), also called water use efficiency, and irrigation water use efficiency (IWUE, kg m^{-3}) were calculated as follows (Mubarak, Janat, 2020):

$$WP = \frac{Yield}{seasonal\ ET_c} \quad (3)$$

$$IWUE = \frac{Yield}{Irrigation\ water\ amount} \quad (4)$$

The measured parameters (FL, FD, FW, Yield, DM, WP and IWUE) were subjected to the analysis of variance (ANOVA) using the DSAASTAT add-in (Onofri, 2007). A combined analysis of data over both years was achieved to identify treatment whose mean effect over years is high and stable (Gomez, Gomez, 1984). Mean separation was conducted after combined analysis using the least significant difference test (LSD) at the 5% level of significance.

Results

No significant interactions were detected between year and treatment, or between planting density and irrigation method. Hence, the effects of studied factors on the measured parameters were averaged over both years (Table 2). Moreover, ANOVA showed that both studied factors significantly influenced the measured parameters, indicating that both factors play an essential role in bottle gourd production in the studied region.

Although both the shape and size of bottle gourd fruits vary widely within or among varieties (Chimonyo, Modi, 2013), the shiny green fruits produced herein were somewhat uniform with long and necked shape. The fruit shape was represented in this study by two indicators: fruit length (FL) and diameter (FD). Both planting densities produced fruits of similar sizes with no significant differences. However, fruits produced under the drip irrigation method were considerably longer by about 16% than those produced under surface irrigation. No significant difference in fruit diameter was detected between both irrigation methods (Table 2). The fruit dimensions recorded herein were in agreement with published data (Sivaraj, Pandravada, 2005). According to Sivaraj and Pandravada (2005), fruit sizes vary from 5 to 40 cm for diameter and from 20 to 90 cm for length.

Table 2. Mean comparisons of crop responses as a function of both planting density and irrigation method

Factor	FL, cm	FD, cm	FW, g	DM, t ha^{-1}	Yield, t ha^{-1}	WP, kg m$^{3-1}$	IWUE, kg m$^{3-1}$
Planting density							
Pd1 (11 111 plant ha^{-1})	17.22^a	4.60^a	288.8^b	1.73^a	44.67^b	8.12^b	7.29^b
Pd2 (5 555 plant ha^{-1})	17.68^a	4.75^a	342.9^a	2.09^a	49.60^a	9.05^a	8.13^a
$LSD_{0.05}$	1.82	0.43	27.7	0.61	3.87	0.76	0.69
Irrigation method							
Drip	18.74^a	4.65^a	358.3^a	2.58^a	61.37^a	11.92^a	10.66^a
Surface	16.16^b	4.70^a	273.3^b	1.24^b	32.90^b	5.25^b	4.76^b
$LSD_{0.05}$	0.97	0.19	22.4	0.68	2.11	0.33	0.31

In each column and for each tested factor, means followed by different letters are significantly different according to LSD test at 5% level. FL = fruit length, FD = fruit diameter, FW = fruit weight, DM = dry matter, Yield = fresh marketable yield, WP = water productivity, IWUE = irrigation water use efficiency.

Regarding the FW, the mean value of FW under drip irrigation (358.3 g) was significantly higher by about 31% than that obtained under surface irrigation (273.3 g) (Table 2). Moreover, lower planting density Pd2 produced fruits significantly heavier by about 19% than those produced under Pd1. The mean values of FW were 288.8 and 342.9 g under Pd1 and Pd2, respectively (Table 2).

Fruit dry matter yields under both planting densities were similar with no significant differences. However,

the DM yield of drip-irrigated fruits (2.58 t ha^{-1}) was higher than that of surface-irrigated fruits (1.24 t ha^{-1}), with a significant increase of more than 100% (Table 2).

Moreover, using the drip irrigation method significantly enhanced the fresh marketable yield with a mean value of 61.37 t ha^{-1}, which represented about twice that obtained using the surface irrigation method (32.90 t ha^{-1}). In addition, the fresh marketable yield under lower planting density Pd2 (49.60 t ha^{-1}) was significantly higher by 11% than that obtained under the higher planting density Pd1 (44.67 t ha^{-1}).

Figure (1) shows fresh marketable yield distribution among the tested treatments. It is evident that fresh marketable yield widely varied from 31.93 to 65.34 t ha^{-1}, according to the studied treatments. The minimum value was found in the treatment combining between the higher planting density (Pd1) and surface irrigation method, while the maximum value was found in the treatment combining between the lower planting density (Pd2) and drip irrigation method.

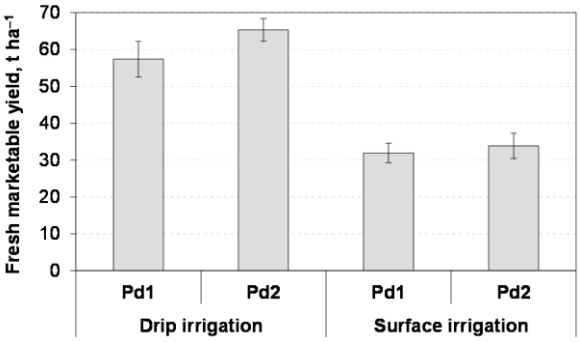

Figure 1. Fresh marketable yield distribution according to the studied treatments for the combined data of both growing seasons. Pd1 and Pd2 represent planting densities. Error bars represent the standard deviations.

As no effective rain precipitated during both cropping seasons, large volumes of irrigation water were added to meet crop water requirements. During the 1st growing season (2019), the seasonal crop evapotranspiration (ETc) calculated using Eq. (1) was about 490 and 589 mm under drip and surface irrigation methods, respectively; while in the 2nd season (2020), they were about 542 and 666 mm, respectively. Respective values for irrigation water amount applied were about 533 and 636 mm in 2019 and 626 and 750 mm in 2020.

The mean values of WP and IWUE under the lower planting density (Pd2) were significantly higher by 11.5% than those obtained under Pd1. In addition, both traits were highly significantly ameliorated using the drip irrigation method with mean values of 11.92 and 10.66 kg m^{-3}, respectively; which corresponded to more than twice those found using the surface irrigation method (5.25 and 4.76 kg m^{-3} for WP and IWUE, respectively). Figure (2) shows all mean values of both parameters (WP and IWUE) obtained for studied treatments. It is clear that WP ranged from 5.09 to 12.69 kg m^{-3} and IWUE ranged from 4.62 to 11.36 kg m^{-3} depending on the tested treatments. The highest values of WP and IWUE were recorded by combining the drip irrigation method and Pd2; while the lowest values were determined under the combination between the surface irrigation method and Pd1. The higher fruit yield resulted in higher WP and IWUE.

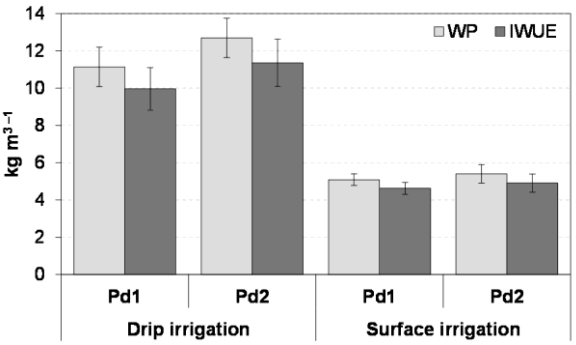

Figure 2. Water productivity (WP) and irrigation water use efficiency (IWUE) distribution according to the studied treatments for the combined data of both growing seasons. Pd1 and Pd2 represent planting densities. Error bars represent the standard deviations.

Discussion

The impacts of different planting densities and irrigation methods on bottle gourd yield were evaluated in an arid Mediterranean environment. Both tested planting densities produced fresh fruits of similar sizes. However, lower planting density (5555 plant ha^{-1}) produced fruits considerably heavier than those produced under the higher density (11 111 plant ha^{-1}). This could be explained by the less competition between the plants as they were widely cultivated. This result was in agreement with those of Jan *et al.*, (2000) and El-Seifi *et al.*, (2015), who reported that the significant highest average fruit weight was related to plants grown at low planting density.

Moreover, the higher yield of fresh marketable fruits obtained under Pd2 could be due to the better use of nutrients and light with less competition between plants. However, this was not in a line with other findings reported by other studies (Shukla, Prabhakar, 1987; Jan *et al.*, 2000; El-Seifi *et al.*, 2015), who reported that the total yield per hectare of bottle gourd was increased with the increase in plant density. This disagreement could be related to the differences in the studied contexts, especially to the differences in planting geometries and harvested fruits. In those studies, the total yield of fruits was determined at the maturity stage with very few harvests at the end of the growing season; while in our study, the young fruits were harvested three times weekly for human consumption in local marketing. Moreover, under the same plant population, planting geometry plays an important role in the total yield of bottle gourd crop. Rosin *et al.*, (2017) evaluated the response of the total yield and irrigation water use efficiency of bottle gourd to three cropping geometries (row spacing×plant spacing): 3×0.5 m, 2×0.75 m, and 1×1.5 m. Even though the planting density was identical, their findings indicated that 3×0.5 m was the most valuable geometry in terms of fruit length, diameter, and weight, the number of

fruits/vine, vine length, total yield, and irrigation water use efficiency. This optimal planting configuration is in agreement with our results, where rows were spaced 3.0 m apart and holes with two plants in each were spaced 120 cm for Pd2. The superiority of lower planting density in the fresh marketable yield could be related to the enhanced stretching (trailing) of bottle gourd plants. This may stimulate roots to grow up and absorb the required nutrients from the soil. This also indicates that the relationship between fresh marketable yield and planting density of bottle gourd is of significant agronomic interest (El-Seifi *et al.*, 2015).

As stated earlier, wide-spaced furrows with an interdistance of 3 m were used as a surface irrigation method. Under such a method, irrigation water amount was not applied on the whole land-area basis, but it was related to the fraction of ground covered by foliage of the plants as in drip irrigation scheduling. So, it was reduced by a reduction factor (k_r) calculated using Eq. (2). This attenuated the huge water amounts usually applied under surface irrigation in the case that the whole land area would be concerned. The evolutions of ground cover under all tested treatments were somewhat similar (data are not shown). Despite that, it is very important to notice that about 20% of the irrigation water amount and seasonal crop water needs were saved when the drip irrigation method was used as compared with the wide-spaced furrow irrigation. Moreover, fruit characteristics, fruit dry matter, fresh marketable yield, WP, and IWUE were considerably increased when using drip irrigation. Considering these results, water might be lost by evaporation and other losses under the surface irrigation method and, therefore, decreasing fresh marketable yield relative to drip irrigation. This confirmed the essential role of drip irrigation in terms of a significant decrease in crop water requirements, even under an arid environment.

Combining the drip irrigation method with the lower planting density produced the highest values of fresh marketable yield, WP, and IWUE, while the lowest values were obtained under the combination between the surface irrigation method and the higher planting density. Furthermore, under the lower planting density, more nutrients, water, and photosynthetically active light could be effectively explored by plants without competing with each other. Nutrients reach roots by mechanisms directly related to the water availability in the soil; therefore, crops express their maximum production potential in a favourable soil-water status (Santos *et al.*, 2018). Again, this proves that the crop production and in-field water use efficiency are enhanced under the drip irrigation method as compared with other methods (Goyal, 2014, 2015; Venot *et al.*, 2017).

WP and IWUE are measures of productivity of water used by plants or added by irrigation. They are vital tools for the assessment of irrigation methods and agricultural water management, especially in arid and semi-arid environments where water is precious. Efficient water management and practices should be considered in this context to produce more yields with less water. Moreover, it is important to notice that the water productivity values of bottle gourd crop found herein (5.02–13.49 kg m^{3-1}) are much higher than those reported for some other crops planted in the same studied climate: 0.02–0.63 kg m^{3-1} for quinoa (Mubarak, Janat, 2020), 0.85–2.18 kg m^{3-1} for sweet corn (Mubarak, 2020), 0.83–6.98 kg m^{3-1} for onion (Mubarak, Hamdan, 2018), 3.84–7.15 kg m^{3-1} for potato (Mubarak *et al.*, 2018), but similar with other crops of its crop family as a cucumber with 5.6–15.3 kg m^{3-1} (Yaghi *et al.*, 2013) and summer squash up to 15.7 kg m^{3-1} (Kuslu *et al.*, 2014). This is related to the higher fresh marketable yield of bottle gourd crop. For that, the potential of bottle gourd, which is considered a neglected and underutilized crop, needs to be unlocked to contribute to food security in the dry Mediterranean area, especially under changing climatic conditions and serious poverty.

Conclusions

Significant effects of different irrigation methods and planting densities on bottle gourd fruit characteristics, dry matter, fresh marketable yield, WP, and IWUEwere found. Seasonal crop water requirements were considerably reduced and dry matter, fresh marketable yield, WP, and IWUE were doubled under the combination of drip irrigation and lower planting density of 5555 plant ha^{-1}.

These findings of high yield and water productivity suggest that bottle gourd can be considered as an alternative crop for food security and economic prosperity of rural communities. Adopting drip irrigation can effectively address water shortage and its consequences and sustain bottle gourd production in the arid Mediterranean area.

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Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

Author contributions

IM (50%) and MJ (50%) – study conception and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision and approve the final manuscript.

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POTATO YIELD FORECAST BY USING GUTTATION TEST METHOD IN HOUSEHOLD LABORATORY CONDITIONS

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ABSTRACT. This paper aims to present the use of the guttation test method to establish the relationship between guttation and potato (*Solanum tuberosum* L.) yield. The laboratory tests (*in vitro* L.) under household conditions were carried out. To assess the state of the potato (variety 'Ando') yield the field (0.07 ha) of the family farm "Miili" were used. Assessment of the state of guttation plant barley (*Hordeum vulgaris* L.) variety 'Anni' by using of hydro-thermostat in conditions adapted hose hold laboratory (according to the generally known a bad epidemiological situation) were carried out. The test sites were located on a light sandy loam of Molli-Calcaric Cambisol (WRB) with areas of the field in the presence of soil samples No. 1 and 2. We have found that the maximum yield of potato 'Ando' on a plot of soil sample No. 1 (15 900 kg ha^{-1} or 1.00 of relative units) was obtained. At the same time, the minimum yield of soil sample No. 2 of the potato tubers was 3900 kg ha^{-1} (0.25 of relative units). To ranking score of the soil fertility level of the studied potato field, additional laboratory experiments were carried out, with extremely humus-rich soil (the guttation droplet imprint on the filter paper was 117.6 mm^2 to that was equated to 1.00 of relative units) and with sand the guttation droplet imprint – 37.0 mm^2 and 0.31 of relative units, respectively. The results of laboratory tests by using of guttation method with barley (variety 'Anni') carried out. The soil samples have been taken from the same areas of the field. In this case, for soil sample No. 1 the average area of the guttation droplet imprint on the filter paper was 55.1 mm^2 to that was equated to 0.47 of relative units and for soil sample No. 2 – 42.9 mm^2 or 0.36 of relative units, respectively. The results of the research have shown that concerning cultivating potatoes (variety 'Ando') and guttation experiments with barley (variety 'Anni') under conditions of soil samples No. 1 and 2 of the potato field a quite reliable relationship between guttation and the yield of potato tubers ($P < 0.001$, $R^2 = 0.98$) was obtained. To assess the different levels of soil fertility for soil samples No. 1 and 2 in the potato field taking as a reference soil with the highest possible fertility (humus-rich soil) and with the lowest possible fertility (clean sand) it was revealed that guttation of soil sample No. 1 of the potato field was 1.6 times inferior according to the results of guttation of the humus-rich soil. Analytical calculations have shown that if we are dealing with a humus-rich soil where potato 'Ando' cultivation would be carried out under the conditions of classical organic farming then the yield of potato tubers would be 22 880 kg ha^{-1} or 30 Mg ha^{-1} rounded. The novelty of our research was the development of a method for assessing the yield of potato by using the guttation test method.

Introduction

It is known that plants are capable of secret guttation fluid only under certain conditions of temperature and relative air humidity (Reppo, 1980). Moreover, not every plant has the inherent ability of guttation. It has been established that taking into account crops only cereal plants have this ability (Goatley, Lewis, 1966; Joachimsmeier, 2011). Unfortunately so far this ability has received too little attention.

One of the main problems is to forecast the yield of the crops (Caldeira *et al.*, 2016). Despite the modern and high level of agriculture, it is very difficult to predict what the upcoming yield will be given the variability of weather conditions (Meng *et al.*, 2017; Saue, Kadaja, 2010). As for potatoes, this crop is considered the most sensitive to soil conditions as well as to the variability of weather conditions in particular.

The guttation method eliminates this drawback since in this case; the soil-plant system functions under conditions of constant air temperature and at optimal soil water content (Jauneau *et al.*, 2020). So climate variability does not count here. Provided that all other conditions such as soil fertility its density and water content are the same as in the field. Moreover, it is natural that these conditions should be optimal.

Referencing the authors (Joachimsmeier, 2011; Singh, Singh., 2013; Singh, 2014), it is noteworthy to note that the guttation method is efficient and was first described in detail back in 1676 by Abraham Munting (Ivanoff, 1963) already. Having such a rich experience in using the guttation method, a justified question arises why this method has not yet found wide application. It seems to us that apparently, this method requires special conditions in a well-equipped laboratory. On the contrary, using the example of this work, we can show that for this it is enough to have any suitable closed container where it will be possible to place any small containers with water and a couple of cylinders with soil where the germinated seeds of grain crops will be introduced. If at the same time it is possible to maintain a constant air temperature in this closed container, which will be different for different crops, then after 52 hours you can begin to collect guttation liquid from plant sprouts. It seems to us that this method is so simple that it can be used by any farmer.

Having already rich experience in the application of the guttation method, we have the right to confirm that using this method, the water constants (Reppo, 1981) of the soil (field capacity (FC) including) can be determined. It is also possible to use this method to determine the level and disposition of the negative impact of heavy agricultural machinery on the soil (Reppo, Nugis, 1983; Kuht, Reintam, 2001; Nugis *et al.*, 2020).

Since, according to the logic of the test crops, they also respond synchronously (through guttation) to soil fertility if certain crops also react to this, then the logical consequence is the forecast of the yield using the guttation method. The purpose of this first-time research on the example of potato cultivation was conducted.

Methods

The studies were carried out in the Lääne-Harju Community quite near of Klooga (N59° 19'; E24° 15') since 2020 on the land use of private family farm 'Miili'. To solve these subjects a specialized test site on an area of 0.07 hectares was organized. Display (by ArcGIS-online) of orthophoto shown in Fig. 1.

Assessment of the state of plant for guttation (spring barley variety 'Anni') by using of hydro-thermostat in conditions adapted hose hold laboratory (according to the generally known a bad epidemiological situation) were carried out. The test sites on a light sandy loam of Molli-Calcaric Cambisol (WRB) were located. For comparison, soil samples No. 1 and 2 within the same potato field were taken. Since it was impossible to determine the level of fertility for soil samples No. 1 and 2 under the conditions of a household, the laboratory tests on guttation with rich humus and cleanly sand were carried out.

In experiments *in vitro,* an old electric stove oven was used as a hydro-thermostat, the container of which was filled with 8 small plastic boxes (1012.5 cm^3 of one box) which also filled with water so that most of the oven would be occupied by these boxes. To provide a constant temperature in such a sealed oven and at the same time to provide the maximum relative humidity of the air, an additional heater with a fan was used. Warm fan air directed towards the oven door kept and in this result the temperature inside the oven constant at 21 °C. According to our preceding investigations in the laboratory of the Estonian Crop Research Institute, the temperature at box TPS-3 as hydro-thermostat was 23 °C (Nugis, Kuht, 2013).

In the same thermostat and under the same conditions of heat and relative air humidity (more than 90%), germination of seeds of barley of the 'Anni' variety was carried out. For this is needed 24 hours only. Germinated (*in vitro L.*) seeds 5 pieces per cylinder (geometric parameters of the cylinder are 270 cm^3 in volume; 6.4 cm in diameter and 8.4 cm in height) were according to the patent (Nugis, Kuht, 2013) to put in the soil of the cylinder which was then placed in a hydro-thermostat.

After 52 hours of keeping the cylinders in the hydro-thermostat, barley sprouts with an average length of 3 cm appeared on which droplets of water appeared. These droplets were collected (after every 3 hours) using dry filter paper pretreated in a 5% solution of copper sulfate. Thanks to this, an imprint or a blot of this droplet appeared on the filter paper. After the process of drying the guttate on filter papers, they were scanned. The scanned imprints were processed on a computer using Foxit Reader.

Previously, soil samples were taken from a potato field after harvesting potatoes, variety 'Ando'. Concerning experiments of guttation soil samples marked with crosses (Fig. 1) five replicates from a potato field with two contrasting soil samples No. 1 and 2 were taken.

The cylinders in which there was soil with the above soil samples (for differing treatment I and treatment II) and where guttational barley plants were grown have had a volume of 271 cm^3. The water content % (kg kg^{-1}) and bulk density (Mg m^{-3}) of the soil was also determined by the above-mentioned cylinders. Wherein, one cylinder was with humus-rich soil (III treatment) where the germinated seeds of 'Anni' barley were to put in soil, and the second – to put in clean sand (IV treatment). Everything else was done in the same way as described above.

Concerning the above, the guttation experiments in three replicates were carried out.

The obtained indicators were used in the implementation of a guttation method, as well as the peculiarities of the young sprout of barley and ways of their discharge of guttation fluid. This was the first time it was implemented in household's conditions.

The statistical estimation of data of the areas of a splotch of the guttation fluid and corresponding yield of potato tubers has been carrying out by Student T-test at 0.05 levels. The least significant difference (LSD) test as of right was used. In addition, the correlation coefficient (r) was calculated through the coefficient of determination (R^2) taking from it the square root.

Figure 1. Orthophoto of test site of a potato field in the private family farm "Miili", borough town Klooga, Lääne-Harju Community

Note: the signs on the orthophoto show the location of soil sample No. 1 and guttation treatment I in the potato field after harvesting the potatoes (variety 'Ando') and at the other end part of the field the soil sample No. 2 and guttation treatment II. (concerning more and less fertile soil, it was an initial valuation only).

Results

As a result of our investigations it was found that after harvesting of potato tubers in the soil sample No. 1 (I treatment) the yield on average was 14 150 ± 800 kg ha^{-1} or 0.89 relative units (highest yield was 15 900 kg ha^{-1} or 1.00 relative units) and on the less fertility part (II treatment) of the field − 6200 ± 1000 kg ha^{-1} or 0.39 relative units (smallest yield was 3900 kg ha^{-1} or 0.25 relative units). As for the area of an imprint of the guttation droplet on the filter paper, these indicators had average values for soil sample No. 1 − 55.1± 3 mm^2 (or 0.47 relative units) and for soil sample No. 2 − 42,9 ± 4,8 mm^2 (or 0.36 relative units), respectively. With all three replications taking into account the growth and development of barley sprouts (variety 'Anni') it was possible to collect water droplets on the barley sprouts only for one and a half days. If the barley sprouts could continue to grow then the plant roots would reach the

bottom of the cylinder and after that, the data of the above-mentioned droplets would have already distorted values. How the relationship between the value of the relative area on the filter paper of the guttation droplet and the potato yield looks can be seen in Fig. 2.

Concerning water content (WC) and bulk density (BD) related above mentioned treatments they are: I treatment WC = 24.4 ± 1.0% (kg kg^{-1}), BD = 1.07 ± 0.04Mg m^{-3}, II treatment WC = 19.8 ± 1.1% (kg kg^{-1}), BD = 1.12 ± 0.05 Mg m^{-3}. At the same time we have had for humus-rich soil (III treatment) WC = 20.5 ± 1.4% (kg kg^{-1}), BD = 0.92 ± 0.06Mg m^{-3} and for clean sand (IV treatment) − WC = 19.2± 1.3% (kg kg^{-1}), BD =1.16 ± 0.03Mg m^{-3}, respectively.

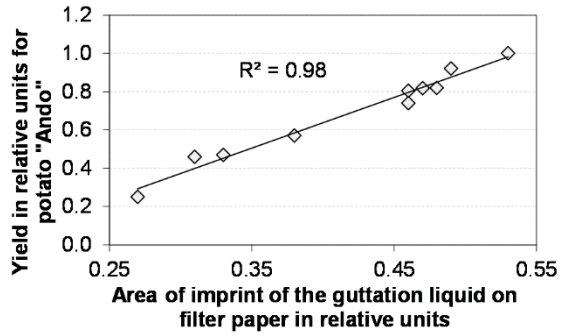

Figure 2. Relationship between the area of the imprint of guttation liquid (in relative units) and yield of potato 'Ando' ($P < 0.001$, $R^2 = 0.98$).Note: concerning yield of potato for soil sample No. 1 soil LSD_{05} = 2 530 kg ha^{-1} (0.16 relative units) and for soil sample No. 2 LSD_{05} = 3170 kg ha^{-1} (0.20 relative units). At the same time concerning the area of the imprint of guttation liquid on filter paper LSD_{05} = 2 mm^2 (0.03 relative units)

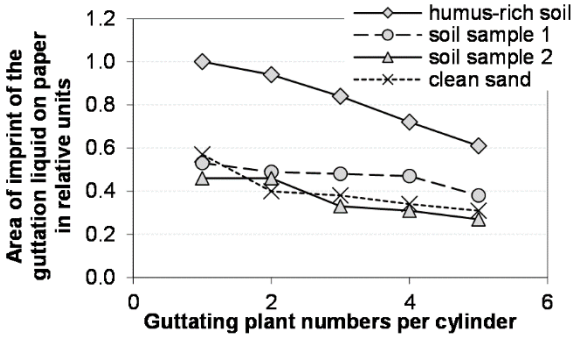

Figure 3. Results of comparing soil sample 1 (treatment I) and soil sample 2 (treatment II) of a potato field with the extreme level of fertility, i.e. humus-rich soil (treatment III) and clean sand (treatment IV)
Note: concerning area of imprint of guttation liquid on filter paper for humus-reach soil LSD_{05} = 73.5 mm^2 (0.63 relative units), but for clean sand − LSD_{05} = 22.1 mm^2 (0.19 relative units)

According to the above task, in connection to compare the degree of soil fertility of parts of a potato field with extreme levels of fertility (humus-rich soil and clean sand); the results are shown in Fig. 3. It can be seen from this figure that the highest value of the guttation droplet imprint on the filter paper was in the humus-rich soil which was equal to 117.6 ± 30.0 mm^2 or 1.00 relative units (average 89.1 ± 3.2 mm^2 or 0.76 relative units), while for pure sand, on the contrary, it was 37± 9 mm^2 or 0.31 relative units (but average 47.1 ± 5.2 mm^2 or 0.40 relative units).

Discussion

When considering the method of gutting the main problem until now was not the yield of this plant, but the description of the process of releasing a droplet of water under certain conditions by this plant (Eaton, 1943; Ivanoff, 1963). In this regard, there are various studies (Takeda *et al.*, 1991; Hughes, Brimblecombe, 1994; Joachimsmeier *et al.*, 2011) on how many guttation droplets are emitted by a plant, while guttation is closely correlated with temperature and humidity. Also, the study of guttation after the germination of spring wheat in dense soil (over 1.4 Mg m^{-3}) showed somewhat lower assimilation of water from the soil. This was revealed in the lower percentage of guttation water that was pressed out of hydathodes by the root pressure of plants (Nugis, 2017; Kuht, Reintam, 2001).

We could thus reach the depths of the xylem structure which is the vascular tissue of the plant that conducts water and dissolved nutrients up from the root to the end of the plant sprout (Komarnytsky *et al.*, 2000). If we go even deeper into the problem of guttation then it is known that the xylem plays a double role (Fisher *et al.*, 1997) providing 1) the gradient of the water potential (transpiration); 2) gradient of osmotic potential (root pressure of the fluid). In this case, this work would have a completely different purpose. Be that as it may, but we decided to use a simple method to determine the relationship between the guttation of the test culture and the potato yield. The fact that such a result was obtained (Fig. 2) and such a close rectilinear relationship ($P < 0.001$, $R^2 = 0.98$), we could not even expect. It can be emphasized here that a large difference in the natural fertility of the soil of the potato field played a certain role here. The natural fertility of the soil of this potato field was formed as a result of the 4-year rest of the field under black fallow. Weed control was the only challenge to keep the black fallow. However, about the marginal part of the potato field (Fig. 1 and 3), as a result of long-standing reclamation work, a large amount of gravel was carried out to the surface where even weeds did not want to grow. Therefore, the soil of this part of the field, as shown by the results of experiments on guttation. It differs little in fertility from clean sand (Fig. 3).

The next problem for us was how to assess the level of soil fertility in a potato field. Since it is known that everything is cognized in comparison it seems to us that in practice the chosen method has fully justified itself in this (Fig. 3). As can be seen from this figure that such part of the soil of the potato field which was soil sample No. 1 (treatment I) is 1.6 times inferior to the humus-rich soil (treatment III) according to the results of guttation which can be compared with chernozem. The fact that single sprouts of 'Anni' barly numbered in the order of decreasing area of the imprint of a droplet of

water on filter paper (Fig. 3) vary relatively more in humus-rich soil (treatment III) than in soil sample No. 1 (treatment I) soil of a potato field which is apparently due to the quality of the seed, which was impossible foresee.

As for the results obtained in relative units, we took as the basis for the potato yield its maximum value of 15 900 kg ha^{-1}, which was equated to one. All other yield data were individually divided by 15 900 and thus a corresponding numerical series of indicators in relative units was obtained. The same was done with the data of guttation where the largest area of the imprint of a droplet of guttation liquid on filter paper was taken 117.6 mm^2 or 1.00 as a relative unit. This largest area of the imprint belonged to humus-rich soil about which all other data on the areas of the imprints of a droplet of guttation liquid on filter paper for experimental treatments I, II, III and IV were calculated (Fig. 2 and 3).

Approaching our main goal which is associated with the forecast of the potato (variety 'Ando') yield if it were cultivated on a highly humus-rich soil then this can be done analytically by calculating through a simple ratio between the above-average indicators of guttation results in mm^2 and the average yield of potato tubers in a field with soil sample (No. 1) then if 14 150 kg ha^{-1} ∗ 89.1 mm^2 / 55.1 mm^2 = 22 880 kg ha^{-1} which would be the logical result. If this figure is rounded up to 30 Mg ha^{-1}, then taking into account the fact that if we are dealing only with black fallow where potato cultivation will be carried out without the introduction of mineral and organic fertilizers then with such classic organic farming. Finally, we can ultimately consider that this hypothetical yield of potato is a very real result.

Conclusions

The results of our research have shown that the guttation method is an economical, cheap and efficient method for predicting potato yield. These studies have shown that taking into account the existing epidemiological situation it is quite feasible to carry out the necessary laboratory experiments in household laboratory conditions. On the example of the cultivation of potatoes (variety 'Ando') and guttation experiments with barley (variety 'Anni') in conditions of soil samples No. 1 and 2 parts of the potato field as a result of laboratory and field experiments a completely reliable ($P < 0.001$, $R^2 = 0.98$) relationship between guttation and the yield of potato tubers was obtained.

To assess the different levels of soil fertility in the potato field taking as a reference soil with the highest possible fertility (humus-rich soil) and with the lowest possible fertility (clean sand) it was revealed that guttation of soil sample No. 1 was 1.6 times inferior according to the results of guttation of the humus-rich soil which can be identified with chernozem. The corresponding analytical calculations showed that if with black fallow we were dealing with humus-rich soil where potato cultivation would be carried out without the introduction of mineral and organic fertilizers which would naturally correspond to the conditions of classical organic farming then the yield of potato tubers is about 22 880 kg ha^{-1} or roughly 30 Mg ha^{-1} which is a probable entirely a real result.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publications of this paper.

Author contributions

EN − 40%, JK − 40%, AK − 20% − study of the concept and design;
EN − 60%, JK − 20%, AK − 20% − data collection;
EN − 40%, JK − 30%, AK − 30% − analysis and interpretation of data;
EN − 60%, JK − 30%, AK − 10% − writing a manuscript;
EN − 30%, JK − 40%, AK − 30% − critical revision and approval of the final manuscript.

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RESEARCH INTO THE PARAMETERS OF A POTATO HARVESTER'S POTATO HEAP DISTRIBUTOR, AND THE JUSTIFICATION OF THOSE PARAMETERS

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ABSTRACT. The low levels of efficiency and general quality when it comes to the use of potato harvesters in difficult soil and climatic conditions substantiate the relevance of the problem which is faced in terms of research by technologically advanced equipment and tools. They are looking to increase the efficiency of potato harvesters. This paper serves to justify the formation of the design and technical parameters of the V-type distributor, which directly acts on the undermined mass to increase the ability of the potato harvester to separate the soil. Preliminary experimental studies have shown that to achieve efficient technological processing in terms of the distribution of the general soil and potato heap, the distributor must possess the appropriate technological and design parameters. Calculations which have been carried out by using as a basis the theoretical dependencies that have been obtained serve to allow us to determine the optimum speed of progress through the heap, using the following design and kinematic parameters: V_{el} = 2.0 m s^{-1}, A = 0.35 m, h_v = 0.22 m, Δ = 0.08 m, b_{el} = 1.2 m. The allowable speed for heap movement will be $[V]$ = 1.62 m s^{-1}, which will ensure the prevention of any heap clogging in front of the distributor. An analysis of the dependencies which have been obtained during the work shows that rational values for the distributor wing's fitting angle fall within the range of α = 40°.

Introduction

Experience in operating potato harvesters shows that they can satisfactorily carry out their required technological processes only when working on favourable soil and in equally favourable climatic conditions (Ruysschaert *et al.,* 2006; Bishop *et al.,* 2012; Ichiki *et al.,* 2013; Lü *et al.,* 2017; Kheiry *et al.,* 2018; Ruzhylo *et al.,* 2020). When working in difficult conditions, harvesters do not provide high-quality potato harvesting and have low levels of efficiency, plus a lack of reliability in terms of the technological processes, and increased losses amongst and damage to tubers, which leads to increased costs in terms of harvesting because the process requires significantly more labour, including the provision of labour input (Misener, McMillan, 1996; Blahovec, Židova, 2004; Bulgakov *et al.,* 2020). Therefore any study of working bodies that can increase the efficiency of potato harvesters is something of an urgent task.

It is possible to improve the quality indicators in potato harvesters by introducing intensifying equipment into the technological scheme, which additionally interacts with the digging layer (Gao *et al.,* 2011; Lü *et al.,* 2015; Wang *et al.,* 2017; Issa *et al.,* 2020). Studies (Feller *et al.,* 1987; Hevko *et al.,* 2016; Feng *et al.,* 2017; Wei *et al.,* 2017; Xin, Liang, 2017; Bulgakov *et al.,* 2018, 2019; Gulati, Singh, 2019) have shown that

destroying the digging potato ridge is advisable to be able to carry out dynamic action processes in the digging zone as, in this case, the tubers are protected by the soil from any mechanical damage. One of the more promising avenues of research involves the vertical rotor (Bulgakov *et al.*, 2021). For example rod drums that have been installed between the digger shovel or two wings and the elevator, which, simultaneously to the feeding of the dug mass onto the elevator, serve to destroy the layer and sift out parts of the soil. Research which has been carried out at the National Scientific Centre, the Institute for Agricultural Engineering and Electrification, have shown that when at work (especially in conditions which involved raised humidity levels) and when fitted with digging and separating equipment which consists of a passive digger shovel and rod drums, the potato harvester has an essential area in which it is lacking: the digging layer is not evenly distributed onto the basic separating bar elevator, instead of coming in the form of a compact longitudinal windrow in the central section of the elevator. During further movement, the swathe is partially distributed across the width of the elevator surface, but not to a great enough degree for quality soil separation. As a result, due to the low level of completeness of separation, the level of clogging of tubers in the container is high, which does not meet current agrotechnical requirements. In addition, at such a distribution level of the pile on the elevator, the process of destroying soil clods by balloon-compactors is somewhat unsatisfactory (Petrov, 2004; Pshechenkov *et al.*, 2018).

Experimental studies (Wei *et al.*, 2019a, b) have shown that the intensification of the separation process utilizing uniform distribution of the heap across the width of the elevator web can improve the quality of the results seen in the use of the potato harvester.

The purpose of this study was to justify the design for and technological parameters of the potato pile distributor, which is part of the potato harvesting machine.

Materials and methods

Experimental studies into the use of the harvesting machine (Fig. 1 and 2) and an analysis of the quality of its working processes have shown that, before feeding it into the main elevator, as the digging layer is well-crushed by the rod drums – in this case for the uniform distribution of the heap – a passive operating tool is promising, with that unit being a V-type distributor which directly acts on the dug mass. It consists of two wings that have been turned at a fitting angle of 2α and are located behind the rod drums, above the main rod elevator (conveyor).

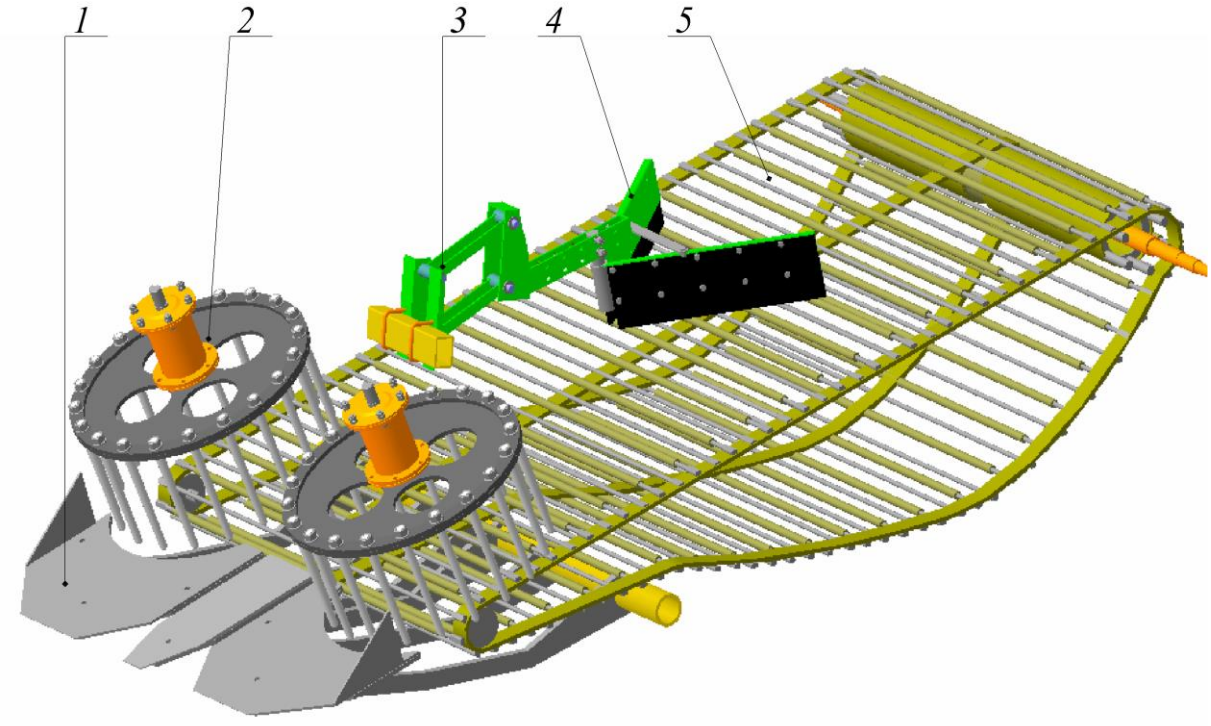

Figure 1. Digging and separating equipment for the potato harvesting machine: 1) digger shovel; 2) rod drum; 3) parallelogram mechanism; 4) distributor; 5) separating elevator

The technological process which is involved in distributor operation is as follows (Fig. 1). The soil and potato heap, undermined by the digger shovel (1), and by bar drums (2), is fed into the elevator (5) and falls onto the distributor (4). Then the flow of the crop moves along the wings of the distributor (4), while some part of the soil will always pass under the distributor because it is set so that it has a gap with respect to the surface of the elevator (5). Therefore,

after the distributor (4), we get a layer of the crop that is uniform across the entire width of the elevator (5).

The distributor (4) must have the appropriate technological and design parameters to be able to qualitatively pass any test of the technological process of soil and potato heap distribution.

Figure 2. General view of the pilot potato harvesting machine

The height of the wing on the distributor h is selected based on prevailing conditions, wanting to ensure that the heap does not overshoot the heap through the distributor, while also depending upon the thickness of the layer of the heap h_b, which comes onto the conveyor after passing the rod drums. The thickness of a layer depends upon the depth of the undercutting process for potatoes h_{gr}, parity of the translation speed of the potato harvesting machine V_M, the circumferential speed of the rotation of the rod drums ωR, and the speed of the rod elevator web V_{el}, and also on the separating ability of the drums and transporter.

When considering an unfavourable case in which separation in the section up to the distributor does not occur and the entire crop is fed into the distributor, the conditions in which the height of the wing h is to be selected, taking into account the gap between the conveyor and the distributor ρ, is as follows:

$$h + \Delta > h_v, \tag{1}$$

where:

$$h > h_v - \Delta. \tag{2}$$

The size of the gap Δ is determined by taking into account the following factors: firstly, it is necessary to ensure the free passage of potato tubers between the elevator surface and the distributor; and secondly, the necessary uniform thickness of the pile flow on the elevator needs to be ensured. At the same time, it is also necessary to take into account the amplitude of oscillations in the elevator surface, which are carried out under the influence of shakers.

When taking into account the fact that potato tubers, while moving on the elevator and trying to take up a stable position when the centre of mass of a tuber takes the lowest position, ie. is placed perpendicular to the surface of the elevator thickness or width of the tuber, then the height of the gap Δ must not be less than the greatest width of the potato tuber b_b:

$$\Delta > b_b. \tag{3}$$

The height of the heap flow h_v which arrives at the distributor we can find by equating the second feed from the coulters and the second capacity of the conveyor:

$$Q_{el} = Q_p, \tag{4}$$

where Q_{el} is the conveyor capacity, kg s^{-1}; Q_p is the feed for the digging and separating equipment, kg s^{-1}.

The second feed rate for the digging and separating equipment for a two-row potato harvester can be found according to the formula:

$$Q_p = 2 \cdot S_p \cdot V_m \cdot \rho_p, \tag{5}$$

where S_p is the cross-section of the digging layer, m^2;
V_m is the speed of the harvester, m s^{-1};
ρ_p is the bulk density of the digging layer, kg m^{-3}.

If the contour of the ridge in the cross-section is taken as a parabola, then the cross-section of the digging layer can be determined as follows:

$$S_p = \frac{2}{3} \cdot h_{gr} \cdot l_{gr}. \tag{6}$$

where h_{gr} is the ridge height, m; l_{gr} is the ridge width, m; then:

$$Q_p = \frac{4}{3} h_{gr} \cdot l_{gr} \cdot V_m \cdot \rho_p. \tag{7}$$

Elevator capacity:

$$Q_{el} = S_v \cdot V_{el} \cdot \rho_p, \tag{8}$$

where S_v is the cross-section of the soil and potato heap on the elevator, also:

$$Q_{el} = h_v \cdot A \cdot V_{el} \cdot \rho_p. \tag{9}$$

where h_v is the height of the heap on the elevator, m; A is the width of the web on the elevator, m.

By equating (7) and (9), after transformations we obtain:

$$h_v = \frac{4 h_{gr} \cdot l_{gr} \cdot V_m}{3 \cdot A \cdot V_{el}}. \tag{10}$$

The length of the distributor wings l must be long enough to distribute the heap across the entire width of the conveyor belt. In addition, a free zone must be provided at the point at which the heap comes off the wing. The length of the distributor wings l can be determined using the expression:

$$l < \frac{b_{el} - A}{2 \sin \alpha}, \tag{11}$$

where b_{el} is the elevator width; α – wing fitting angle.

The distance between the distributor and the drums affects the technological process because if the distance is too short, the heap will accumulate, blockages may occur, and the heap will not pass through; on the other hand, if the distance is too great, the distribution of the crop starts later, and the separation efficiency levels in the first conveyor will decrease. The greatest size of the heap will form at the beginning of the arrival of the heap onto the wings of the distributor. From Fig. 3 we have:

$$L > h_v \cdot \mathrm{ctg}\left(\varphi_v - \beta_{el}\right), \qquad (12)$$

where φ_v is the angle of the stall of the heap, β_{el} – is the angle of inclination of the elevator.

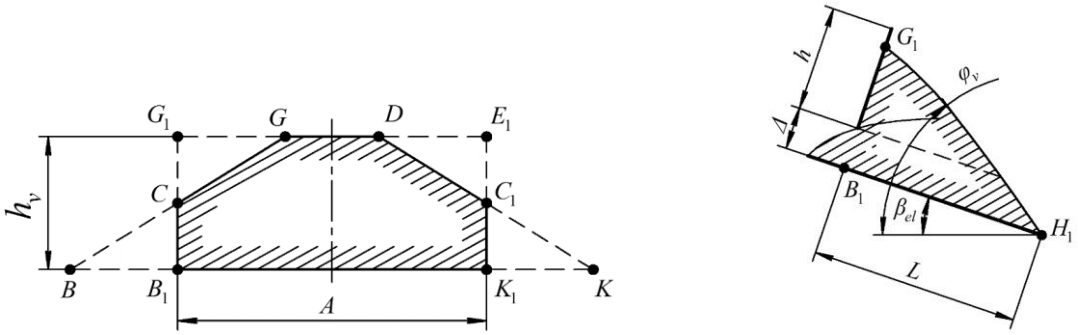

Figure 3. Diagram for calculating the parameters of the distributor: a) the shape of the heap flow coming onto the distributor; b) the shape of the heap flow which moves along the wing of the distributor: *BGDK* is the actual shape of the heap flow; and $B_1G_1E_1K_1$, $B_1G_1H_1$ is calculated from of heap flow

By substituting numerical values into expressions (10), (11), (12) we obtain the following values for the distributor parameters $h > 130$ mm, $l < 400$–500 mm, $\Delta > 80$ mm, $L > 540$ mm.

The main parameter for the distributor is the angle of the wings. Any incorrect choice of angle will lead either to a bunching of the heap in front of the distributor and, as a consequence, to a failure of the technological processes involved in automated operations, or to the fact that the crop will not be distributed across the width of the conveyor belt. To justify the angle being used in the distributor solution, a theoretical analysis needs to be carried out of crop movement on the working surfaces of the distributor and the separating conveyor.

Consider the interaction of a particle of the flow of the soil and potato heap, which moves along on the conveyor belt, with the wing of the distributor (Fig. 4). From this, a coordinate system can be drawn up, the beginning of which is connected with the nose of the distributor. The *Ox*-axis is directed in a direction that is perpendicular to the surface of the distributor; the *Oy*-axis is directed perpendicularly to the plane of the conveyor belt; the *Z*-axis is directed along the direction of flow of the crop through the distributor. On the proposed particle, the following forces will act: the gravitational force *G* (weight) of the particle on the heap, the normal reaction N_1 of the conveyor surface, the normal reaction N_2 of the spreader surface, the friction force F_1 in terms of sliding the particle along on the conveyor, the combined friction force F_2 in terms of sliding the particle on the heap along on the spreader on the axis *Oy*, and with F_3 being the combined friction-force-sliding of the heap particles on the spreader along the *Oz*-axis so that the force *P* comes from surface vibrations of the conveyor due to the shaking mechanism.

The equations regarding motion in the heap particle in vector form will be as follows:

$$m\bar{a} = \bar{G} + \bar{N}_1 + \bar{N}_2 + \bar{F}_1 + \bar{F}_2 + \bar{F}_3 + \bar{P}\,, \qquad (13)$$

where $G = mg$ is the gravity of a particle of the heap mass *m*;

$N_1 = mg \cdot \cos\beta_{el}$ is the normal reaction of the elevator surface;

N_2 is the normal reaction of the distributor surface;

$F_1 = N_1 \cdot f_1$ is the sliding friction force of the heap particle on the elevator, where f_1 is the coefficient of the sliding friction of the soil and potato heap on the elevator surface;

$F_2 = N_2 f_2$ is the component of the particle's sliding friction force on the distributor along the *Oy* axis, where f_2 is the coefficient of friction of the heap sliding along on the surface of the distributor;

$F_3 = N_2 \cdot f_2$ is the component of the sliding friction force of the heap particle on the distributor along the axis *Oz*;

$P = m \cdot \omega_{el}^2 \cdot A_{el} \cdot \sin\left(\psi_0 + \psi_f + \omega_{el} t\right)$ is the force from the vibrations of the conveyor belt when the shaking mechanism has been activated (we assume that harmonic vibrations occur perpendicular to the surface of the conveyor belt, while the amplitude of vibrations along the entire length of the conveyor belt is the same), where A_{el} is the vibration amplitude of the conveyor, and ψ_0 is the initial position relative to the zero amplitude (min); ψ_f is the phase shift (angular parameter), and ω_{el} is the frequency of elevator oscillations, rad s^{-1}.

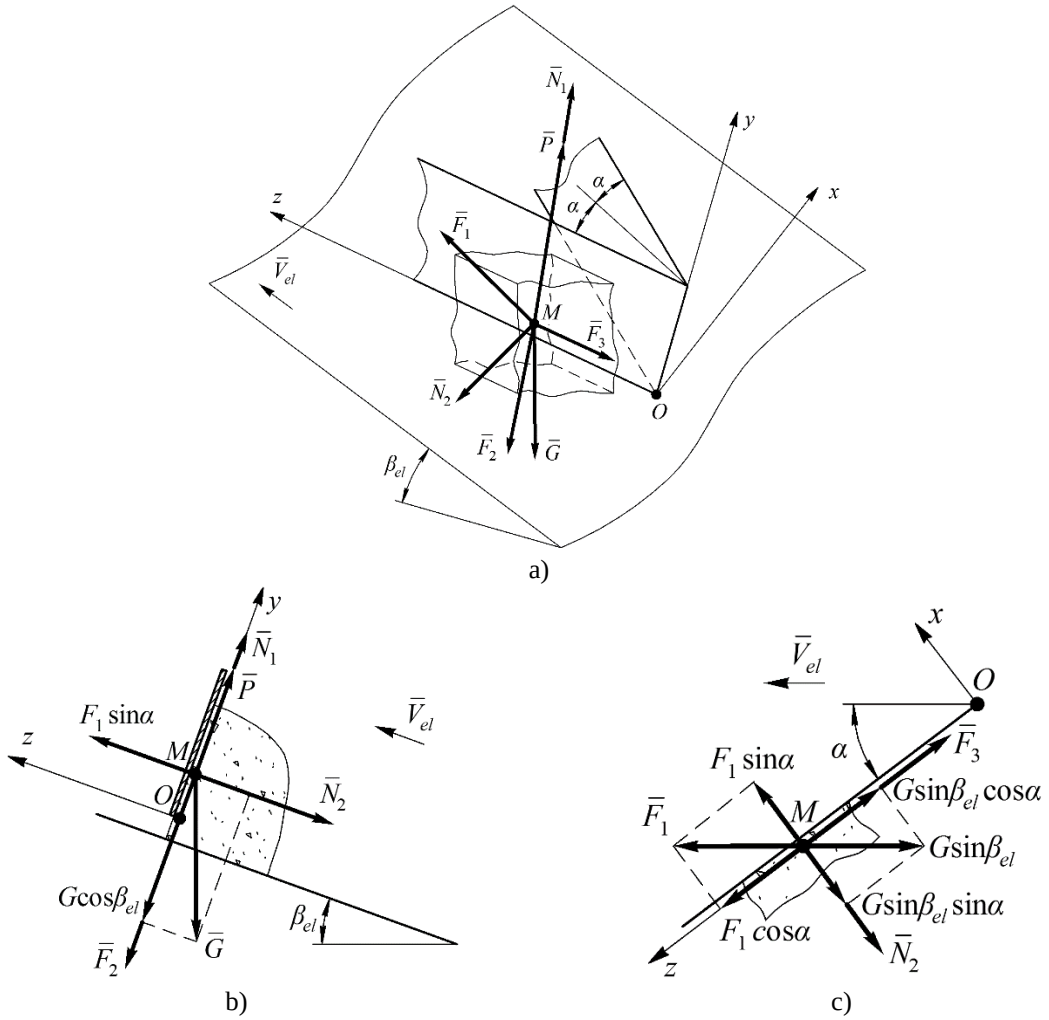

Figure 4. Schematic of interaction between the heap and the distributor: a) general view; b) view along the *yOz* plane; and c) view along the *zOx* plane

In projections onto the axes of the chosen coordinate system, the equations look like this:

$$m\ddot{x} = -N_2 + F_1 \cdot \sin\alpha - G \cdot \sin\beta_{el} \cdot \sin\alpha\,, \tag{14}$$

$$m\ddot{y} = N_1 + P - G \cdot \cos\beta_{el} - F_2\,, \tag{15}$$

$$m\ddot{z} = -F_3 + F_1 \cdot \cos\alpha - G \cdot \sin\beta_{el} \cdot \cos\alpha\,, \tag{16}$$

while after the transformation the result is this:

$$m\ddot{x} = -N_2 + f_1 \cdot mg \cdot \cos\beta_{el} \cdot \sin\alpha - mg \cdot \sin\beta_{el} \cdot \sin\alpha\,, \tag{17}$$

$$m\ddot{y} = mg \cdot \cos\beta_{el} + m \cdot \omega_{el}^2 \cdot A_{el} \cdot \sin\left(\psi_0 + \psi_f + \omega_{el} \cdot t\right) - mg \cdot \cos\beta_{el} - f_2 \cdot N_2\,, \tag{18}$$

$$m\ddot{z} = -f_2 \cdot N_2 + f_1 \cdot mg \cdot \cos\beta_{el} \cdot \cos\alpha - mg \cdot \sin\beta_{el} \cdot \cos\alpha\,, \tag{19}$$

In this system of differential equations, the unknown value of the reaction is N_2.

Since there is no motion along the *Ox*-axis, that is $\ddot{x} = 0$, then:

$$0 = -N_2 + f_1 \cdot mg \cdot \cos\beta_{el} \cdot \sin\alpha - mg \cdot \sin\beta_{el} \cdot \sin\alpha\,, \tag{20}$$

from:

$$N_2 = f_1 \cdot mg \cdot \cos\beta_{el} \cdot \sin\alpha - mg \cdot \sin\beta_{el} \cdot \sin\alpha\,. \tag{21}$$

After the substitution of force values, plus simplifications and transformations, the equations (14–16) look like this:

$$\ddot{x} = 0, \tag{22}$$

$$\ddot{y} = \omega_{el}^2 A_{el} \cdot \sin\left(\psi_0 + \psi_f + \omega_{el} \cdot t\right) - f_2 \cdot g \cdot \sin\alpha\left(f_1 \cdot \cos\beta_{el} - \sin\beta_{el}\right), \tag{23}$$

$$\ddot{z} = g\left[f_1 \cos\beta_{el} \cos\alpha - \sin\beta_{el} \cos\alpha - f_2 \sin\alpha\left(f_1 \cos\beta_{el} - \sin\beta_{el}\right)\right]. \tag{24}$$

The particle velocity projections of the heap will be found by integrating equation (22–24) under initial conditions t = 0: $\dot{x} = 0$, $\dot{y} = 0$:

$$V_0 = 0, \tag{25}$$

$$V_y = -f_2 \cdot g \cdot \sin\alpha\left(f_1 \cdot \cos\beta_{el} - \sin\beta_{el}\right) \cdot t - \omega_{el} \cdot A_{el} \cdot \cos\left(\omega_{el} \cdot t\right), \tag{26}$$

$$V_z = g \cdot t\left[\left(\cos\alpha - f_2 \cdot \sin\alpha\right) \cdot \left(f_1 \cdot \cos\beta_{el} - \sin\beta_{el}\right)\right] + V_{el} \cdot \cos\alpha. \tag{27}$$

The particle speed of the heap along the distributor's wing is:

$$V = \sqrt{V_x^2 + V_y^2 + V_z^2}. \tag{28}$$

The displacement of the heap particle can be found by integrating equations (25-27) under initial conditions t = 0: $x = 0$, $y = 0$, $z = 0$:

$$X = 0, \tag{29}$$

$$Y = -f_2 \cdot g \cdot \sin\alpha\left(f_1 \cdot \cos\beta_{el} - \sin\beta_{el}\right) \cdot \frac{t^2}{2} - \omega_{el} \cdot A_{el} \cdot \sin\left(\omega_{el} \cdot t\right), \tag{30}$$

$$Z = \frac{g \cdot t^2}{2}\left[\left(\cos\alpha - f_2 \cdot \sin\alpha\right) \cdot \left(f_1 \cdot \cos\beta_{el} - \sin\beta_{el}\right)\right] + V_{el} \cdot \cos\alpha \cdot t. \tag{31}$$

Results

Using the obtained expressions (25–27) and (29–31), we can describe the motion of the heap on the distributor's wing.

Using expression (28), we plotted the time dependence of the heap speed V on the distributor's wing at different values of the wing fitting angle α (Fig. 5).

An analysis of graphic dependences shows that the speed of the soil and potato heap movement along the wing decreases with an increase of the angle α. When speed V decreases less than the permissible value [V], the heap will unload onto the distributor, which will result in a violation of the machinery's technological process, that is, the condition must be fulfilled $V \geq [V]$.

The allowable speed of rotation of the heap, taking into account the process of the distribution of the heap across the width of the elevator web, can be found from the condition of equality in the heap flow into the distributor and its descent from the distributor:

$$\Delta \cdot b_{el}\left[V\right] = A \cdot h_v \cdot V_{el}, \tag{32}$$

where [V] is the allowable speed of heap movement along the distributor's wing, m·s^{-1}.

Consequently, the speed of the soil and potato heap along the distributor's wing should not be less than the value:

$$\left[V\right] = \frac{A \cdot h_v \cdot V_{el}}{\Delta \cdot b_{el}}. \tag{33}$$

At the given V_{el} = 2.0 m·s^{-1}, A = 0.35 m, h_b = 0.22 m, Δ = 0.08 m, b_{el} = 1.2 m the permissible speed of soil and potato heap movement is [V] = 1.62m·s^{-1}.

An analysis of the obtained dependencies shows that the rational values of the distributor's wing fitting angle fall within the range α = 40°.

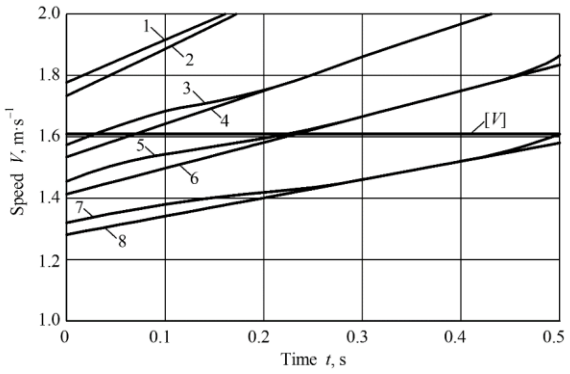

Figure 5. Dependence of heap speed along the distributor's wing V on time t at different values of the wing fitting angle α (at $f_1 = f_2 = 0.55$, $\beta_{el} = 22°$):
1 – $\alpha = 30°$ ($\omega_{el} = 4$ rad s^{-1}, $A_{el} = 0.05$ m);
2 – $\alpha = 30°$ ($\omega_{el} = 0$, $A_{el} = 0$);
3 – $\alpha = 40°$ ($\omega_{el} = 4$ rad s^{-1}, $A_{el} = 0.05$ m);
4 – $\alpha = 40°$ ($\omega_{el} = 0$, $A_{el} = 0$);
5 – $\alpha = 45°$ ($\omega_{el} = 4$ rad s^{-1}, $A_{el} = 0.05$ m);
6 – $\alpha = 30°$ ($\omega_{el} = 0$, $A_{el} = 0$);
7 – $\alpha = 50°$ ($\omega_{el} = 4$ rad s^{-1}, $A_{el} = 0.0$ m);
8 – $\alpha = 50°$ ($\omega_{el} = 0$, $A_{el} = 0$)

Conclusions

1. The analytical dependences are obtained, which allow a determination to be made of the basic design and technological parameters of the distributor which is part of the digging and separating equipment of a potato harvesting machine.

2. Modelling the processes of soil and potato heap movement concerning the real design and kinematic parameters made it possible to determine the allowable speed of heap movement $[V] = 1.62$ m s^{-1}, which ensures the prevention of heap clogging in front of the distributor.

3. As a result of the application and analysis of the graphical dependencies for the mathematical model which has been obtained, it was determined that the rational values for the wing fitting angle of the distributor fall within the range of $\alpha = 40°$.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

Author contributions

VA, JO, VK – study conception and design;
JO, VK – drafting of the manuscript;
VA, VK, JO, RI – analysis and interpretation of data;
VK, VM, HK, YI – acquisition of data;
JO – critical revision and approval of the final manuscript.

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EFFICACY AND SELECTIVITY OF PRE-EM HERBICIDE ON DEPENDENCE OF SOIL TYPES AND PRECIPITATION IN SUNFLOWER CROP

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ABSTRACT. During the growing seasons in 2018 and 2019, two field trials were conducted to estimate how precipitation affects the efficacy of PRE-em herbicides in sunflower crop grown on different soil types. Both regions were naturally infested with a high population of *Polygonum aviculare* L., *Solanum nigrum* L., *Chenopodium album* L., *Amaranthus retroflexus* L., *Portulaca olearacea* L. and *Echinochloa crus-galli* (L.) P. Beauv. Efficacy of PRE-em herbicides varied among weed species, treatments, periods of efficacy estimation, regions and years, respectively. Overall performances of the PRE-em herbicides were correlated with the weather and soil properties. Humid April in Bitola region in 2018, particularly the first week after application (34 mm) before weed emergence caused herbicide leaching from the soil surface, which probably was the most likely reason for the lower efficacy of PRE-em herbicides in 2018, compared to their application in 2019. In 2018 precipitation above 30 years average were recorded in the Titov Veles region as well, but due to their equal occurrence particularly during the first and second week after application, as well as soil type properties (higher content of clay and organic matter) leaching did not occur and efficacy was good to excellent. Contrary, the limited precipitation after PRE-em application (five, nine, and eight mm during the first week before application, first and second week after application) may have contributed to the poor performance of PRE-em herbicides in the Titov Veles region in 2019 compared with 2018. Heavy precipitation directly following PRE-em application caused sunflower injury in the Bitola region in 2018, which ranged from 9–28% across PRE-em treatments seven days after application. Injures of oxyfluorfen and dimethenamid were more serious (24 and 28%, respectively). Sunflower yields for each treatment in both region s generally reflected overall weed control and crop injury.

Introduction

Sunflower (*Helianthus annuus* L.) is one of the four most important annual crops in the world grown primarily for edible oil and is cultivated on all continents (De la Vega, Hall, 2002). It is gaining importance for oil production due to its photo insensitivity, short duration, low water requirement, drought tolerance and wide range of adaptability to various agro-climatic conditions (Reddy, 2005). Sunflower is an important oil crop in North Macedonia and is mainly grown following winter wheat or barley in non-irrigated cropping systems (Egumenovski *et al.*, 2003). Despite the adoption of good management practices, the productivity of sunflower in North Macedonia has been low, with average productivity of 1 440 kg ha^{-1} (Anonymous, 2019a), which is very much lower than the EU average of 2 210 kg ha^{-1} (Anonymous, 2019b), indicating wider scope for improving the yield potential. Weed competition has long been known to decrease sunflower yield (Johnson, 1971). Sunflower is usually planted in rows spaced 76 cm apart at lower densities than some other crops. Consequently, weeds that emerge during this time thrive in the wide interrow spaces. The season-long weedy conditions caused a 25.7% reduction in seed yield of sunflower (Wanjari *et*

al., 2000). The uncontrolled weed growth during the entire crop growth season caused an 83% reduction in seed yield of sunflower (Khan *et al.*, 1988; Legha *et al.*, 1992). Lewis, Gulden (2014) showed that sunflower yield was reduced by up to 76% when *Kochia scoparia* emerged at about the same time as the sunflower crop. Sunflower yield loss ranged from 35–54% under competition with *Avena fatua*, (Chubb, Friesen, 1985), *Sesbania herbacea* (Woon, 1987), *Orobanche cumana* (Grenz *et al.*, 2008), and mixed weed species (Reddy *et al.*, 2008). When *Artemisia biennis* emerged at about the same time as the sunflowers, the yield was reduced by up to 46% (Lewis *et al.*, 2016), whereas Johnson (1971) found that a combination of *Digitaria sanguinalis*, *Eleusine indica*, *Cassia obtusifolia*, *Ipomea purpurea*, *Ipomea hederacea*, and *Amaranthus retroflexus* decreased sunflower yield by 62% when the weeds competed with sunflower for the entire growing season. Therefore, weed control during the first 50–60 days after sunflower sowing is essential for high yield (Wanjari *et al.*, 2000). The outcome of crop-weed competition should be practised as early as possible to allow time for weed control measures (Kneževič, 2000). Concerning weed control, due to its sowing period (mid-March to mid-April), this crop is very often characterized by a complex specific weed flora, composed of grass and broad-leaved weeds (Fried *et al.*, 2006). This weed flora has been traditionally controlled with PRE-em herbicide applications, due to the scarce availability of POST-em herbicides (di Rapparini, 1996). The use and norms of PRE-em herbicides on the sunflower vary depending on the type of herbicides and their combination (Jursik *et al*., 2015; Simić *et al*., 2011).

PRE-em herbicides are intended to be applied to the soil, and many require activation by rainfall and irrigation (Rainbow, Derpsch, 2011; Haskins, 2012). The activity of PRE-em herbicides applied to soil surface depends not only on the physicochemical properties of the herbicides, but the soil organic matter and clay content, and the period before the first rainfall event after application and the duration of following rainfall events (Lamoreaux *et al.*, 1993; Rodrigues, 1993; Watts, Hall, 1996). For most PRE-em herbicides precipitation is required within 7–14 days after application to dissolve the herbicide in soil water solution so that it can be taken up by the emerging weeds after germination (Buhler, Werling, 1989; Buhler, 1991; Novosel *et al.*, 1998; Chomas, Kells, 2004). It is widely known that PRE-em herbicides, such as *S*-metolachlor and dimethenamid-P require precipitation within 7–10 days after the application for proper movement into the active zone of weed seed germination (Steckel *et al.*, 2002; Anonymous, 2008). Inadequate or delayed precipitation can reduce herbicide effectiveness and decrease weed control (Armel *et al.*, 2003; Lyon, Wilson, 2005; Loux *et al.*, 2008). In addition, it is reported that different meteorological conditions influenced the activity of the soil-applied herbicides in sunflower (Simić *et al.*, 2011). Depending on soil type, high amounts of precipitation (*i.e.* greater than 25 mm), especially immediately after application, can cause herbicides to leach through the soil profile and consequently reduce efficacy (Reddy, Locke, 1996; Ferrell *et al.*, 2004; Boerboom *et al.*, 2006). Pendimethalin is an example of an herbicide that is more persistent in the soil under dry conditions and can affect rotational crops but is easily leached when soil conditions are wet (Savage, 1978; Lee *et al.*, 2000). Furthermore, pendimethalin's weed spectrum is reduced, especially the control of annual grasses, when soil conditions are dry up to 3 weeks after application (Bond, Griffin, 2005). It is well known that PRE-em herbicide sorption is highly dependent on soil organic matter, organic manure and soil pH value (Rouchaud *et al.*, 1998; Mitra *et al.*, 1999).

Taking into consideration that PRE-em herbicides can decrease and delay susceptible annual weed emergence and establishment, reduce subsequent growth, and minimize weed/crop interactions (Adcock, Banks, 1991; Black, Dyson, 1993), the main objectives were (i) to estimate the efficacy of PRE-em herbicides in sunflower depends on precipitations and soil types, and (ii) to evaluate their injury effect and influence on the sunflower yield. This research will help many farmers to use PRE-em herbicides at the right time depending on climatic conditions with special emphasis on rainfall and soil type.

Material and methods

The field trials were carried out during two sunflower growing seasons in 2018 and 2019 on commercial sunflower fields in the Bitola 41° 34' 52" N, 21° 39' 54" E and Titov Veles 41° 12′ 23″ N, 21° 21′ 32″ E sunflower growing regions in south-western and central Macedonia on Molic-vertic gleysol and Vertisol, respectively (Filipovski, 2006) (Table 1).

Table 1. Soil characteristics in the sunflower-growing regions

Region	Soil	Coarse sand %	Fine sand %	Clay + Silt %	Organic matter %	pH-water
Bitola	Molic-vertic gleysol	31.10	50.30	18.60	1.56	6.10
Titov Veles	Vertisol	3.50	34.20	60.30	2.40	7.20

The sunflower *(Helianthus annuus* L.) was grown following conventional tillage practices. The soil was tilled with a field cultivator before sowing. Nitrogen, phosphorus and potassium were applied as per soil test-based recommendation. The field trials were carried out with "Surimi CL" and "Driver CL" sunflower hybrids sowed in a well-prepared soil at a seeding rate of 60 000 seeds ha^{-1} and 58 000 seeds ha^{-1} on 17th April 2018 and 11th April 2019 in the Bitola region, and on 8th April 2018 and 3rd April 2019 in the Titov Veles region respectively. The trials were conducted in two different regions of the same commercial sunflower fields. The area of the main plots was 21 m^2 (5 m long and 4.2 m wide, *i.e.*, seven sunflower rows). At

harvesting time, the sunflower grain yield (adjusted to 9% of moisture content) was determined by hand-harvesting the central part of each plot 3.5 m^2 (1.4 m × 2.5 m). The weedy control plots were left untreated during the entire experimental period. Weed-free control was maintained by hand weeding. Hand-weeding was initiated at weeds emergence and continued as needed to maintain weed-free plots.

The experimental design was a randomized complete block with four replicates. Treatments included Stomp Aqua (pendimethalin 455 g l^{-1}, BASF Agro B.V Arnhem (NL), Zweigniederlassung Zürich, Switzerland) at 3.0 l ha^{-1}; Proman (metobromuron 500 g l^{-1}, Belchim Crop Protection N.V./S.A. Londerzeel, Belgium) at 3.0 l ha^{-1}; Goal (oxyfluorfen 240 g l^{-1}, Dow AgroScience LLC, Indianapolis, IN) at 1.25 l ha^{-1}; Challenge 600 EC (aclonifen 600 g l^{-1}, Bayer Crop Science AG51368 Leverkusen, Germany) at 4.0 *l* ha^{-1}; Dual Gold 960 (S-metolachlor 960 g l^{-1}, Syngenta International, Basel, Switzerland) at 1.5 l ha^{-1} and Frontier 900 EC (dimethenamid-P 900 g l^{-1} BASF, Ludwigshafen am Rhein, Germany) at 1.7 l ha^{-1}. Untreated and weed-free controls were included in the studies, as well. All tested herbicides are registered for weed control in sunflower in the Republic of North Macedonia.

Herbicides were applied with a CO_2-pressurized backpack sprayer calibrated to deliver 300 l ha^{-1} aqueous solution at 220 kPa. Herbicides were applied at the dry seed – beginning of seed imbibitions sunflower growing stage (BBCH 00–01). Weeds at the time of treatment were in the same growth stages as sunflower (BBCH 00–01). Weed control efficacy was estimated 28 days after applications (DAA) after weed emergence (four true sunflower leaves, BBCH 14; the first assessment) and 56 DAA shortly before canopy closure (BBCH 30–32; the second assessment) by the weed plants counting from 1 m^2 area within each plot, and herbicide efficacy was calculated by equitation (Chinnusamy *et al.*, 2013):

$$W_{CE} = \frac{W_{up} - W_{tp}}{W_{up}} \times 100 \quad (1)$$

where:
W_{CE} – weed control efficiency
W_{up} – number of weeds in the untreated plots
W_{tp} – number of weeds in the treated plots

Sunflower injury was visually evaluated based on a 0–100% rating scale, where 0 is no injury to sunflower plants and 100 is complete death of sunflower plants (Frans *et al.*, 1986). Visual estimates of per cent sunflower injury were estimated 7 and 21 days after emergence (DAE), based on chlorosis and necrosis for each plot at both localities during the two-year experimental period. The yield was determined after harvest based on the weights of the grain containing 9% moisture.

The data were tested for homogeneity of variance and normality of distribution (Ramsey, Schafer, 1997) and were log-transformed as needed to obtain roughly equal variances and better symmetry before ANOVA was performed. Data were transformed back to their original scale for presentation. Means were separated by using the LSD test at 5% of probability.

Results and discussion

The general assessment of weed control

The efficacy of PRE-em herbicides varied among weed species, treatments and periods of efficacy estimation, regions and years, respectively. Overall performances of the PRE-em herbicides were correlated with the weather and soil conditions. Inconsistent weather patterns between the two years of the study likely influenced the weed control. Humid April in 2018 (Table 2), particularly 1st WAA (34 mm) before weed emergence, caused herbicide leaching from soil surface which probably was the most likely reason for lower efficacy of PRE-em herbicides 2018 compared to their application in 2019 in the Bitola region (Table 4). Precipitations 1st WBA and 2nd WAA in 2018 were in line with the average for Bitola region, but 1st WAA was unusually wet, particularly the 2nd, 3rd, and 6th day of the week, as well as 1st day of the 2nd WAA.

Table 2. Mean weekly temperatures (T) and total weekly rainfall (P) 1 week before (WBA) and 4 weeks after PRE-em applications, respectively at Bitola and Titov Veles region in 2018 and 2019

Weeks	Bitola region				Titov Veles region			
	2018		2019		2018		2019	
	P, mm	T, °C	P, mm	T, °C	P, mm	T, °C	P, mm	T, °C
1st WBA	16	12	9	16	14	14	5	17
1st WAA	34	9	18	14	22	11	9	15
2nd WAA	13	14	8	18	16	16	8	18
3rd WAA	7	17	17	13	5	18	15	15
4th WAA	4	15	9	17	1	18	13	18
Sum of P	74	–	61	–	77	–	50	–
Average of T		13.4		15.6		15.4		16.6

Abbreviations: WBA – a week before application; WAA – a week after application; P – precipitations, T – temperature.
PRE-em treatments in both years were applied at times when herbicide applications typically occur in North Macedonia sunflower production and are thus representative of producer practices and label recommendations.

In the Titov Veles region for the same year precipitations occurred in the 1st WBA, 1st WAA and 2nd WAA were 45% above the 30 years average for this region (38 mm). In 2019, precipitation occurred in the 1st WBA, and 2nd WAA were scarce for the Bitola region, while rainfall in the 1st WAA and 3rd WAA was in the line with the average for this region (41 mm). In the Titov Veles region, in the same year, the period during the1st WBA, 1st WAA and 2nd WAA was very dry (5, 9 and 8 mm). It rained on the third and fourth days at intervals throughout the 3rd WAA and 4th WAA, respectively (Table 2). Also, one week before and four weeks after PRE-em applications temperatures particularly in 2019 for both regions were a little bit above the average and that was attributed to favourable environmental conditions associated with non-frost night time during the estimated 1st WBA and 4 week

period after PRE-em applications, respectively. Usually, higher amounts of precipitation and heavy rains immediately after PRE-em application, particularly on sandy soils with low organic matter (Inoue *et al.*, 2010; Shaner, 2014) may cause leaching of herbicides through the soil profile below the weed seed-germinating zone and consequently decrease weed control efficacy (Heatherly, Hodges, 1998 Ferrell *et al.*, 2004). In 2018 precipitation above 30 years average were recorded in Titov Veles, as well, but due to their equal occurrence particularly during the 1st WAA and 2nd WAA, as well as soil type characteristics (higher content of clay and organic matter) leaching did not occur and efficacy was good to excellent. It is reported that higher soil organic matter content results in a higher herbicide efficacy (Xing, 2001). Opposite, the limited precipitation after PRE-em application (five, nine, and eight mm, during the 1st WBA, 1st and 2nd WAA) may have contributed to the poor performance of PRE-em herbicides in the Titov Veles region in 2019 compared with 2018 (Table 4). Since many of the PRE-em herbicides can volatilize and photodegrade on the soil surface over time, rainfall is needed to move these herbicides into the zone where weed seeds germinate (Wilcut *et al.*, 1994; Janak, Grichar, 2016) which explains the inconsistent control of predominant weeds noted with PRE-em herbicides under the drought conditions observed at Titov Veles region in early spring 2019. However, in both regions, regardless of year and herbicide treatments, the efficacy of PRE-em herbicides was insignificantly lowered by 56 DAA, due to new weed emergence occur between two estimation periods (Table 4 and 5).

Pendimethalin

PRE-em treatment with pendimethalin resulted in two distinct control years in both regions, but it did not significantly differ among periods of efficacy estimation by year. In 2018, in the Bitola region, 28 DAA weed control efficacy was ranged from 65% *Solanum nigrum* L. (SOLNI) to 77% *Chenopodium album* L. (CHEAL). Further decreasing in pendimethalin efficacy was recorded 56 DAA (between 54% SOLNI and *Echinochloa crus-galli* L. P. Beauv. (ECHCG), and 72% CHEAL. Pendimethalin efficacy was significantly improved in 2019. However, 28 DAA *Polygonum aviculare* L. (POLAV) was fully controlled (100%). Except for SOLNI (85%), the rest of the weeds were controlled between 96 and 97%. Negligible weed control decreasing occurred 56 DAA (Table 4). Unlike the Bitola region, in 2018, in the Titov Veles region efficacy of pendimethalin was substantially higher. 28 DAA weed control efficacy was ranged from 93% *Portulaca oleraceae* L. (POROL) to 96% *Amaranthus retroflexus* L. (AMARE). Only SOLNI was controlled <90%. Insignificantly lower efficacy (between 87% CHEAL and 91% AMARE and POROL pendimethalin provided 56 DAA. Significantly lower efficacy of this herbicide (78%) was recorded in control of SOLNI. In 2019 pendimethalin provided no more than 74% and 57% weed control 28 DAA and 56 DAA, respectively (Table 5). In the investigation of Pannacci *et al.*, (2007) pendimethalin applied at 921 g a.i. ha^{-1} (grams of active ingredient per hectare) in sunflower controlled AMARE between 88% and 100%, ECHCG between 94 and 100%, and CHEAL 100%. Similar, in the sunflower crop pendimethalin effectively controlled CHEAL more than 95%, while the efficacy on ECHCG ranged between 85 and 98% (Jursík *et al.*, 2015). In the same study, the efficacy of pendimethalin on *Solanum physalifolium* was significantly lower on plots without irrigation (≥ 67% compared to irrigated plots (≥85%).

Metobromuron

A significant treatment by year interaction resulted in two distinct years for metobromuron weed control in both regions, but metobromuron weed control did not significantly differ among periods of efficacy estimation by year. In the Bitola region, in 2018 metobromuron provided poor control of investigated weeds. 28 DAA efficacy was ranged between 55% ECHCG and 68% SOLNI. Lower efficacy from 46% CHEAL to 63% SOLNI was recorded 56 DAA. Metobromuron efficacy was significantly improved in 2019. The 28 DAA, metobromuron controlled CHEAL, POLAV and AMARE >95%, while significantly lower efficacy was recorded in control of SOLNI and ECHCG (78 and 82%, respectively). Decreasing of metobromuron efficacy for a few per cent in control of all weeds was noticed 56 DAA (Table 4). Metobromuron provided effective weed control in the Titov Veles region in 2018. AMARE and CHEAL were controlled 96%, POROL 91%, while SOLNI was controlled only 71%, 28 DAA. Metobromuron efficacy was slightly reduced by 56 DAA. Opposite, in 2019, due to dry soil conditions, control of weeds was less than 66% and 63%, 28 and 56 DAA, respectively (Table 5). Similar to these results of Bergmann (2016), in field trials conducted from 2009 till 2012 concluded that Proman (metobromuron) applied at 3 l ha^{-1} provided 90% control of POLAV, 93% of CHEAL), 89% of AMARE, but only 70% on SOLNI and 63% on ECHCG.

Table 3. Weed population (species and number of weeds) in sunflower at Bitola and Titov Veles region in 2018 and 2019

Weed species	Bitola region		Titov Veles region	
	2018	2019	2018	2019
Polygonum aviculare L.	33	14	–	–
Chenopodium album L.	24	21	54	27
Solanum nigrum L.	27	13	38	16
Amaranthus retroflexus L.	18	17	66	40
Echinochloa crus-galli (L.) P. Beauv.	16	14	–	–
Galinsoga parviflora Cav.	4	2	–	–
Abutilon theophrasti Medic.	2	3	2	1
Diplotaxis muralis (L.) D.C.	2	–	3	3
Portulaca olearacea L.	1	4	48	29
Digitaria sanguinalis (L.) Scop.	–	–		9
Total weed species	9	8	6	7
Total weeds (No. m^{2-1})	127	88	212	125

Table 4. Efficacy of PRE-em herbicides (%), 28 and 56 DAA in sunflower in 2018 and 2019 in Bitola regions [a-d]

Bitola region																								
Treatments	Pendimethalin 3.0 l ha^{-1}				Metobromuron 3.0 l ha^{-1}				Oxyfluorfen 1.25 l ha^{-1}				Aclonifen 4.0 l ha^{-1}				S-metolachlor 1.5 l ha^{-1}				Dimethenamid 1.7 l ha^{-1}			
	2018		2019		2018		2019		2018		2019		2018		2019		2018		2019		2018		2019	
Weed species	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA
POLAV	74^{a}	69^{a}	100^{a}	98^{a}	63^{ab}	61^{a}	98^{a}	95^{a}	71^{ab}	68^{a}	98^{ab}	95^{a}	68^{a}	66^{a}	100^{a}	97^{a}	65^{ab}	61^{ab}	99^{a}	97^{a}	60^{bc}	57^{b}	100^{a}	94^{a}
SOLNI	65^{b}	54^{b}	85^{c}	81^{c}	68^{a}	63^{a}	78^{c}	66^{c}	75^{a}	75^{a}	98^{ab}	92^{abc}	64^{a}	53^{c}	76^{c}	63^{c}	71^{a}	65^{a}	95^{b}	89^{c}	75^{a}	68^{a}	96^{abc}	87^{b}
CHEAL	77^{a}	72^{a}	96^{b}	89^{b}	59^{bc}	46^{b}	95^{ba}	90^{a}	67^{b}	55^{bc}	100^{a}	90^{bc}	64^{a}	56^{bc}	95^{b}	91^{b}	61^{b}	50^{c}	97^{ab}	92^{b}	56^{c}	42^{c}	95^{bc}	88^{b}
AMARE	73^{a}	61^{a}	97^{a}	90^{b}	65^{ab}	58^{a}	100^{a}	92^{a}	70^{ab}	61^{b}	97^{b}	93^{ab}	68^{a}	62^{ab}	98^{ab}	91^{b}	63^{b}	56^{bc}	95^{b}	87^{c}	60^{bc}	58^{b}	94^{c}	89^{ab}
ECHCG	69^{ab}	54^{b}	96^{b}	89^{b}	55^{c}	49^{b}	82^{c}	75^{b}	69^{b}	52^{c}	97^{b}	88^{c}	70^{a}	57^{bc}	98^{ab}	90^{b}	67^{ab}	62^{ab}	100^{a}	93^{b}	64^{b}	54^{b}	100^{a}	92^{ab}
LSD 0.05	7.17	6.74	3.26	5.22	6.25	5.81	4.23	7.52	5.08	7.43	2.54	4.73	6.65	6.48	4.03	5.26	6.36	7.86	3.68	2.51	6.90	6.08	4.19	5.03
Random effect interactions																								
PRE-em herbicides treatment × year	*				*				*				*				*				*			
PRE-em herbicides treatment × PEE	NS		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS	

[a]Abbreviation: PRE-em; DAA – days after application; POLAV – *Polygonum aviculare;* SOLNI – *Solanum nigrum*; CHEAL – *Chenopodium album*; AMARE – *Amaranthus retroflexus*; ECHCG – *Echinochloa crus-galli;* PEE – periods of efficacy estimation; NS – not significant; * Significant at the 5% level according to a Fisher's protected LSD test at P < 0.05

[b]PRE treatments were applied in the same growth stages as sunflower (at the dry seed – beginning of seed imbibitions of sunflower growing stage – (BBCH 00-01).

[c]Weed control efficacy was estimated at 28 DAA and 56 DAA

[d]Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at P < 0.05

Table 5. Efficacy of PRE-em herbicides (%), 28 and 56 DAA in sunflower in 2018 and 2019 in Titov Veles region [a-d]

Titov Veles region																								
Treatments	Pendimethalin 3.0 l ha^{-1}				Metobromuron 3.0 l ha^{-1}				Oxyfluorfen 1.25 l ha^{-1}				Aclonifen 4.0 l ha^{-1}				S-metolachlor 1.5 l ha^{-1}				Dimethenamid 1.7 l ha^{-1}			
	2018		2019		2018		2019		2018		2019		2018		2019		2018		2019		2018		2019	
Weed species	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA	28 DAA	56 DAA
AMARE	96^{a}	91^{a}	68^{b}	57^{a}	96^{a}	89^{a}	60^{ab}	51^{b}	94^{ab}	90^{a}	66^{a}	54^{b}	96^{a}	87^{a}	66^{a}	60^{a}	93^{a}	87^{a}	63^{b}	53^{b}	93^{ab}	85^{b}	58^{b}	49^{b}
CHEAL	94^{a}	87^{b}	64^{bc}	55^{ab}	96^{a}	87^{a}	53^{b}	48^{b}	94^{ab}	85^{ab}	59^{b}	52^{b}	94^{a}	87^{a}	57^{b}	52^{b}	90^{ab}	81^{ab}	55^{c}	46^{bc}	91^{ab}	83^{b}	53^{b}	44^{c}
POROL	93^{a}	91^{a}	74^{a}	51^{bc}	91^{b}	82^{b}	66^{a}	53^{b}	90^{b}	80^{b}	58^{b}	49^{b}	93^{a}	83^{a}	68^{a}	55^{ab}	85^{b}	78^{b}	53^{c}	42^{c}	88^{b}	79^{c}	57^{b}	46^{bc}
SOLNI	84^{b}	78^{c}	61^{c}	49^{c}	78^{c}	71^{c}	66^{a}	63^{a}	96^{a}	90^{a}	70^{a}	68^{a}	62^{b}	58^{b}	55^{b}	52^{b}	93^{a}	88^{a}	71^{a}	68^{a}	94^{a}	90^{a}	73^{a}	70^{a}
LSD 0.05	3.30	1.87	5.60	5.59	3.42	4.36	7.35	7.08	4.30	7.01	4.56	6.40	6.01	5.00	8.20	7.51	6.77	8.03	6.94	7.67	5.82	3.92	6.83	4.53
Random effect interactions																								
PRE-em herbicides treatment × year	*				*				*				*				*				*			
PRE-em herbicides treatment × PEE	NS		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS	

[a]Abbreviation: PRE-em; DAA – days after application; AMARE – *Amaranthus retroflexus;* CHEAL – *Chenopodium album*; POROL – *Portulaca oleracea;* SOLNI – *Solanum nigrum*; PEE – periods of efficacy estimation; NS – not significant; * Significant at the 5% level according to a Fisher's protected LSD test at P < 0.05

[b]PRE treatments were applied in the same growth stages as sunflower (at the dry seed – beginning of seed imbibition of sunflower growing stage – (BBCH 00-01)

[c]Weed control efficacy was estimated at 28 DAA and 56 DAA

[d]Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at P < 0.05

Oxyfluorfen

PRE-em application of oxyfluorfen resulted in two distinct years for its efficacy in both regions. However, weed control did not significantly differ among periods of efficacy estimation by year. In the Bitola region, in 2018, oxyfluorfen controlled weeds between 67% CHEAL and 75% SOLNI 28 DAA, and 52% ECHCG and 75% SOLNI 56 DAA. Significantly increasing in weeds control oxyfluorfen provided in 2019. 28 DAA, the CHEAL was fully controlled (100%), and the rest of the weeds were controlled between 97 and 98%. Except for ECHCG which was controlled 88%, the rest of the weeds were controlled between 90 and 95%, 56 DAA (Table 4). In the Titov Veles region, in the first experimental year (2018), oxyfluorfen effectively controls all predominant weeds. Efficacy was ranged from 90% POROL to 96% SOLNI, and 80% POROL to 90% SOLNI, 28 and 56 DAA, respectively. In the second experimental year (2019), oxyfluorfen efficacy substantially decreased. Efficacy of oxyfluorfen gave only marginal control (<70% and <68%, 28 and 56 DAA, respectively) of predominant broadleaf weeds (Table 5). Oxyfluorfen applied at 240 a.i. ha^{-1} in sunflower controlled AMARE, ECHCG and CHEAL 100% (Pannacci *et al.*, 2007). In the study of Jursík *et al.* (2015) efficacy of oxyfluorfen in sunflower, the crop was very good on AMARE (control greater than 95%) and was not affected by soil moisture conditions in any trial year, but oxyfluorfen was not as effective on CHEAL under non-irrigated conditions. In leek, oxyfluorfen at 360 g a.i. ha^{-1} controlled SOLNI 96% (Karkanis *et al.*, 2012).

Aclonifen

A significant treatment by year interaction resulted in two distinct years for aclonifen weed control in both regions, but aclonifen weed control did not significantly differ among periods of efficacy estimation by year. In 2018, in the Bitola region, 28 DAA weed control efficacy was ranged from 64% SOLNI and CHEAL to 70% ECHCG. Decreasing of aclonifen efficacy was recorded 56 DAA. The herbicide provided control between 56% SOLNI, and 66% POLAV. Next 2019 efficacy of aclonifen was significantly improved. 28 DAA, unless SOLNI which was poorly controlled (76%), the rest of the weeds were nearly fully controlled (>98%). Decreasing aclonifen weed control for few percents occurred 56 DAA (Table 4). Unlike the Bitola region, in 2018, in the Titov Veles region efficacy of aclonifen was substantially higher. Weed control efficacy was ranged from 93% POROL to 96% AMARE, 28 DAA, and 83% POROL to 87% AMARE and CHEAL 56 DAA. Poor aclonifen efficacy (only 62 and 58%) was noted in control of SOLNI during both estimation periods. In 2019 aclonifen provided no more than 68% and 60% weed control 28 DAA and 56 DAA, respectively (Table 5). In banded herbicide application in a conventional sunflower production system aclonifen applied at 0.75 kg a.i. ha^{-1} controlled CHEAL between 84 and 89% **(**Serim *et al.*, 2018). In the study of Jursík *et al.* (2015) aclonifen controlled AMARE and CHEAL with efficacy greater than 97%, regardless of irrigation. In the same study, aclonifen controlled ECHCG (efficacy over 80%), but only when irrigation was applied or natural precipitation at the beginning of the growing season was sufficient. On the other side, regardless of irrigation *Solanum physalifolium* was controlled between 52 and 56%. Also, in the investigation of Pannacci *et al.* (2007) aclonifen applied at 900 g a.i. ha provided poor control of SOLNI (33%–67%).

S-metolachlor

A significant treatment by year interaction resulted in two distinct years for S-metolachlor weed control in both regions, but S-metolachlor weed control did not significantly differ among periods of efficacy estimation by year. In the Bitola region, in 2018 S-metolachlor provided inadequate control of investigated weeds. 28 DAA efficacy was ranged between 61% CHEAL and 71% SOLNI. Further decreasing in efficacy from 50% CHEAL to 62% ECHCG was recorded 56 DAA. S-metolachlor efficacy was significantly increased in 2019. 28 DAA, S-metolachlor fully controlled 100% ECHCG, while the rest of the weeds were controlled between 95 and 99%. A negligible few per cent decreasing of S-metolachlor efficacy in the control of predominant weeds was recorded 56 DAA (Table 4). S-metolachlor provides effective weed control in the Titov Veles region in 2018. During the first estimation period 28 DAA, AMARE and SOLNI were controlled 96%, CHEAL 90%, while POROL was controlled <90%. Insignificantly lower efficacy between 78% POROL and 88% SOLNI S-metolachlor provided 56 DAA. Contrary, in 2019, due to dry soil conditions, control of weeds was less than 71% and 68%, 28 and 56 DAA, respectively (Table 5). S-metolachlor in irrigated sunflower plots nearly completely controlled AMARE and ECHCG (efficacy 93–100%). However, on treatment without irrigation, the efficacy of S-metolachlor on AMARE decreased by 8%, and the efficacy on ECHCG decreased significantly by 13% (Jursík *et al.*, 2015). In banana pepper S-metolachlor at 534 g a.i. ha^{-1} provided control of CHEAL of 99% (2 WAT) and 85 (4 WAT), while S-metolachlor at 1 070 g a.i. ha^{-1} provided control of CHEAL of 96 (2 WAT) and 90 (4 WAT). At the same rates, POROL was controlled between 61 and 67% (Mohseni-Moghadam, Doohan, 2015). Opposite, in spinach S-metolachlor at rates ≥0.56 kg ha^{-1} provided >95% control of POROL (Fennimore *et al.*, 2001).

Dimethenamid-P

PRE-em application of dimethenamid-P resulted in two distinct years for its efficacy in both regions. However, weed control did not significantly differ among periods of efficacy estimation by year. In the Bitola region, in 2018, dimethenamid controlled weeds between 56–42% CHEAL, and 75–68% SOLNI 28 and 56 DAA, respectively. Significantly increasing in weeds control dimethenamid provided in 2019. 28 DAA, POLAV and ECHCG were fully controlled (100%), and the rest of the weeds were controlled

between 94 and 96%. The SOLNI and CHEAL were controlled <90%, while POLAV and ECHCG controlled >90%, 56 DAA (Table 4). In the Titov Veles region, in the first experimental year (2018), dimethenamid-P effectively control all predominant weeds (>91% and >83%), except POROL (88% and 79%), 28 and 56 DAA, respectively. In the second experimental year (2019), dimethenamid-P efficacy substantially decreased. Efficacy of dimethenamid gave only marginal control (<73% and <70%, 28 and 56 DAA, respectively) of predominant broadleaf weeds (Table 5). In potato crop dimethenamid-P has provided greater than 96% control of SOLNI, CHEAL, and AMARE in Idaho research trials at rates of 1.1 to 1.7 kg ha^{-1} (Tonks *et al.*, 1999). Similar, in Idaho field research trials Alvarez, Hutchinson (2005) and Hutchinson *et al.* (2005) confirmed that dimethenamid-P provided acceptable season-long SOLNI control (>88%). Dimethenamid-P applied alone gave excellent control (>98%) of AMARE and SOLNI in dry bean (Arnold *et al.*, 2012). An evaluation of PRE herbicides for weed control in pumpkin found that 21 days after treatment dimethenamid-P at 2.24 kg ha^{-1} resulted in 81–100% control of AMARE (Brown, Masiunas, 2002). In the investigation of Yamaji *et al.* (2016) dimethenamid-P at 1138 g a.i. ha^{-1}, provided control of ECHCG greater than 90%. Similar, in sugarbeet AMARE and CHEAL control with dimethenamid-P, applied at 0.84 kg ha^{-1} was 99% and 91% (Bollman, Sprague, 2007).

Sunflower injury

PRE-em herbicides were applied at the time when herbicide applications typically occur in North Macedonia sunflower production and are thus representative of producer practices and label recommendations. However, in 2018 in the Bitola region, heavy precipitation occurred in the 1st WAA, which caused the leaching of herbicides through the soil profile. Possible that sunflower injury was due to higher amounts of rain (34 mm) directly following PRE-em herbicide treatments. It ranged from 9 to 28% across PRE-em treatments seven days after emergence (DAE). Injures of oxyfluorfen and dimethenamid-P were more serious (24 and 28%, respectively). Oxyfluorfen showed phytotoxicity symptoms like slight bleaching, leaf tip burn, and stunting of sunflower growth. Stunting of sunflower growth was recorded in plots treating with dimethenamid-P, as well. Injuries caused by other PRE-em herbicides decreased by seven and 21 DAA (Table 6). However, sunflower injuries of oxyfluorfen and dimethenamid were still evident at 21 DAE. In the same line are investigations by Jursík *et al.* (2015) who concluded that the sunflower phytotoxicity caused by oxyfluorfen was the highest (25–47%) without the effect of irrigation. Sunflower growth was inhibited and regeneration was slow; however, the seed yield was not significantly reduced in any year. Similar, in the study of Andr *et al.* (2017) the highest level of sunflower injury was recorded on plots treated by oxyfluorfen (18%). The injury caused by oxyfluorfen on sunflower was mainly caused by raindrops bouncing from the soil surface, which contaminated leaves and caused necrosis and leaf deformation. Further, the sunflower tolerance to dimethenamid-P was good (phytotoxicity less than 7%), except in the year when sunflower injury ranged from 10–12% across irrigation treatments. On the other side, the sunflower injury caused by pendimethalin, aclonifen, and S–metolachlor was minimal (between 5 and 7%) (Jursík *et al.*, 2015).

Sunflower yield

Sunflower grain yields for each treatment in both regions generally reflected overall weed control and crop injury (Table 6). Comparison of weed and weed-free control indicated that weeds reduced sunflower grain yield by 72–75% in the Bitola region, and 72–76% in the Titov Veles region for both years, respectively (Table 6). Similar, Jaykumar *et al.* (1988), Elezovic *et al.* (2012), and Alves *et al.* (2013) reported the yield reduction due to weeds in sunflower is estimated to be between 70 and 81%. A significant treatment by year interaction resulted in two distinct years for sunflower grain yield in the Bitola region. In both years, the lowest sunflower grain yield was recorded in untreated control plots (980 and 850 kg ha^{-1}, respectively). The lowest yield between PRE-em herbicides in 2018 was obtained in plots treated with aclonifen (2 030 kg ha^{-1}). No one of PRE-em applied herbicides yielded higher than the weed-free control, because sunflower yields were more closely related to the per cent of weeds control. In 2019 the effective removal of the competitive effect of the weeds led in an increase of the sunflower yield in all PRE-em herbicide treatments significantly increased and resulted in yields similar to that of the weed-free control (Table 6). A significant treatment by year interaction resulted in two distinct years for sunflower yields in the Titov Veles region with PRE-em herbicides, as well. In 2018 sunflower yields was on the line with that of weed-free control. It was ranged from 810 to 3 680 kg ha^{-1}. Aclonifen was the lowest-yielding herbicide treatment with 3 505 kg ha^{-1}, whereas oxyfluorfen was the highest yielding herbicide treatment (3 680 kg ha^{-1}). In 2019 sunflower yields following all PRE-em applied herbicides were significantly lower (between –610 and –760 kg ha^{-1}) than weed-free control (Table 6). In investigation of Pannacci *et al.* (2007) the highest average sunflower yields among PRE-em treatments were obtained in plots treated with S-metolachlor + oxyfluorfen (720 + 168 and 960 + 144 g a.i. ha^{-1}), S-metolachlor + aclonifen (960 + 720 g a.i. ha^{-1}) and pendimethalin + imazamethabenz (768 + 400 g a.i. ha^{-1}). Sunflower yield in pendimethalin and oxyfluorfen treated plots was 46 and 63% higher than in weedy control (Narender *et al.*, 2017). Regardless of irrigation and sunflower injury, in all investigated PRE-em herbicides yield was significantly higher in comparison to untreated control plots (Jursík *et al.*, 2015).

Table 6. Sunflower plant injury as influenced by PRE-em applied herbicides, and yield of sunflower in Bitola and Titov Veles region in 2018 and 2019 [a-d]

Treatments	Rate (l ha^{-1})	Bitola region						Titov Veles region					
		Sunflower injury				Grain yield, kg ha^{-1}		Sunflower injury				Grain yield, kg ha^{-1}	
		2018		2019				2018		2019			
		7 DAE	21 DAE	7 DAE	21 DAE	2018	2019	7 DAE	21 DAE	7 DAE	21 DAE	2018	2019
Weedy control		0	0	0	0	980^{d}	850^{e}	0	0	0	0	810^{d}	950^{d}
Weed-free control		0	0	0	0	3490^{a}	3340^{abc}	0	0	0	0	3670^{a}	3410^{a}
Pendimethalin	3.0	11	7	0	0	2320^{b}	3390^{a}	0	0	0	0	3620^{ab}	2740^{bc}
Metobromuron	3.0	14	9	0	0	2090^{c}	3220^{bcd}	0	0	0	0	3540^{bc}	2620^{c}
Oxyfluorfen	1.25	24	20	0	0	2170^{bc}	3330^{abc}	0	0	0	0	3680^{a}	2800^{b}
Aclonifen	4.0	9	6	0	0	2030^{c}	3145^{d}	0	0	0	0	3505^{c}	2650^{c}
S-metolachlor	1.5	15	11	0	0	2270^{bc}	3350^{ab}	0	0	0	0	3580^{abc}	2700^{bc}
Dimethenamid	1.7	28	22	0	0	2080^{c}	3210^{cd}	0	0	0	0	3540^{bc}	2780^{b}
LSD 0.05						195.96	170.50					99.52	146.55
Random effect interactions PRE-em herbicides × year			*				*		NS				*

[a]Abbreviation: PRE-em; DAA – days after application; NS – not significant; * Significant at the 5% level according to a Fisher's protected LSD test at $P < 0.05$

[b]PRE treatments were applied in the same growth stages as sunflower (at the dry seed – beginning of seed imbibitions of sunflower growing stage – BBCH 00–01).

[c]Sunflower injury was estimated 7 and 21 days after emergence (DAE)

[d]Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at $P < 0.05$

Conclusion

The efficacy of the PRE-em herbicides was correlated with the weather and soil conditions in both regions. The humid April in Bitola region in 2018, before weed emergence, caused herbicide leaching from soil surface which probably was the most likely reason for the lower efficacy of PRE-em herbicides in 2018 compared to their application in 2019. However, in the region of Titov Veles due to equal precipitation, particularly a few weeks after herbicide application and soil type characteristics leaching did not occur and efficacy was good to excellent. Opposite, the limited precipitation after PRE-em application contributed to the poor performance of PRE-em herbicides in the Titov Veles region in 2019 compared with 2018. The sunflower injury occurred due to heavy precipitations in the Bitola region in 2018. Sunflower grain yields for each treatment in both regions generally reflected overall weed control and crop injury. Based on the results we can recommend to the producers that the use of PRE-em herbicides in sunflower crop should be based on climatic conditions with special emphasis on rainfall and soil type.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author contributions

ZP, AM – contributed to the preparation, creation and/or presentation of the manuscript.

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THE IMPACT OF NUTRITION OPTIMIZATION ON CROP YIELD AND GRAIN QUALITY OF SPRING BARLEY VARIETIES (*Hordeum vulgare* L.)

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ABSTRACT. The research is aimed to determine the yield and quality of spring barley grain depending on the varietal characteristics and optimization of nutrition. Methods. The experimental studies were conducted during 2013–2018. on the research field of Mykolayiv National Agrarian University (Ukraine). Results. The highest grain yield of spring barley varieties in all years of research is determined to be formed by growing the crop on the background of applying $N_{30}P_{30}$ and carrying out two foliar top dressing of crops with Escort-Bio or Organic D2 preparations. So, on average, over the years of research on the variety factor, the grain yield was 3.41 and 3.37 t ha^{-1}, which exceeded its level in the non – fertilized control by 26.7–28.2%. The optimization of nutrition affected the grain quality indicators of the studied varieties of spring barley significantly as the maximum values of grain nature (606.2 up to 611.2 g l^{-1}, depending on the variety) were reached by applying $N_{30}P_{30}$ before sowing and double applying of Escort-bio to the crops. The protein content in the grain and digested protein in this nutrition option was also determined to be the maximum as 12.5 up to 13.1% and 61.0 up to 63.8 g kg^{-1}, respectively, depending on the variety. Slightly higher grain quality indicators among the spring barley varieties taken for the study were formed by the 'Aeneas' variety. The highest protein content in the spring barley grain of 'Stalker' and 'Vakula' varieties was accumulated in 2018, and the last one was in 2016. The amount of protein in both varieties increased under the influence of nutrition optimization and, on average, it increased from 10.8% in the control up to 11.3–11.6% in the variants with fertilizing in 'Stalker' variety and from 10.7 up to 11.3–11.6% in 'Vakula' variety during three years.

Introduction

Barley (*Hordeum vulgare* L.) is the fourth cereal crop in the world and it is a secondary small grain crop cultivated in Europe. The most adaptable: there are barley varieties suited to temperate climatic conditions, subarctic climatic conditions as well as subtropical climatic conditions. For achieving good yields of spring barley, the best environment is a temperate moist climate with a growing period of at least 90 days (Daničić *et al*., 2019). Barley is one of the major crops, its grain is widely used for food and feed purposes (Lapa *et al*., 2016).

Increasing the yield and quality of grain crops, including spring barley, is the basis for the economic stability of agricultural enterprises. The steady growth of grain production today is associated with the intensification of the technological process of cultivation, aimed at creating highly productive sowings, improving the quality of grain while maintaining environmental safety, reducing resource and energy costs (Kalenskaya *et al*., 2015; Novotna *et al*., 2015; Povilaitis *et al*., 2018). It is known that for growing stable yields of spring barley with high grain quality indicators, it is important for the plants to be provided with nutrients from the very beginning of the growing season by means of mineral fertilizers. It is possible to compensate the lack of nutrition in subsequent phases of growth and development of spring barley by carrying out foliar top dressing of plants with growth-stimulating preparations (Panfilova *et al*., 2019). Due to synthetic preparations the resistance of plants to adverse weather conditions and to crop infestation

increases, thus, the yield and quality of grain, etc. increases (Kolesnikov *et al*., 2016; Panfilova *et al*., 2020). Nowadays, there are a large number of preparations affecting the growth and development of plants but their impact on the yield and quality of spring barley grain has not been sufficiently studied and covered in the world scientific literature.

Material and methods

Experimental researches were carried out during 2013–2018. on the research field of Mykolayiv National Agrarian University, Ukraine.

We studied the following varieties of spring barley. The technology of growing spring barley in the experiment, except for the studied factors, was generally accepted to the existing zonal recommendations for the southern steppe of Ukraine, which has a temperate continental climate and chernozem soils (black soil of the South, light clay-loam soil on loess). Spring barley was sown in the third decade of March and harvested in the first decade of July. During spring barley vegetation, the temperature of the air exceeded the average annual parameters by 0.3–1.4 °C, depending on the year. The only exception was 2016, where the temperature of the air during the vegetation period was +14.9 °C, which was somewhat lower than the long-term figures. During the vegetation of spring barley, depending on the year of the study, the precipitation fell as 95.8–189.5 mm. At the same time, in 2015 and 2016, the largest precipitation was 189.5 and 179.0 mm respectively, which exceeded the average annual figures by 15.1–19.8%.

Two experiments were conducted:

Experiment 1

The experiment scheme included the following variants:

Factor A – variety: 1. 'Adapt'; 2. 'Stalker'; 3. 'Aeneas'.

Factor B – plant nutrition: 1. Control (water treatment); 2. $N_{30}P_{30}$ – under pre-sowing cultivation; 3. $N_{30}P_{30}$, Urea K1 (1 l ha^{-1}); 4. $N_{30}P_{30}$, Urea K2 (1 l ha^{-1}); 5. $N_{30}P_{30}$, Escort-bio (0.5 l ha^{-1}); 6. $N_{30}P_{30}$, Urea K1, Urea K2 (0.5 l ha^{-1}); 7. $N_{30}P_{30}$, Organic D2 (1 l ha^{-1}). The fertilization of crops by fertilizers was carried out at the beginning of the phases of the spring barley stooling and earing.

Experiment 2

The scheme of the experiment included the following variants:

Factor A – variety: 1. 'Stalker'; 2. 'Vakula'.

Factor B – plant nutrition: 1. Control (water treatment); 2. Fresh Florid (200 g ha); 3. Fresh Florid (300 g ha); 4. Fresh energy (200 g ha); 5. Organic D2–M (1 l ha); 6. Escort-bio (500 g ha). The standard working solution was 200 l ha. The crops were treated in three phases of vegetation: tillering, stooling and beginning of earing. In all three of these periods, one, two- or three-drug treatments were performed.

The yield was determined by the method of continuous harvesting of each registration area (Sampo - 130 combine harvester). Technological indicators of spring barley grain quality intended for food needs use were established by DSTU 3769-98 'Spring Barley'. Protein content and digestible protein content were determined by the Kjeldahl method (DSTU ISO 5983:2003).

The statistical analysis of the data experiment was performed using the Statistica 6.0 application package (Ermantraut *et al.*, 2007).

Results and discussion

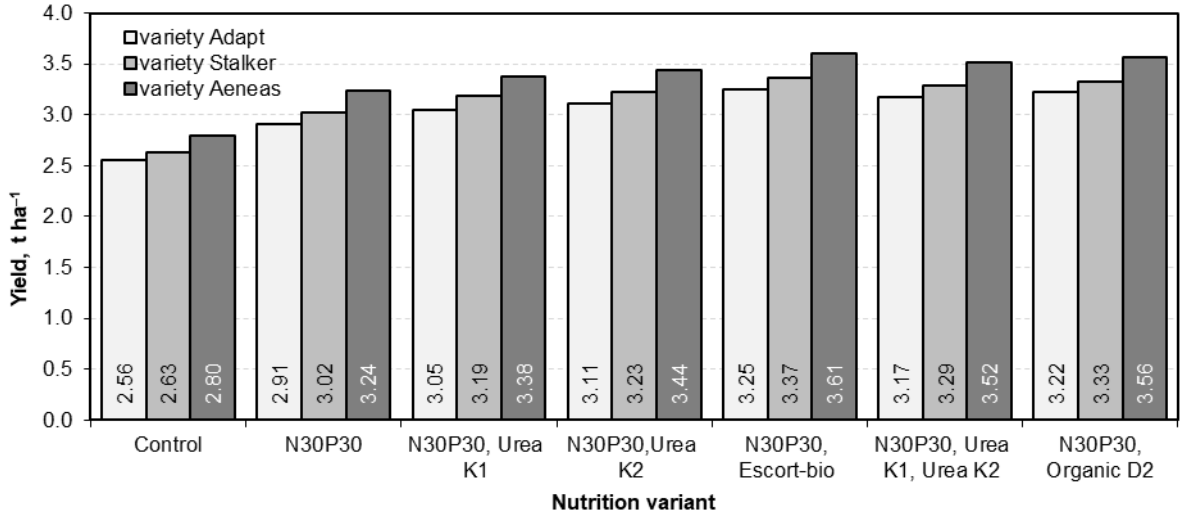
Our studies found the yield of spring barley grain changed under the influence of varietal characteristics, nutrition background and temperature regime during the vegetation season. Thus, the lowest yield of spring barley was formed in the dry year 2013. Favourable weather conditions in 2016 during the growing season of plants ensured the highest grain yield, regardless of the factors studied. It should be noted that on average, over the years of research, the nutrition factor had a greater impact on the formation of the yield of spring barley grain (Fig. 1).

Barley yield generally varies less under changing weather conditions relative to the other small grains (Newton *et al*., 2011). Concerning different cardinal temperature thresholds for different phenological processes, the crop's response to a high temperature, in general, depended on the character of the temperature increase as well as the phonological stage of the crop (Porter *et al.*, 1999). However, its yield and biomass may be hindered by a high temperature and a severe water deficiency (Araya *et al*., 2010) if these occur during the flowering and grain-filling phase. The lowest yield of spring barley grain from all the years of the research was in the control version of the experiment as 2.56 up to 2.80 t ha^{-1}, depending on the variety. Carrying out foliar top dressing of plants with growth-stimulating preparations during the growing season contributed to an increase in grain yield, especially with the Escort-Bio application variant. Thus, the grain yield of the plants of 'Adapt' variety in this variant of the experiment increased by 21.2%, in the variant of 'Stalker' variety, it increased by 22.0%, and in the variant of 'Aeneas' variety, it increased by 22.4% compared to the control. The results were similar when using Organic D2.

According to the results of our research, it was established that, in addition to weather conditions and plant nutrition variants, the variety played an important role in the formation of spring barley yield and other cereals (Panfilova, Mohylnytska, 2019). So, on average, over the years of research, the highest yield was formed by the variety 'Aeneas' as 2.8 up to 3.61 t ha^{-1}. At the same time, it was the highest when using Organic D2 and Escort-Biogrowth-stimulating preparations. The same trend was observed when growing 'Adapt' and 'Stalker' varieties, but the yield was lower compared to 'Aeneas' variety by 6.5 up to 9.5 and 6.6 up to 10.0%, depending on the preparation.

Studies of many scientists (Laidig *et al*., 2017; Peltonen-Sainio *et al*., 2009; Rijk *et al*., 2013) also confirmed that in recent decades a significant impact on the increase of grain yields was made by the selection and genetic improvement of modern varieties.

Plant nutrition options had a positive effect on the quality indicators of spring barley grain (Table 1). In particular, on average during the years of research, the nature of grain of non-fertilized plants of 'Aeneas' and 'Stalker' varieties was from 6.1 to 6.4 g l^{-1}greater in comparison with the variant of fertilizing with the dose of $N_{30}P_{30}$. The foliar application in the phases of the spring barley stooling and earing contributed to the increase of this indicator by 1.4 up to 4.1 and 1.2 up to 2.3% depending on the studied varieties in comparison with the control, respectively. In the years of the research, a little more nature of grain is determined in the variety 'Adapt' as 601.3 up to 611.2 g l^{-1}.



$LSD_{0.5}$**:** Factor A (variety): 2–8. Factor B (plant nutrition): 1–4.

Figure 1. The yield of barley depending on the varietal characteristics and nutrition, t ha^{-1} (average for 2013–2017)

Table 1. Grain quality of spring barley varieties depending on nutrition optimization (average for 2013–2017)

Nutrition variant	Indicators		
	protein content, %	nature of the grain, g l^{-1}	digestible protein, g kg^{-1}
	'Adapt'		
Control	10.3	601.3	56.5
$N_{30}P_{30}$	11.2	605.5	58.9
$N_{30}P_{30}$, Urea K1	11.5	607.1	60.2
$N_{30}P_{30}$, Urea K2	11.7	608.3	60.9
$N_{30}P_{30}$, Escort-bio	12.6	611.2	63.1
$N_{30}P_{30}$, Urea K1, Urea K2	12.0	609.7	61.6
$N_{30}P_{30}$, Organic D2	12.2	610.5	62.4
	'Stalker'		
Control	10.4	593.4	54.5
$N_{30}P_{30}$	11.5	599.8	56.1
$N_{30}P_{30}$, Urea K1	11.9	600.5	57.6
$N_{30}P_{30}$, Urea K2	11.9	601.9	58.1
$N_{30}P_{30}$, Escort-bio	12.5	607.2	61.0
$N_{30}P_{30}$, Urea K1, Urea K2	12.1	604.9	58.7
$N_{30}P_{30}$, Organic D2	12.3	606.7	59.3
	'Aeneas'		
Control	10.9	581.4	57.8
$N_{30}P_{30}$	11.8	587.5	59.2
$N_{30}P_{30}$, Urea K1	12.4	589.6	60.9
$N_{30}P_{30}$, Urea K2	12.5	590.3	61.6
$N_{30}P_{30}$, Escort-bio	13.1	606.2	63.8
$N_{30}P_{30}$, Urea K1, Urea K2	12.7	603.6	62.7
$N_{30}P_{30}$, Organic D2	12.9	605.8	63.3
$LSD_{0.5}$: Factor A	0.2–0.3	11.2–13.5	10.2–12.5
Factor B	0.1–0.2	10.6–11.8	10.9–12.9

Carrying out of foliar fertilizing of plants during the vegetation season on the background of fertilizing with a dose of $N_{30}P_{30}$ also increased the content of digestible protein in the grain.

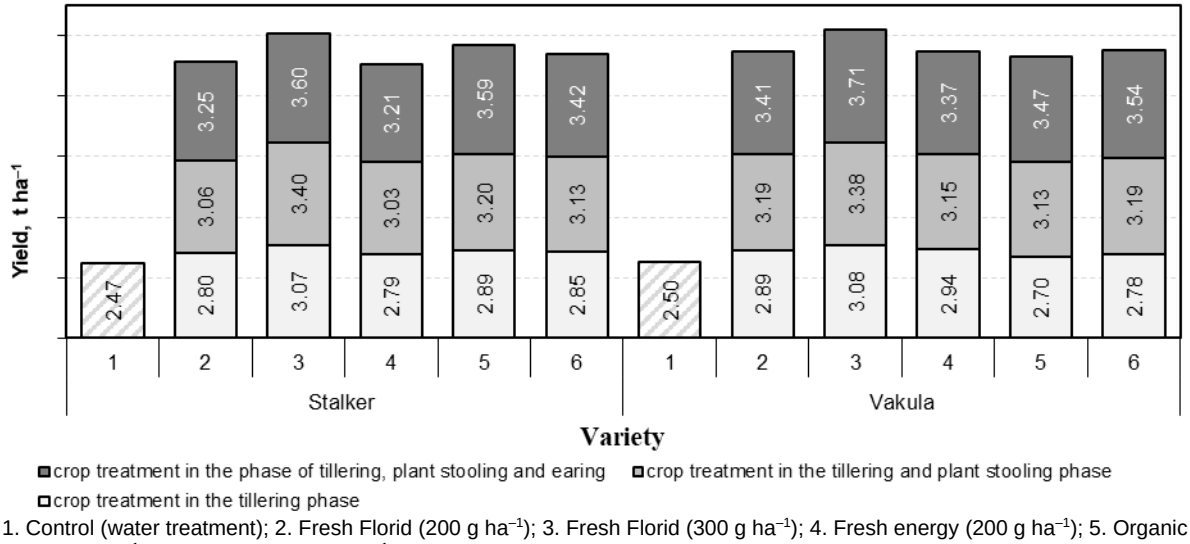
On average over the years of the research, where the Escort-bio preparation was used, this indicator increased by 9.4 up to 10.7% depending on the variety. On average by the nutrition factor, the digestible protein content in 'Aeneas' grain variety was slightly more: by 1.3 up to 5.5% than such content of 'Stalker' and 'Adapt' varieties.

To some extent, nutritional variants influenced the protein content in the grain of spring barley varieties. The application of $N_{30}P_{30}$ provided increasing of the protein content depending on the variety by 7.6–9.6 v. p., and carrying out on their background foliar feeding increased it by 10.4–18.3; 12.6–16.8 and 12.1–16.8 v. p. depending on the variety.

The barley protein content is highly dependent on the variety (Qi *et al.*, 2006) and differs in growth conditions, particularly in the rate and timing of nitrogen fertilization (Duffus *et al*., 1993). The higher crude protein content in barley was usually accompanied by lower contents of starch and dietary fibre (Biel *et al*., 2013). Some investigations showed that an increase in protein content was accompanied by a decrease in essential amino acids, mainly lysine (Arendt *et al*., 2013).

Better grain was formed by spring barley plants when processing crops with Escort-Bio. The digestible protein content of the spring barley grain for this variant was 61.0 up to 63.8 g kg^{-1}, the protein content was 12.5 up to 13.1%, and the conditional protein yield from 1 hectare of sowing area was 0.41 up to 0.47 t depending on the varieties studied.

The high efficiency of modern growth-regulating substances application was also determined in another experiment with spring barley, in which 'Stalker' and 'Vakula' varieties were grown after peas. It was found that the grain productivity of both varieties under the influence of crop treatment with the studied preparations in the main periods of vegetation increased significantly in comparison with the control and depended on the number of top fertilizations, the preparation and its dose (Fig. 2).



1. Control (water treatment); 2. Fresh Florid (200 g ha^{-1}); 3. Fresh Florid (300 g ha^{-1}); 4. Fresh energy (200 g ha^{-1}); 5. Organic D2–M (1 l ha^{-1}); 6. Escort-bio (500 g ha^{-1})

$LSD_{0.5}$: Factor A (variety): 0.1–0.2. Factor B (plant nutrition): 0.2–0.3.

Figure 2. The yield of spring barley varieties depending on non-root fertilizing with bio preparations, t ha^{-1} (average for 2016–2018)

The research found that both varieties of spring barley formed the highest yield when three-fold processing of crops in the main phases of vegetation was carried out. On average, the maximum level of grain yield of 'Stalker' varieties, over the years of research, was provided by fertilizing plants with Organic D2–M as 3.59 t ha^{-1}, and the level of grain yield of 'Vakula' was provided by fertilizing plants with Escort-bio as 3.54 t ha^{-1}. This indicates almost identical grain productivity in the context of varieties.

The optimization of nutrition and the number of foliar fertilization carried out on crops also affected individual indicators of the quality of spring barley grain (Table 2).

Table 2. Grain quality of spring barley varieties depending on nutrition optimization (average for 2016–2018)

Nutrition variant (factor B)	Variety (factor A)			
	'Stalker'		'Vakula'	
	protein content, %	nature of the grain, g l^{-1}	protein content, %	nature of the grain, g l^{-1}
Control (water treatment)	10.8	588.1	10.7	543.0
Fresh Florid (200 g ha^{-1})	11.0	590.2	10.9	551.1
Fresh Florid (300 g ha^{-1})	11.3	591.4	11.3	553.7
Fresh energy (200 g ha^{-1})	11.0	593.0	11.0	551.1
Organic D2–M (1 l ha^{-1})	11.5	596.9	11.5	569.4
Escort-bio (500 g ha^{-1})	11.6	599.8	11.6	567.4
LSD0.5: Factor A	0.1–0.2	11.9–14.7		
Factor B	0.1–0.3	11.9–12.2		

The varieties of spring barley taken for research did not differ in the protein content of the grain. Both of them responded equally to nutrition optimization by increasing its content. Thus, the maximum protein content was accumulated during the three-time treatment of crops with the preparation Escort-bio, when this indicator for both varieties was 11.6%. The increase in the amount of protein compared to the control for 'Stalker' variety was 0.8%, and the increase in the amount of protein in 'Vakula' variety was by 0.9%, or by 7.4 and 8.4 relative points, respectively. Organic D2–M also had a positive effect on the grain protein content.

The nature of the grain was also greater in 'Stalker' variety. Maximum values of nature were reached on the background treatment of crops with Escort-bio in the amount of 500 g ha^{-1} to 599.8 g l^{-1} for the 'Stalker' variety, and 567.4 g l^{-1} for the 'Vakula' variety with

indicators without fertilizing (with water treatment of plants) of 588.1 and 543.0 g l^{-1}, or increased by 2.0 and 4.5%, respectively, compared to the control variant.

Conclusions

In the South of Ukraine, optimizing spring barley plants nutrition based on the principles of resource conservation provides an increase in grain yield and its quality. It was determined that the double application of top fertilization with modern complex organic-mineral fertilizers for foliar top dressing of plants in the main phases of vegetation on the background of $N_{30}P_{30}$ allows optimizing the nutrition regime of spring barley. Thus, the use of $N_{30}P_{30}$ and Escort-bio for the growth of spring barley 'Aeneas' provides an increase in grain yield by 22.4%, the content of protein in the grain increases by 2.2%, the nature of the grain increases by 4.1%, and the content of digestible protein increases by 9.4%.

The treatment of spring barley crops of 'Stalker' and 'Vakula' varieties in the earing phase with Organic D2–M and Escort-bio preparations provides an increase in the protein content in the grain by 6.1 up to 7.0 and 6.9 up to 7.8 percentage points, respectively. At the same time, the grain yield in these variants of the experiment was the highest one and it increased to 3.47 up to 3.59 and 3.42 up to 3.54 t ha^{-1}, respectively.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author contributions

AP − study conception and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript;
VG − drafting of the manuscript, critical revision and approval of the final manuscript;
NP − analysis and interpretation of data.

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CHEMOTACTIC RESPONSES OF SWEET FLAG (*Acorus calamus* L.) ROOT EXUDATES AND EVALUATION OF INOCULATION EFFECTS ON ITS GROWTH

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ABSTRACT. Root exudate is an important source of nutrients for microorganisms in the rhizosphere and it plays a major role in the early colonization including chemotactic responses and adsorption of rhizospheric bacteria. In this study, we characterized the root exudates from sweet flag under hydroponic conditions and assessed their effect on plant growth. In the present study, the crude root fractions of sweet flag recorded a maximum yield of 520.6 µg plant^{-1} followed by cationic, anionic and neutral fractions. Among the qualitative and quantitative analysis of different fractions, the cationic fraction recorded a maximum of 90 µg plant^{-1} for glutamic acid, followed by aspartic acid, glycine, serine and proline. In the anionic fraction, malic acid recorded a maximum of 78.0 µg plant^{-1} followed by oxalic, succinic, citric and glutamic acid fractions. The neutral fractions included different saccharides, among which, fructose recorded a maximum of 42.5 µg plant^{-1}, followed by glucose, maltose, ribose and arabinose. The relative chemotactic response (RCR) of PGPB (plant growth-promoting bacteria) strains towards different root exudate fractions of *Acorus calamus* was recorded and it was observed that the combination of Anionic + Cationic + Neutral fraction recorded maximum chemotactic response for PGPR strains. The adsorption of PGPR strains in the root of the *Acorus calamus* was recorded in three different phases of growth and among these, Log phase bacterial cells exhibited maximum colonization of 7.65×10^{-6} cells g^{-1} with *A.venilandii* (ACAzt-2). Inoculation effect of PGPB strains on the root exudate of *Acorus calamus* and its growth was evaluated and it was observed that the treatment T_5 – Consortium recorded maximum plant height and root growth of *Acorus calamus*, followed by T_2. Our results indicate that sweet flag root exudates induce chemotactic responses of PGPR strains and promoted their growth.

Introduction

The rhizosphere is best defined as the volume of rhizome around living roots, which is influenced by root activity and root exudate quantity and the number of individual components of the root exudates (Hiltner, 1904). This is a densely populated area in which plant roots must compete with the invading root systems of neighbouring plants for space, water and mineral nutrients, and with other soil-borne organisms, including bacteria for their energy requirements. Plants mediate both positive and negative interactions in the rhizosphere via root exudates (Bais *et al.*, 2006; Philippot *et al.*, 2013). (Ryan, Delhaize, 2001; Bais *et al.*, 2004). The ability to secrete a wide range of compounds in the rhizosphere is one of the most remarkable metabolic features of plant roots, with around 5–21% of total photosynthetically fixed carbon being transferred into the rhizosphere through root exudates (Whipps, 1990; Marschner, 1995; Derrien *et al.*, 2004). Friman *et al.* (2020) reported that the plant interaction with the above and below ground plants parts results in changes in the traits of the plant in terms of defence response against the insect herbivorous.

Root exudation includes the secretion of a diverse array of carbon-containing primary metabolites, such as saccharides, amino acids and phenolic acids, as well as more complex secondary compounds that are involved in plant defence and in stimulatory or inhibitory interactions with other soil organisms (Bertin *et al.,* 2003; Jones *et al.,* 2004; Bais *et al.,* 2006). Soil microorganisms play a crucial role in the sustainability and functioning of soil-based ecosystems because of their involvement in key processes, such as mineral nutrition cycling, organic matter turnover, soil structure formation and toxins removal (van Elsas *et al.,* 2006; Brussaard *et al.,* 2007). Root exudates are considered as one of the most important factors that affect soil microorganisms (Bais *et al.,* 2006; Yao, Allen, 2006). In continuous monocropping systems, crop roots repeatedly release the same types of exudates for many years, occasionally resulting in significant colonization and infection by certain beneficial or pathogenic microorganisms that utilize these substrates. Thus, root exudates persisting during the planting season of a monoculture crop could be responsible for soil sickness, and their allelopathy should be better understood in terms of soil microbial ecology (Inderjit, 2005; Kaur *et al.,* 2009). Root exudates are one of the most poorly quantified compounds of the belowground C cycle as they only occur in the narrow rhizosphere and are rapidly absorbed by different soil components and/or assimilated by soil microorganisms (Neumann *et al.,* 2009; Paterson, 2003; Phillips *et al.,* 2008; Pavankumar *et al.* 2019). Sharma *et al.* (2020) reported that the root exudates play a major in plant growth-promoting bacterial biofilm formation and colonization in *Brachypodium.*

Root exudate collection presents significant challenges due to difficulties associated with i) accessing the rhizosphere without disturbance or damage to plant roots as a result of the collection system; ii) selecting a suitable collection medium that does not affect root physiology and exudate recovery; and iii) spatial and temporal variations in root and rhizosphere environment (Phillips *et al.,* 2008). Various approaches have been used to collect exudates either directly from nutrient solutions, where plants are grown or through accumulation in solid media (generally sand or glass beads) or recovery through different flushing or extraction procedures (Gransee, Wittenmayer 2000; Sandnes *et al.,* 2005; Tang, Young 1982). However, the recovery of exudates by such approaches exert various physiological effects on the plant and incomplete leaching or adsorption of exudates by the solid media (Gransee, Wittenmayer 2000; Neumann, Römheld, 2007; Sandnes, *et al.* 2005).

Since root exudates are mainly derived from photosynthesis, this results in significant carbon (C) cost for plants as root exudates are believed to have important functions in the regulation of plant growth (both directly or indirectly), although most of these functions are just beginning to be investigated (Bertin *et al.,* 2003; Walker *et al.,* 2003).

In this study, the quantification of different fractions of the root exudates and chemotactic responses of the *Acorus calamus* medicinal plant and their effects of plant growth-promoting rhizobacteria on its growth was evaluated.

Material and methods

Collection of root exudates from *Acorus calamus*

For the collection of root exudates of *Acorus calamus* Linn., the wide-mouthed glass bottles with an aluminium mesh at the bottom for supporting the seeds were used. The lids were provided with holes fitted with a cotton plug for easy flow of filtered air. About 500 ml of Fahraeus nutrient solution of 1 mM $CaCl_2$, 2 mM K_2SO_4, 1 mM $MgSO_4$, 1 mM Fe(III)-citrate, 1 mM KNO_3, and the microelements (Fåhraeus, 1957) was added in the bottle and the entire set was wrapped in papers, followed by sterilization in an autoclave.

Five *Acorus calamus* transplanted plant showing no contamination on nutrient soft agar were transferred to the bottles and placed in the solution. The bottles were kept in the sunlight for three weeks and afterwards, the plants were removed and the solution containing root exudates was carefully collected, centrifuged at 300 g for 10 min and filtered through a 0.45 μm fritted glass filter, freeze-dried and weighed.

Fractionation of purified root exudates

The purified freeze-dried crude root exudate was dissolved in sterile distilled water and made up to the original volume. Then it was passed through 2.5 × 1.0 cm columns of Dowex-1 (200–400 μm mesh, formate form) and Dowex-50 (200–400 mesh, chloride form) (Sigma Chemical Company, USA) resins respectively to fractionate the exudates into anionic, cationic and neutral fractions.

The Dowex-50 cation exchange resin column was eluted with 1 M NH_4OH to obtain the cationic fraction of *Acorus calamus* root exudates and the eluate was evaporated to dryness until no ammonia odour was detected. The Dowex-1 anion exchange resin column was eluted with glacial acetic acid to get the anionic fraction of the same. The effluent that passed through the cation and anion exchange resin column constituted the neutral fraction. The different fractions obtained were freeze-dried and weighed.

Qualitative and quantitative analysis of the cationic fraction of the root exudates

The freeze-dried, cationic fraction of the root exudate was redissolved to the original volume in sterile distilled water and bidimensional paper (Whatman No. 1) chromatography was carried out to separate the amino acids using the following solvent systems:

- Butanol : acetic acid : water (4:1:5 v/v),
- Phenol : water (3:1 w/v).

Aliquots of the cationic fraction (20 μL) were spotted on two papers separately and both papers were subjected to chromatography under identical conditions. After developing the paper in the above-mentioned solvent system for 14 hr, the positions of amino acids were determined by spraying one paper

with ninhydrin reagent (2 g of ninhydrin in 25 ml acetone: 25 ml of 0.2 M acetate buffer in pH 5.5). The spots were identified by co-chromatography with authentic amino acids.

On the other paper, positions corresponding to the ninhydrin positive spots were marked, cut into several small segments and eluted into 8 ml of 80 per cent ethanol for 45 min., two ml of the ninhydrin reagent were added to the eluant (8 ml) and the tubes were kept in boiling water bath for 15 min. The pink colour developed was measured by Spectronic 20 colourimeter at 550 nm. The concentration of each amino acid present in the cationic fraction was determined by comparing their absorbance against the standard graph prepared with known quantities of different amino acids.

Qualitative and quantitative analysis of the anionic fraction of the root exudates

The freeze-dried anionic fraction of the root exudates was redissolved in the original volume of sterile distilled water and one-dimensional paper (Whatman No. 1) chromatography was carried out to separate the organic acids using the following solvent system: (n-pentanol: 5M formic acid 1:1, v/v). Aliquots of the anionic fraction (about 20 µl) were subjected to chromatography under identical conditions. After developing the paper for 15 hr, the position of organic acids was determined by spraying with bromothymol blue reagent (100 mg of bromothymol blue dissolved in 1 ml of 0.02 M NaOH and then raised to 250 ml with distilled water). The spots were identified by using reference chromatograms, with known organic acids, developed in the same solvent system. On the other paper, positions corresponding to these bromothymol blue positive spots were marked, cut out into smaller segments and eluted in 8 ml of distilled water and the concentration of each organic acids present in the anionic fraction was determined by titrating the eluant against 0.0144 M NaOH with phenolphthalein as the indicator.

Qualitative and quantitative analysis of the neutral fraction of the root exudate

The freeze-dried neutral fraction of the root exudate was redissolved to the original volume in sterile distilled water and bidimensional paper (Whatman No. 1) chromatography was carried out to separate the saccharides using the following solvent system:

- 1. n-propanol : Ethylacetate : water (70:10:20, v/v),
- 2. n-butanol : Acetic acid : water (100:22:50,v/v).

Aliquots of 10 µl of neutral fraction were spotted on two papers separately and both papers were subjected to chromatography under identical conditions. After developing the papers for 40 hr, the positions of saccharides were ascertained by spraying one paper with aniline hydrogen phthalate reagent (1.7 g phthalic acid; 1 ml aniline, 90 ml ethanol; 5 ml glacial acetic acid, 5.0 ml 40 per cent trichloroacetic acid). The spots were identified by chromatography with authentic saccharide. On the other paper, positions corresponding to these spots were marked, a paper was cut into smaller segments and eluted in 8 ml distilled water in a test tube. Reducing saccharides were estimated by the Somsgyi-Nelson method (Somogyi, 1952) and fructose (after hydrolysis with 0.1 M oxalic acid) by the method of Bacon and Bell (1948).

Chemotaxis by capillary assay

In vitro chemotaxis was assayed according to the method described by Rao and Johri (1989) with a slight modification. The root exudate was introduced into capillary tubes of 15 mm length and 0.25 mm diameter and tubes were placed into the cell suspensions of each of *Azospirillum*, *Azotobacter*, *Bacillus* and *Pseudomonas* isolate for 30 minutes. Then capillaries were rinsed with distilled water and the content of capillaries was ejected onto plates containing the yeast extract glucose agar medium. Five replicates were maintained for each kind of organisms. The relative chemotactic response was calculated by the number of cfu per capillary containing the root exudate divided by the number of cfu in capillaries containing sterile distilled water (Eq. 1).

Determination of the number of cells adhered to the root

Acorus calamus plants were surface sterilized by immersion into 95 per cent ethanol for 1 min., followed by incubation for 20 min in 1 per cent of NaOCl. After rinsing five times with sterile distilled water, the sterilized seeds were placed on the surface of 1 per cent water agar on Petri plates (9 cm diameter at the rate of five transplanted plant exudates plated). Plants were incubated in an inverted position for 10 days at room temperature to allow growth. The plates were sealed with wax to avoid agar drying during germination. After transplanting, the seedlings were transferred onto semisolid Weaver's medium maintained in a test tube, at the rate of one seed per tube. The PGPB isolates, namely *A. lipoferum* (ACAzs-5), *A. venilandii* (ACAzt-2), *B. cereus* (ACPb-3) and *P. fluorescens* (ACPf-4), were harvested at lag, log and stationary phases, separately. One ml of a culture of each of the three different growth phases was inoculated in tubes maintained at 30 ± 2 °C alternating 10 hr in light and 14 hr in darkness for 48 hr. Five replication were maintained for each treatment.

After 48 h of inoculation, the roots from each treatment were excised separately into 10 ml of sterile distilled water. To enumerate the rhizoplane population of the root, serial dilutions were made and an indirect viable count was made on nitrogen-free malate agar, Waksman's base medium 77, Pikovskaya's and King's B media to enumerate populations of *A. lipoferum* (ACAzs-5), *A. venilandii* (ACAzt-2), *B. cereus* (ACPb-3) and *P. fluorescens* (ACPf-4) strains, respectively.

$$Relative\ chemotactic\ response = \frac{The\ number\ of\ colonies\ per\ capillary\ tube\ with\ root\ exudates}{The\ number\ of\ colonies\ per\ capillary\ tube\ with\ distilled\ water} \quad (1)$$

Evaluation of its growth parameters in *Acorus calamus* as influenced by Inoculation of PGPR strains

The *A. calamus* plants were raised in wide-mouthed glass bottles and inoculated with PGPR strains such as *A. lipoferum* (ACAzs-5), *A. venilandii* (ACAzt-2), *B. cereus* (ACPb-3) and *P. fluorescens* (ACPf-4) at 5 ml of each and consortium is the combination of all organisms. The plant growth-promoting effect of the PGPR strains was evaluated by determining their plant height and root length were recorded on the 21st day after planting.

Statistical analysis

Experimental results were analysed by one or two-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). Significant difference among different treatments was considered at $P < 0.05$.

Results

Collection and fractionation of the root exudates of *Acorus calamus*

The root exudates of *Acorus calamus viz.* were collected and the constituents of different fractionations are studied as shown in Fig. 1. The crude root exudate of *Acorus calamus* (520. 6 µg plant^{-1}), the cationic fraction (198.3 µg plant^{-1}), the anionic fraction (145.2 µg plant^{-1}) and the neutral fraction (102.8 µg plant^{-1}). The quantity of cationic fraction was higher than that of anionic and neutral fractions ande the anionic fraction was weightier than the neutral fraction in the root exudate of the *Acorus calamus*. The cationic fraction included different amino acids, the anionic fraction included different organic acids and the neutral fraction included different sugars.

Quantitative and qualitative analysis of different root exudates of *Acorus calamus*

Five different amino acids, *viz.* aspartic acid, glutamic acid, glycine, serine and proline were detected in the cationic fractions of the root exudates of *Acorus calamus*. The relative occurrence of amino acids was in the descending order of content: glutamic acid > aspartic acid > glycine > serine > proline (Table 1).

Five organic acids, *viz.*, malic acid, oxalic acid, succinic acid, citric acid and glutaric acid were detected in the anionic fractions in the root exudates of *Acorus calamus*. The organic acids present were in the descending order of content: malic acid > oxalic acid, > succinic acid > citric acid > glutaric acid.

Five different saccharides, *viz.*, fructose, glucose, maltose, ribose and arabinose were detected in the neutral fractions of the root exudates of *Acorus calamus*. The saccharides present were in the descending order of content: fructose > glucose > maltose > ribose > arabinose (Table 1).

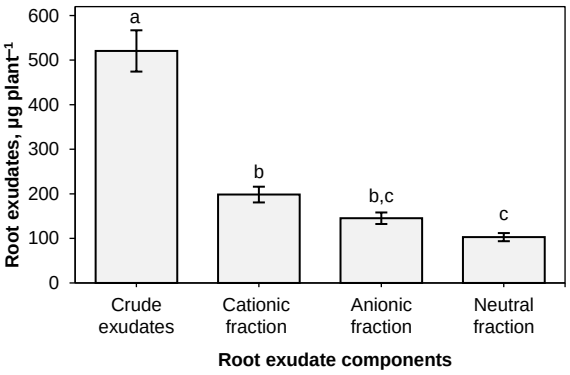

Figure 1. Fractions of the root exudates of *Acorus calamus*. Values represent a mean of six replications ± SD; Standard deviation. Different letters (a-c) after values indicate a significant difference among treatments at ($P < 0.05$)

Table 1. Qualitative and quantitative analysis of different fractions of the root exudates of *Acorus calamus*

Cationic fraction		Anionic fraction		Neutral fraction	
Amino acid	Quantity, µg plant^{-1}	Organic acid	Quantity, µg plant^{-1}	Sugar	Quantity, µg plant^{-1}
Aspartic acid	57.5 ± 3.1^{b}	Malic acid	78.0 ± 4.0^{a}	Fructose	42.5 ± 1.5^{a}
Glutamic acid	90.0 ± 5.0^{a}	Oxalic acid	39.5 ± 4.5^{b}	Glucose	24.5 ± 2.5^{b}
Glycine	23.5 ± 4.5^{c}	Succinic acid	17.5 ± 3.5c	Maltose	15.0 ± 2.0^{c}
Serine	16.5 ± 3.5^{d}	Citric acid	13.5 ± 2.5^{c}	Ribose	12.5 ± 2.0^{c}
Proline	10.5 ± 3.7^{d}	Glutaric acid	6.5 ± 1.5^{d}	Arabinose	7.5 ± 1.5^{d}

Values represent a mean of six replications ± SD (standard deviation).
Different letters after values indicate a significant difference among treatments at ($P < 0.05$)

Table 2. Relative chemotactic response of PGPR strains towards different root exudate fractions of *Acorus calamus* Linn.

Fraction of root exudates	RCR root exudates fraction of *Acorus calamus* PGPR strains*			
	ACAzs-5	ACAzt-2	ACPb-3	ACPf-4
Crude	1.0 ± 0.16^{a}	3.8 ± 0.22^{a}	2.4 ± 0.13^{b}	3.0 ± 0.10^{a}
Anionic	0.5 ± 0.25^{b}	3.0 ± 0.22^{b}	1.5 ± 0.86^{bc}	2.5 ± 0.26^{ab}
Cationic	0.4 ± 0.66^{b}	1.5 ± 0.15^{c}	1.0 ± 0.40^{c}	1.3 ± 0.15^{b}
Neutral	0.4 ± 0.35^{b}	2.0 ± 0.26^{c}	1.5 ± 0.42^{bc}	1.7 ± 0.42^{b}
Anionic + Cationic	0.8 ± 0.46^{ab}	3.4 ± 0.42^{b}	2.1 ± 0.76^{b}	3.0 ± 0.68^{a}
Anionic + Neutral	0.8 ± 0.20^{a,b}	3.2 ± 0.75^{b}	2.0 ±0.45^{b}	2.7 ± 0.65^{a,b}
Cationic + Neutral	0.6 ± 0.50^{b}	3.0 ± 0.05^{b}	1.8 ± 0.65^{b}	2.3 ±0.87^{a,b}
Anionic + cationic + Neutral	1.0 ± 0.89^{a}	4.3 ± 0.19^{a}	3.1 ± 0.97^{a}	3.8 ± 0.42^{a}
Control	0.01^{c}	–	–	0.01^{c}

A. lipoferum ACAzs-5; 2- *A. venilandii* ACAzt-2; 3- *B. cereus* ACPb-3; 4- *P. fluorescens* AC*Pf*-4.
Values represent a mean of six replications ± SD (standard deviation).
*At 1×10^{6} CFU ml^{-1} inoculant level. Different letters after values indicate a significant difference among treatments at $P < 0.05$. RCR-Relative chemotactic response.

Relative response of PGPR strains towards different root exudate fractions of *Acorus calamus*

Relative chemotactic responses of PGPR strains of *Acorus calamus viz.* were used as such (crude) as well as their fractionated compounds either singly or in combinations to study their chemotactic activity and to determine the relative chemotactic response of four selected efficient PGPR strains *viz.*, *A. lipoferum* ACAzs-5, *A. venilandii* ACAzt-2, *B. cereus* ACPb-3 and *P. fluorescens* AC*Pf*-4 (Table 2).

Among the three fractions tested individually, all the four strains showed higher and lower RCR values to anionic, cationic and neutral fractions followed by crude root exudate as such and a combination of the fraction of any two and individual fractions. Chemotactic response of *A. calamus* to all the four-strain although higher towards recombined fractions than the other fraction, the RCR value obtained was in the order of *A. lipoferum* ACAzs-5 (1.0 ± 0.89), *A. venilandii* ACAzt-2 (4.3 ± 0.19), *B. cereus* ACPb-3 (3.1 ± 0.97) and *P. fluorescens* AC*Pf*-4, (3.8 ± 0.42). Chemotactic response to all the four strains although lowest towards cationic fractions than the other fraction, the RCR value obtained was in the order of *A. lipoferum* ACAzs-5 (0.4 ± 0.66), *A. venilandii* ACAzt-2 (1.5 ± 0.15), *B. cereus* ACPb-3 (1.0 ± 0.40) and *P. fluorescens* AC*Pf*-4, (1.3 ± 0.15) Table 3.

Table 3. Adsorption of PGPR strains to the roots of *Acorus calamus*

Crop	Growth phase	Root PGPR isolates*			
		A. lipoferum ACAzs-5	*A. venilandii* ACAzt-*2*	*B. cereus* ACPb-3	*P. fluorescens ACPf-4*
Acorus calamus	Lag	3.00 ±0.03^{b}	4.32±0.23^{a}	3.22±0.43^{b}	3.30±0.43^{b}
	Log	6.24±0.32^{ab}	7.65±0.34^{a}	6.36±0.56^{b}	6.70±0.56^{ab}
	Stationary	3.56±0.26^{a}	4.00±0.60^{a}	3.66±0.54^{a}	3.85±0.25^{a}

*1×10^6 cells g^{-1} of the root. Values represent a mean of six replications ± SD (standard deviation).
Different letters after values indicate a significant difference among treatments at $P < 0.05$.

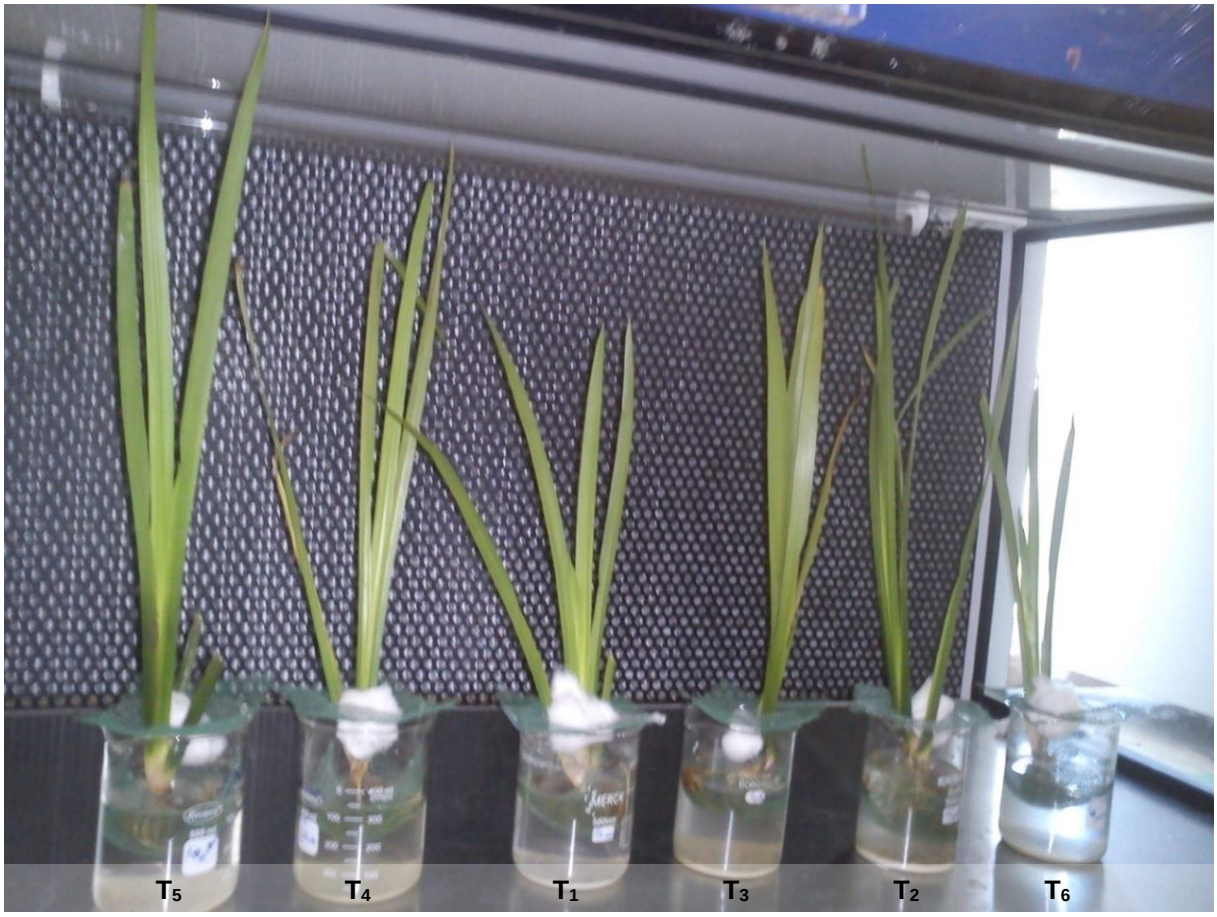

Figure 2. Plant growth as influenced by PGPR strains on the root exudates of *Acorus calamus* (T_1-*Azospirillum*, T_2-*Azotobacter*, T_3-*Bacillus* T_4-*Pseudomonas*, T_5-Consortium and T_6-Control)

Adsorption of PGPR strains to the roots of *Acorus calamus*

The adsorption of PGPR strains namely *A. lipoferum* ACAzs-5, *A. venilandii* ACAzt-2, *B, cereus* ACPb-3 and *P. fluorescens* ACPf-4 to Acorus calamus root as influenced by lag, log and stationary growth phase cells was studied and the results are presented in Table 2. Irrespective of strains, the log phase cells recorded the highest number of adsorbed bacteria to *A. calamus* roots. A log phase 6.24×10^6 for *A. lipoferum* ACAzs-5, 7.65×10^6 for *A. venilandii* ACAzt-2, 6.36×10^6 for *B. cereus* ACPb-3, and 6.70×10^6 for *P. fluorescens* ACPf-4, respectively were recorded in *A. calamus* roots.

Effect of PGPR strain on the growth of *A. calamus* plants

There were significant differences ($P < 0.05$) in plant heights of *Acorus calamus* plants treated with *Azospirillum*, *Azotobacter*, *Bacillus* and *Pseudomonas* strains. The plant height of *Acorus calamus* plants increased significantly due to the inoculated PGPR strains on all 7, 14 and 21 (DAP). The maximum plant height of T_5 – consortium (29.0 ± 1.7 cm plant^{-1}) was followed by T_2. The minimum plant height was recorded in T_1 – *Azospirillum* (22.8 ± 1.7 cm plant^{-1}) Table 4, on 21 DAP when compared to the uninoculated control. (Figs. 2, 3).

Figure 3. Root growth of T_5 (consortium) as influenced by PGPR strains.

Table 4. Effect of PGPR strains on the growth of *A. calamus* in terms of a) plant height (cm) and b) root length (cm). Values represent a mean of six replications ± SD; Standard deviation. Different letters after values indicate a significant difference among treatments at $P < 0.05$.

Treatment	Plant height, cm	Root Length, cm
T_1 – *Azospirillum*	22.8 ± 1.7^b	17.0 ± 2.1^b
T_2 – *Azotobacter*	27.4 ± 2.3^a	23.9 ± 2.2^a
T_3 – *Bacillus*	26.7 ± 2.7^a	24.2 ± 1.7^a
T_4 – *Pseudomonas*	24.3 ± 3.2^{ab}	25.0 ± 3.2^a
T_5 – Consortium	29.0 ± 1.7^a	25.4 ± 2.9^a
T_6 – Control	18.6 ± 1.2^c	13.8 ± 1.9^c

There was a significant difference in root lengths of *Acorus calamus* plants of five replications treated with *Azospirillum*, *Azotobacter*, *Bacillus* and *Pseudomonas*, obtained from the maximum plant height at all sampling periods of root exudates. The root length of the *Acorus calamus* significantly increased due to the inoculated PGPR strains on 7, 14 and 21 DAP. The treatment T_5 – was significantly over other treatments. The maximum root length of T_5 – consortium, (25.4 ± 2.9 cm plant^{-1}) followed by T_2 – *Azotobacter venilandii* (23.9 ± 2.2 cm plant^{-1}) was recorded. The minimum root length was recorded in T_1 – *Azospirillum* (17.0 ± 2.1 cm plant^{-1}).

Discussion

In the root exudates, fructose constitutes over 80% of carbohydrates, indicating that this saccharide residue is the main source of carbon in the rhizosphere of young *A. calamus* plants. Similar findings have been obtained during the growth of wheat, rice and *Catharanthus roseus* seedlings (Jones, Darrah 1993; Přikryl, Vančura, 1980; Bacilio-Jimenez *et al.* 2001; Karthikeyan *et al*, 2007; Karthikeyan *et al.* 2012; Karthikeyan *et al.* 2013).

The cationic fraction of *Acorus calamus* root exudate contained 90.0 µg of glutamic acid, 57.5 µg of Aspartic acid, 23.5 µg of glycine, 16.5 µg of serine and 10.5 µg of proline plant^{-1}. These appreciable quantities of secreted amino acids can initiate the chemotaxis of the PGPR strains. Somers *et al.* (2004) have shown that the amino acids and carbohydrates of the root exudates play a major role in chemotaxis on root surfaces. In the present study, different saccharides *viz.*, fructose, glucose, maltose, ribose and arabinose were obtained at varying quantities from the neutral fraction of the root exudate. These primary metabolites were found responsible for the chemotactic attraction of the plant growth-promoting bacteria.

The anionic fraction of *Acorus calamus* root exudate contained five organic acids *viz.*, malic, oxalic, succinic, citric and glutamic acid. Kloss *et al* (1984) reported the predominance of malic acid in the root exudates of a C4 plant.

The chemotactic response of PGPR strains was in the order of *A. venilandii* ACAzt-2 > *P. fluorescens* ACPf-4 > *Bacillus cereus* ACPB-3 > *A. lipoferum* ACAzs-5. The RCR values for all the PGPR strains towards recombined fraction were significantly higher ($P < 0.05$) than those of crude root exudates.

The *Azotobacter choroococcum* was reported to show *a* stronger response to sugars and amino acids, but weaker towards organic acids (Sood, 2003). Kloss *et al*, (1984) reported that the *P. fluorescens* was strongly attracted towards citric and malic acids, which were predominant in the root exudates of the tomato plant. Root exudates modulate the interaction between plant and plant growth-promoting rhizobacteria (Deweart *et al.* 2002). Mark *et al.* 2005 reported that behavioural changes in the bacteria were a result of altered gene expression elicited by the compounds present in root exudates. Vora *et al.* (2021) reported differential chemotaxis and biofilm formation behaviour in plant

growth-promoting rhizobium strains based on studies on inter- and monocropped plants.

The adsorption of PGPR strains in the roots of *Acorus calamus* was also recorded. *A. venilandii* recorded maximum populations in the logarithmic phase than other phases. The mucilages of the root secretions play a major role in enhancing of adsorption of the cells.

The migration of two rhizosphere beneficial bacteria in the soil towards living wheat plants or towards synthetic attractants is known to occur *in vivo* (Rovira, 1969), and the migration of the *A. brasilense* and *Pseudomonas fluorescens* towards wheat roots occur in the soil (Bashan, 1986). Furthermore, results from soft-agar, capillary tube, and soil chemotaxis assays indicate the attraction of rhizobacteria to seed and seedling root exudates (Begonia, Kremer, 1999). *A. brasilense* exhibits positive chemotaxis towards a large number of organic compounds such as amino acids, saccharides, organic acids, typical for plant root exudates. Malate, succinate, and fructose have been the most effective attractants for *A. brasilense* (Zhulin *et al.*, 1988). The better colonization by *A. brasilense* and *Bacillus* sp., to plant roots may be attributed to chemical effectors, which might have favoured better root colonization. The capacity of root exudates to attract bacteria could be attributed to some of their components (Jin *et al.*, 2019). Rekha *et al.* (2020) reported the role of *B. subtilis* RR4 in the enhancement of root exudation of malic acid and salicylic acid, which serve as plant growth promoters and stress alleviators in rice plant.

Several bacteria were described as presenting positive chemotaxis toward different molecules exuded by plants, including sugars, amino acids, various dicarboxylic acids such as succinate, malate and fumarate, and aromatic compounds such as shikimate, quinate, protocatechuate, vanillate, acetosyringone, gallate, catechol and luteolin (Brencic, Winans, 2005).

Root exudates could supply rhizobacteria with precursors needed for phytohormone synthesis. An interesting report describes the mapping of sugar and amino acid availability in *Avena barbata* root exudates (Jaeger *et al.*, 1999). This study highlighted the availability of tryptophan mainly in the root tip region. Tryptophan is the precursor for indole 3-acetic acid, a major auxin, suggesting that rhizobacteria could exploit root exudate pools for various growth regulator precursors (Zhang *et al.*, 2019).

Plants also exude aminocyclopropane-1-carboxylic acid (ACC), which is an ethylene synthesis precursor and can be used as carbon and nitrogen sources by rhizobacteria, as recently shown by acdS expression mainly by root exudates assimilating bacteria and those inhabiting root tissue (Haichar *et al.*, 2012; Karthikeyan *et.al.*, 2012). The use of ACC by ACC-deaminase-producing rhizobacteria reduces the amount of ACC outside the plant and equilibrated the ACC level outside and inside. Plants release more ACC and therefore produce less ethylene, which inhibits root elongation (Glick *et al.*, 1998).

As mentioned above, plants exude a high variety of saccharides, such as glucose, fructose and sucrose, which are also suggested to be involved in the production of exopolysaccharides (EPSs) by the rhizospheric bacteria. The EPSs are the main contributors in legume-rhizobia interactions, leading to nodulation and nitrogen fixation. They have also other functions such as root-adhering soil structuring, non-legume plant growth promotion or evasion from the legume defence response during crack entry in roots (Alami *et al.*, 2000).

The root exudation can have a major impact on the nutrient acquisition by plants. Further, the PGPR strains are inoculated on the plants and were analysed for their growth parameters. The treatment T_5 (consortium) was recorded the maximum plant height and root length of *Acorus calamus* on all three sampling periods. This was due to the action by PGPR strains, which enhance the plant growth-promoting activity. In this study, we found that the composition and concentration of sugars, amino acids, and organic acids from sweet flag root exudates exert great attraction of PGPR strains. This may provide them with a clear advantage over PGPR strains. Our results strongly suggest that the chemoattractant characteristics of PGPR strains probably favour the *Acorus calamus* growth and could induce the PGPR strains colonies to improve the plant growth.

Conclusions

This study leads to the conclusion that concerning the characterization of root exudates and chemotactic responses from the sweet flag on their effects of PGPR inoculations. The rhizosphere microbes play an important role in improving the medicinal values of medicinal plants. This increases the interest in the research of interaction between medicinal plants and the rhizosphere microbes for the improvement in terms of plant growth and yield of phytochemical constituents of medicinal plants. The inoculation of PGPR is a technology to enhance the quantity and quality of medicinal compounds. Therefore future research is recommended for a better understanding of the diversity and functions of rhizosphere bacteria to improve the yield of *Acorus calamus.*

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

PP – study conception and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision and approval of the final manuscript;
BK – study conception and design, acquisition of data, analysis and interpretation of data, critical revision and approval of the final manuscript;
MMJ – study conception and design, critical revision and approval of the final manuscript.

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EFFECT OF DIFFERENT SUBSTRATE STERILIZATION METHODS ON PERFORMANCE OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

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ABSTRACT. Proper sterilization of substrates is an indispensable step in oyster mushroom cultivation. Oyster mushroom growers in Nepal usually follow three different substrate sterilization methods; however, their comparative effectiveness is vastly unexplored. Thus, these experiments were carried out at the Institute of Agriculture and Animal Science (IAAS), Lamjung Campus, Lamjung, Nepal from January to March, in the years 2017 and 2019. The objective of these experiments was to identify the most appropriate method of sterilization. Three different types of sterilization methods viz chemical sterilization (formaldehyde + carbendazim), steam sterilization, and hot-water sterilization were evaluated for the growth parameters and productivity of oyster mushroom cultivated on rice straw. The experiments were laid out on Completely Randomized Design (CRD) with ten replications. The results showed that the spawning rate was 3.2% of the wet substrate. Data were collected until the third flush. A significantly longer duration to colonize the substrate (29.7 days) was observed under chemical sterilization. The oyster mushroom performed best under steam sterilization as it took the shortest time for pinhead formation (34.30 days), fruiting body formation (43.60 days), cropping duration (89.30 days), and produced the highest mushroom yield (1401.9 g per 4 kg bag), and consequently, the highest biological efficiency (101.38%). Average pileus diameter and stipe length were statistically indifferent among the treatments suggesting the significant effect of sterilization methods on the yield of oyster mushroom but not on its morphological attributes.

Introduction

The use of mushrooms as food is probably as old as civilization itself. Over 200 mushroom species have long been used as functional foods around the world (Kalač, 2013), but only about 35 species have been commercially cultivated (Aida *et al.*, 2009). Over time, an increase in awareness about mushrooms nutritive and medicinal value has enhanced their consumption (Chang, 1999).

Among the cultivated mushrooms, oyster mushroom (*Pleurotus* species) is flourishing in the temperate and sub-tropical environment due to its excellent flavour and taste (Ganeshan *et al.,* 1989). *Pleurotus ostreatus* is the second-largest commercially cultivated mushroom species in the world (Royse, 2013). Oyster mushroom can be grown on various substrates due to their strong enzymatic features (Atila, 2016). *Pleurotus ostreatus* is easier to cultivate, favourable to eat, and grow economically on different kinds of organic waste raw materials and different climatic conditions (Kong, 2004; Sitaula, 2018; Tekeste *et al.*, 2020).

The extensive cultivation of oyster mushroom is also because of its simplistic cultivation, high biological efficiency, and greater nutritional significance (Singh *et al.,* 1990). Nutritionally, the oyster mushroom is an ideal food for humans. It is rich in proteins (30.4%), fat (2.2%), carbohydrates (57.6%), and fibre (8.7) with

345 Kcal energy in 100 g of mushroom on a dry weight basis (Pandey, Ghosh, 1996).

Oyster mushrooms, having a considerable economic, medicinal and nutritional value, are most commonly and commercially cultivated on the non-composted lignocellulosic substrates (Savoie *et al.,* 2007) due to which the agriculture, industrial, and forest wastes can be efficiently utilized (Sanjel *et al.,* 2021). Various substrates, including paddy straw, maize stalks/cobs, vegetable plant residues, and bagasse, are extensively used in its cultivation (Sher *et al.,* 2011). Successful cultivation of oyster mushroom on the various substrates, including sawdust, chopped office papers, cardboard, and plant fibres, are also reported from multiple research works (Mandeel *et al*., 2005). Dubey *et al.* (2019) found that rice straw was found most favourable for mushroom cultivation rather than wheat straw, sugarcane bagasse and banana leaves.

Growing *P.ostreatus* requires sterilization of substrates, which means pre-treatment of the substrates to eliminate pathogenic and competitive micro-organisms, and to enhance the mycelial growth of mushroom. Sterilization of the substrates for the production of mushrooms is carried out mainly to avoid the presence of pathogens that appear to compete for the nutrients available in the unsterilized substrates. It is one of the crucial steps in oyster cultivation, which can determine the success of the cultivation (Ali *et al.*, 2007). Three different treatments, viz. steam sterilization, immersion in hot water, and chemical treatment, are explained in the literature (Mejía, Albertó, 2013). However, there are not enough works comparing the effectiveness of these sterilization methods influencing the quality and quantity of the mushroom produced. Therefore, the present research was undertaken to find out the most appropriate sterilization method, which will be helpful for further improvement in the yield of mushrooms.

Material and methods

Two independent experiments were conducted in Institute of Agriculture and Animal Science (IAAS), Lamjung Campus, Lamjung, Nepal, from January to March, in the year 2017 and 2019, to explore the best sterilization method, *i.e*., chemical, steam and hot-water sterilization in the production of oyster mushroom. Rice straw was taken as the substrate and subjected to hand-chopping to the required length of 5 cm.

Preparation of substrates

Chemical sterilization was carried out as explained by Siddhant *et al.* (2014)**.** The chopped straw was dipped into a 200 L metal drum containing 125 ml formalin mixture and 15 g Carbendazim per 100 L of water. The mixture was stirred with the help of a wooden pole. The drum was made airtight, and the substrate was allowed to soak for 18 hours in the solution. Then, the solution was drained out and the moisture content of about 65% was maintained in the wet substrate before spawning. For steam sterilization, the chopped substrate was soaked in water for 12 hours before sterilization. Presoaked substrate was exposed to steam for about 15–20 minutes, and cooled before spawning, as explained by (Gowda, Manvi, 2019).

We followed the Kalita (2015) method for hot water sterilization. The presoaked substrate was kept in the drum and boiled for 30 minutes, and the solution was drained before spawning. The experiment was set up using a complete randomized design (CRD) with three treatments and ten replications.

Spawning and incubation

Spawning was carried with fresh grain spawn on a 3.2% (weight/weight) rate based on the wet substrate. On every layer of around 4 inches of sterilized straw in polybags, spawn was placed near the periphery forming a circle, and bags were pressed to compact. Three layers of spawning were done to make balls of 4 kg, and perforation of the bag was done after completion of packing by making 8–10 holes of size 8 mm for aeration. Cotton was plugged into each hole to prevent contamination as well as entry of insects. Then, the bags were suspended on strings in a dark and pre-fumigated room with no ventilation. Thus, prepared each ball represented one replication. The temperature of the room was maintained at 13–14 °C, and relative humidity was 80%. The continuous observation was done till the full spawn run, and then the bags were cut from two sides for fructification. Ventilation with diffused light was provided with two gentle irrigations per day and other necessary crop management. Watering was stopped 24 hours before harvest.

Data collection and analysis

Harvesting was done when the majority of caps attained their maximum size. The experiment was terminated after three flushes from each replication. Data were recorded periodically on different parameters, including the number of days to full colonization, the number of days to first fruiting, harvesting duration (day), and mushroom yield in every flush (g), pileus diameter (cm), stipe length (cm), and biological efficiency (BE). The fresh mushroom yield on each harvest was measured, then total fresh yield and biological efficiency were calculated (Eq. 1).

$$BE = \frac{Yield\ of\ fresh\ mushrooms,\ g}{Total\ weight\ of\ dry\ substrate\ used,\ g} \times 100 \quad (1)$$

Statistical analysis

Data were analyzed using RStudio 1.2.5033. The treatment means were separated using Least Significant Difference (LSD) at the 5% level of significance.

Results and discussion

Vegetative and reproductive growth

The time required for full colonization of substrate, also known as spawn run duration, and was compared between three methods of sterilization. In our experiment, a significantly prolonged duration (29.70 days) for complete mycelial growth was observed in the chemically sterilized substrate (Fig. 1**)**.

Hot-water sterilization took the shortest duration (26.20 days), which was statistically at par with steam sterilization. The pattern was similar for days taken for the first pinhead formation and the cropping duration, reflecting the significant effect of the method of sterilization used. Chemical sterilization took the longest duration of 38.20 days for the appearance of the first pinhead, whereas steam sterilization took the shortest duration of 34.30 days. In all the treatments, the pinheads were formed between 8–9 days after the full colonization of the substrate. A significantly longer cropping duration of 96.1 days was recorded in chemical sterilization.

In oyster mushroom cultivation, the above parameters reflect the speed of vegetative and reproductive growth of the mushroom. Rapid growth and development are desirable for the profitable business; however, multiple factors, including ambient environmental condition, substrate physical and chemical properties, and the presence of competitive and pathogenic organisms, and antigrowth substances, will influence the rate of growth. Generally, the oyster mushroom takes up to three weeks for a full spawn run in its optimal environment (Shah *et al.*, 2004; Kalita, 2015). However, we observed a delay of 6–10 days, reflecting the suboptimal temperature (13–14 °C) for mycelia growth in our experiments. Atila (2016) reported that 7–12 days is taken from full spawn run to pinhead formation which is by our result. The fruiting bodies appeared on substrates sterilized by different methods 17–20 days later after a full spawn run. Our results are similar to Tan (1981), who reported that *P. ostreatus* took 14–21 days for fruiting body formation after the full spawn run.

The increased cropping duration in chemical sterilization is probably due to the anti-fungal effect of fungicides that hindered the mycelial growth of the mushroom, which subsequently delayed all the later developmental stage up to the third harvest. A similar delay in cropping duration was seen by Ali *et al.* (2007) where all the species of Pleurotus treated with formalin took long to complete the mycelial growth.

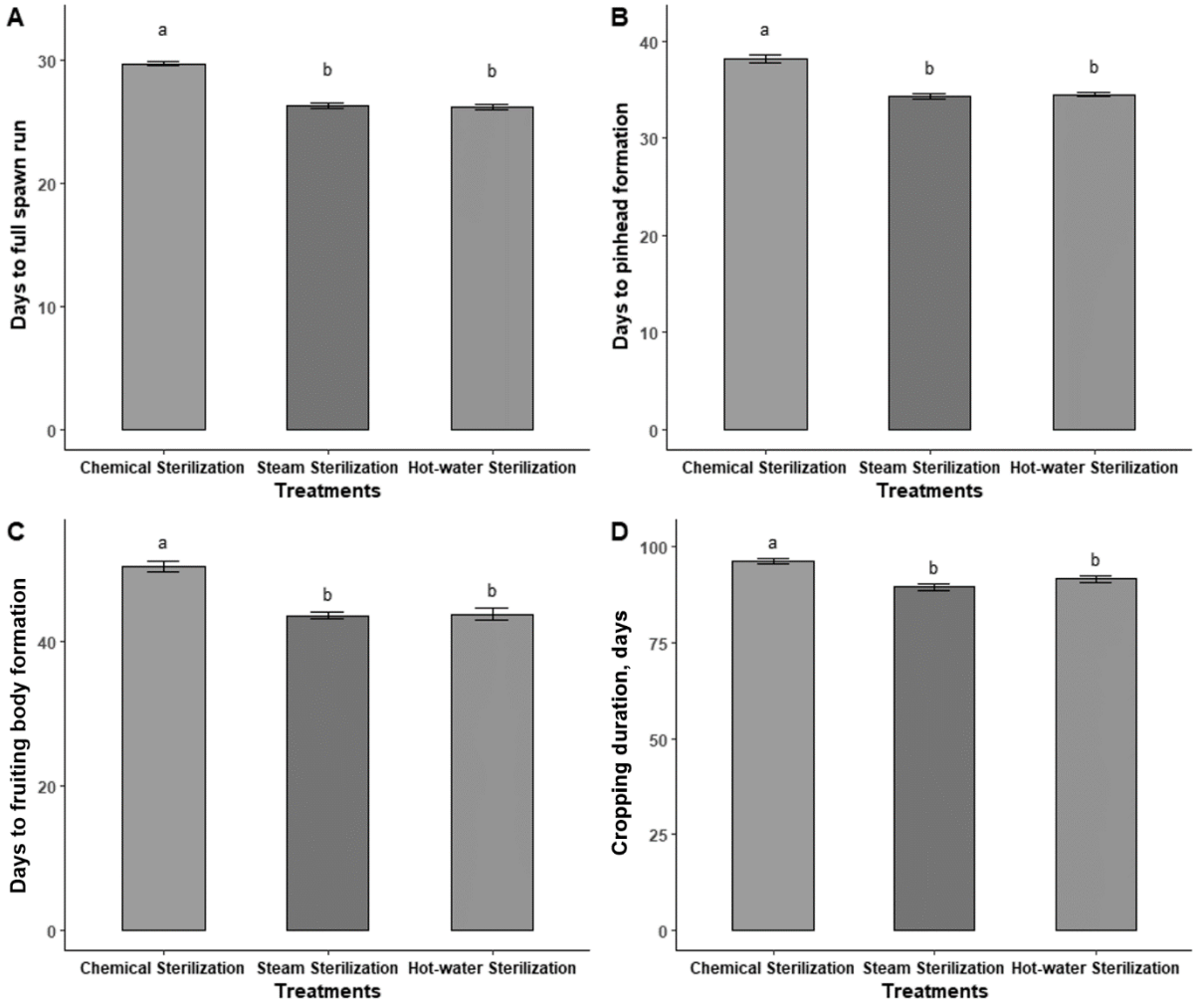

Figure 1. Bar plots showing the effect of sterilization methods on the days taken for full spawn run, pinhead formation, fruiting body formation, and cropping duration of *P. ostreatus*. Values in a bar with the same letter(s) are not significantly different at P = 0.05, according to LSD (least significant difference) test. The black bars in the plots indicate the standard error of the mean

Mushroom morphology

There was no significant difference between the treatments for pileus diameter and stipe length. The stipe lengths ranged from 7.0 to 7.1, and the diameters of pileus ranged from 9.3 to 9.6 (Table 1). It is reasonable that sterilization methods had no impact on the morphological properties of the mushroom. Such parameters highly depend on the mushroom strain and the nutrient factor of substrates. In our experiment, the substrate was the same in all treatments; thus, no variation was observed. The Pileus diameter of *P. ostreatus* was recorded in different growing substrates between 4.0–10 cm (Yildiz *et al.*, 2002). The variation in their work may be due to the variation in nutrient factors of multiple substrates.

Table 1. Pileus diameter and stipe length of *P. ostreatus* ± standard error of the mean, as affected by different sterilization methods

Treatment	Diameter of pileus, cm	Stipe length, cm
Chemical sterilization (T1)	9.408 ± 0.36	7.187 ± 0.19
Steam sterilization (T2)	9.638 ± 0.40	7.07 ± 0.25
Hot-water sterilization (T3)	9.303 ± 0.53	7.027 ± 0.27
F test	NS	NS
LSD (0.05)	0.943	0.711
CV (%)	10.877	10.915

Values in a column with the same letter(s) are not significantly different at P = 0.05 according to LSD (least significant difference). CV: coefficient of variation. NS: not significantly different at P < 0.05

Mushroom yield

There were significant effects of the methods of sterilization on the fresh mushroom yield of *P. ostreatus*. The highest total yield of 1.40 kg ball^{-1} was obtained in steam sterilization, which was statistically at par with hot-water sterilization (Fig. 2). The lowest total yield (1.02 kg ball^{-1}) was obtained in chemical sterilization. Ali *et al.* (2007) found that steam sterilization produced the highest total yield than all other methods which is by our results. The superiority of the steam sterilization might be due to the generation of optimal physical and chemical property of substrate for the mycelium growth. The biological efficiency also varied significantly among the methods of sterilization. The highest biological efficiency (BE) of 101.38% was observed in steam sterilization, and the least BE (79.75%) was observed in chemical sterilization (Table 2).

Two different dominant contaminants viz *Trichoderma* spp. and *Coprinus* spp. were found during the cultivation period in the experiments. The microbial contamination was observed the highest in hot water sterilization (16.00%) and the lowest in chemical sterilization (11.75%) (Table 2). Ashraf *et al.* (2007) found that the fungus associated with oyster mushroom growing media (compost) such as *Aspergillus, Trichoderma, Mucor, Penicillium, Alternaria, Cladosporium, Monilia, Helminthosporium, Coccidioides* and *Scedosporium*. Sharma *et al.* (2007) in the results of his research in India showed that the fungus *Aspergillus* spp., *Aspergillus niger*, *Fusarium* spp., *Mucor* spp. and *Trichoderma* spp. were competitor fungus or may cause disease in cultivated mushrooms such as oyster mushrooms. Lopez-Arevalo *et al.* (1996) also found that *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp. and *Cunninghamella* sp. a fungal contaminant in the tropical country of Mexico.

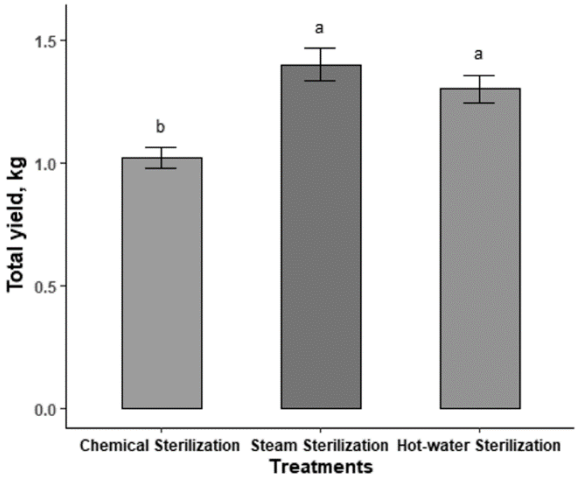

Figure 2. Bar plot showing the effect of sterilization methods on the total yield of *P. ostreatus*. Values in the bar with the same letter(s) are not significantly different at P = 0.05, according to LSD (least significant difference) test. The black bars in the plots indicate the standard error of the mean.

BE measures the ratio of the weight of fresh mushroom to the weight of dry substrate used in its production. In our laboratory, we have observed the BE for oyster mushroom up to 140%. BE in oyster mushroom production varies from 75.5 to 128.8% (Zhang *et al.*, 2002). In the real ground, the BE may be affected by several factors as substrate composition, ambient environment, mushroom strain, disease pest and other multiple management factors. BE of around 90% in hot water sterilization and 80% in chemical sterilization is an acceptable level. However, with the increase in room temperature, competitive moulds and microbes may reduce the BE of the mushroom crop (Biswas, 2014). Thus, we suggest all oyster mushroom growers wisely choose the sterilization techniques to obtain the economically profitable level of BE under suboptimal growing conditions.

Three substrate sterilization methods were evaluated for their effect on the growth and productivity parameters of oyster mushroom. There were significant differences in all the growth and yield parameters, but the morphological attributes were indifferent. Steam sterilization produced the highest fresh mushroom yield of 1409.1 g per bag of 4 kg wet substrate resulting in the highest biological efficiency of 101.3%. Chemical and hot water sterilization resulted in an acceptable production level with BE of around 80 and 90%, respectively. A Significantly longer cropping duration of 96.1 days was seen in chemical sterilization than hot water and steam sterilization. The same pattern was seen for other growth parameters, including days taken for full spawn run and fruiting body formation.

Table 2. Effect of the sterilization methods on the quantity harvests and biological efficiency of *P. ostreatus*.

Treatment	Fresh weight of mushrooms by flushes (g)			BE (%)	Incidence of microbial contamination (%)		
	First	Second	Third		*Trichoderma* spp.	*Coprinus* spp.	Average
Chemical sterilization	729.8 ± 0.03^{b}	214.9 ± 0.01^{c}	76.2 ± 0.02^{b}	79.75	13.75	9.75	11.75
Steam sterilization	890.8 ± 0.02^{a}	412.0 ± 0.03^{a}	160.1 ± 0.01^{a}	101.38	14.50	11.50	13.00
Hot-water sterilization	879.2 ± 0.09^{a}	309.9 ± 0.02^{b}	130.6 ± 0.02^{a}	90.39	16.50	15.50	16.00
F-test	***	***	**	***	**	**	**
LSD (0.05)	0.062	0.074	0.048				
CV (%)	7.9	25.1	42.5				

Values in a column with the same letter(s) are not significantly different at P = 0.05, according to LSD test.
CV: coefficient of variation; *** and ** significant different at P < 0.001 and P < 0.01, respectively

Conclusions

Proper sterilization of substrates is important for the effective and smooth cultivation of mushrooms. In our study, the different methods of sterilization have influenced the vegetative growth, morphology, cropping duration, mushroom yield and biological yield of oyster mushroom, however, the steam sterilization method was found more efficient with a shorter cropping duration, higher yield and higher biological efficiency than other methods. Thus, stem sterilization was found the best method of sterilization for the cultivation of oyster mushroom.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

SS – lead investigator and responsible for literature search and write-up.
SB, RKS, JS – responsible for the literature review and provided critical feedback on the manuscript.
All the authors read and approved the final manuscript.

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ANTIFUNGAL ASSESSMENT OF PLANT EXTRACTS, BIOCONTROL AGENTS AND FUNGICIDES AGAINST *Fusarium verticillioides* (Sacc.) CAUSING EAR ROT OF MAIZE

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ABSTRACT. Ear rot is a prominent biotic threat of maize causing significant yield loss and poor quality of grains. The antifungal activity of aqueous extract of *Acorus calamus* L., *Xanthoxylum armatum* DC., *Azadirachta indica* A. Juss., *Lantana camera* L. and *Artemisia indica* Willd at three different concentrations (1, 2 and 3% W/V), four chemical fungicides viz., Dithane M-45 (Mancozeb 75% WP), SAAF (Carbendazim 12% + Mancozeb 63% WP), ACME-COP (Copper oxychloride 50% WP) and Bavistin (Carbendazim 50% WP) at (500, 1000 and 1500 ppm) and three biocontrol agents (BCA) namely *Trichoderma viride, Trichoderma harzianum* and *Trichoderma koningii* were investigated against *Fusarium verticilliodes* (Sacc.) causing ear rot of maize. The experiment was carried out by poisoned food and dual culture techniques in a completely randomized design with five replications under laboratory conditions at National Maize Research Program, Rampur, Chitwan during the summer season of 2019. *F. verticillioides* showed significant growth inhibition in all the treatments compared to control. The *A. calamus* even at a lower dose (1% W/V on PDA) was able to check completely the growth of pathogen (4.00 mm). The mycelial growth inhibition per cent of *A. calamus*, *L. camera*, *X. armatum*, *A. indica*, and *Artemisia indica* at 3% W/V was 95.50, 51.13, 45.50, 42.12 and 35.36% respectively. In the case of fungicides, at 1500 ppm, the maximum antifungal potential was observed with SAAF (86.32%) followed by Dithane M-45 (80.27%), Bavistin (64.80%) and ACME-COP (59.42%). Antagonist *Trichoderma viride* completely overgrows *F. verticillioides* and covers the entire medium surface and exhibit more than 60% inhibition on the 7th day of incubation. The antifungal components from these plant extracts, fungicides and antagonists explored in this study need to be tested further in field experiments to control the ear rot of maize.

Introduction

Fusarium verticillioides Sacc. is the most commonly reported fungal species causing ear rot of maize (*Zea mays*). The species is known as *Gibberella fujikuroi* species complex and described as mating population A of the *Fusarium fujikuroi* species complex (Seifert *et al.*, 2003). The fungus on infected kernels emits the mutagenic chemical compound fusarin C (Gelderblom *et al.*, 1984), as well as a collection of disease-causing mycotoxins known as fumonisins (Ortiz *et al*., 2015). Among the various ear rotting organisms, *Fusarium verticillioides* Sacc. was found most common and destructive to the maize crop in Nepal (Subedi, 2015). Although Fusarium ear rot occurs throughout the country, it is particularly common in places with high rainfall, moist and foggy weather conditions, and long crop durations in the field, all of which give favourable conditions for disease incidence and severity (Subedi, 2015). Symptoms of the disease include ears with red tip discolouration and light weight, as well as tightly adhered husk leaves during harvest (NMRP, 2015). Multiple minute black fruiting bodies of the fungus, a

Gibberella phase, can sometimes be found on husk covering or other damaged plant parts (Agrios, 2005). The fungus is commonly found in wounded areas caused by ear/kernel/silk-cut insects or birds, as well as in germinating kernels of ears in stuck plants. The disease also appears later on harvested ears. The pathogen can also infect seeds, seedlings, and roots, causing discolourations, rots, and blights in the field during germination, especially at high altitudes with cold and foggy weather (NMRP, 2016). Because it may produce trichothecenes, deoxynivalenol, and nivalenol mycotoxin on mouldy kernels, the pathogen is also significant for maize quality. Trichothecenes mycotoxins were found in 16% of the 74 maize samples collected from various locations of Nepal (Desjardins *et al*., 2000). The toxic consequences of chemical pesticides on individuals and the environment, on the other hand, pushed the search for the development of environmentally sustainable fungus control alternatives. Many synthetic chemicals are known to cause carcinogenicity, teratogenicity on non-target species and pollute the environment, soil and groundwater due to their residual toxicity and non-biodegradable nature (Pimentel, Levitan, 1986). Furthermore, the use of many synthetic fungicides has been limited due to unfavourable characteristics such as high and acute toxicity, a long degradation time and accumulation in the food chain, and an unfavourable extension of their ability to kill useful microorganisms (Subedi *et al*., 2015). Alternative fungal control approaches are being explored owing to issues like chemical residues, biodegradation, phytotoxicity, and pollution connected with chemical control measures. The study primarily aimed to evaluate the efficacy of locally available botanicals and antagonists for their ability to check the growth of *F. verticillioides* as an alternative to the chemical fungicides. In this study, the effect of plant extracts, bio-control agents and chemical fungicides against the growth of *Fusarium verticillioides* Sacc. causing ear rot of maize was investigated under *in vitro* condition.

Material and methods

Isolation of the pathogen

The antifungal assessment of plant extracts, fungicides and biocontrol agents against *Fusarium verticillioides* Sacc. was done at the laboratory of the National Maize Research Program (NMRP), Rampur, Chitwan during the summer season of 2019. To isolate the pathogen, samples of ear rot disease specimens were collected from maize growing fields of NMRP, Rampur during the harvesting stage. Infected ears were brought to a plant pathology laboratory and *F. verticilliodes* was isolated under aseptic conditions and the pure culture colonies of the pathogen purified by single spore isolation method were subcultured aseptically for further study.

Plant extracts

The aqueous extract of *Acorus calamus* L., *Xanthoxylum armatum* DC., *Azadirachta indica* A. Juss., *Lantana camera* L. and *Artemisia indica* Willd at three different concentrations (1%, 2% and 3% W/V) were prepared as described by Subedi *et al*. (2019). The extracts were exposed to UV light for further sterilization.

Fungicides

The chemical fungicides used for the experiment were Dithane M-45 (Mancozeb 75% WP), SAAF (Carbendazim 12% + Mancozeb 63% WP), ACME-COP (Copper oxychloride 50% WP) and Bavistin (Carbendazim 50% WP). The concentration level maintained was 500, 1000 and 1500 ppm.

Bio-control agents

The bio-control agents used for the experiment were laboratory isolated culture of *Trichoderma viride, Trichoderma harzianum* and *Trichoderma koningii*.

***In vitro* test**

The experiment was carried out by poisoned food and dual culture techniques in a completely randomized design (CRD) with five replications. A cork borer cut a four-mm-diameter piece of test pathogen from a one-week-old culture which was picked up with an inoculating needle and put in the centre of the plate. To allow for better and faster pathogen-media contact, a cut piece of the test pathogen was placed upside down in the PDA plate supplemented with the various above-mentioned treatments. The plates were put in incubation at 25 °C for up to 7 days (Subedi *et al*., 2019).

Similarly, the antagonistic effect of bio-control agents against *Fusarium verticillioides* Sacc. was evaluated following the dual culture technique. A four-millimetre-diameter cut piece of test antagonists and pathogen from a one-week-old culture was placed on PDA medium in an equidistant with an inoculating needle under aseptic condition and incubated for seven days at 25 °C. The colony diameter of both the test fungus and bioagent on different days of incubation up to 7 days were measured and per cent inhibition was calculated.

Observation

The average radial growth (mm) of the test pathogen was measured using a measuring scale from the bottom of the Petri-plates on different incubation dates. The percentage of mycelial growth inhibition was determined using the formula given below.

$$MGI, \% = \frac{(dc - dt)}{dc} \times 100 \quad (1)$$

where MGI – mycelial growth inhibition; dc – average diameter of fungal colony in the control and dt – average diameter of fungal colony in the treatment group.

Statistical analysis

The recorded observations were analyzed statistically using Genstat 18th edition computer package program and treatment means were compared using Duncan's Multiple Range Test (DMRT) and Least Significance Difference (LSD) test at 1% levels of significance.

Results

The mycelial growth of *Fusarium verticillioides* Sacc. differed significantly over the control among the treatments at various concentrations (Table1). The sweet flag (*Acorus calamus* L.) even at a lower dose (1% W/V on PDA) was able to check completely the growth of the pathogen (4.00 mm). With the increase in concentration (3% W/V), the mean colony diameter of the *Fusarium verticillioides* Sacc. in the plate amended with lantana (*Lantana camera* L.) and prickly ash (*Xanthoxylum armatum* DC.) was significantly lower *i.e.* 43.40 mm and 48.40 mm (Table 1) respectively than the diameter recorded at control plate (88.80 mm).

Table 1. Effect of plant extracts incorporated PDA on the growth of *Fusarium verticillioides* Sacc. in different concentration level at 25 °C

Treatments	Mean colony diameter, mm		
	1% W/V	2% W/V	3% W/V
Acorus calamus	4.00^{e}	4.00^{e}	4.00^{f}
Xanthoxylum armatum	60.40^{d}	52.60^{d}	48.40^{d}
Azadirachta indica	74.60^{b}	55.40^{c}	51.40^{c}
Lantana camera	61.00^{d}	51.60^{d}	43.40^{e}
Artemisia indica	64.00^{c}	61.80^{b}	57.40^{b}
PDA only	89.40^{a}	88.80^{a}	88.80^{a}
Grand mean	58.90	52.37	48.90
P-value	<0.001	<0.001	<0.001
LSD (0.01)	2.16	2.08	1.97
CV,%	2.00	2.20	2.20

Means in column with the same superscript are not significantly differed by DMRT ($P \leq 0.01$). W/V – weight by volume, PDA – potato dextrose agar

The mycelial growth inhibition per cent of *A. calamus*, *L. camera*, *X. armatum*, *A. indica*, and *Artemisia indica* at the concentration of 3% W/V on PDA was 95.50, 51.13, 45.50, 42.12 and 35.36% respectively (Table 2).

Table 2. Effect of plant extracts incorporated PDA on mycelial growth inhibition of *Fusarium verticillioides* Sacc. in different concentration level at 25 °C

Treatments	Mycelial growth inhibition %		
	1% W/V	2% W/V	3% W/V
Acorus calamus	95.53	95.50	95.50
Xanthoxylum armatum	32.44	40.77	45.50
Azadirachta indica	16.55	37.61	42.12
Lantana camera	31.77	41.89	51.13
Artemisia indica	28.41	30.41	35.36
PDA only			

W/V – weight by volume, PDA – potato dextrose agar

All the tested fungicides incorporated PDA had a significant ($P < 0.01$) effect on the growth of the *Fusarium verticillioides* Sacc. at 25 °C as compared to control at different concentrations up to the 7th day of incubation (Table 3). The mean colony diameter of the pathogen in the plate amended with SAAF (Carbendazim 12% + Mancozeb 63% WP) and Dithane M-45 (Mancozeb 75% WP) was significantly lower ie 12.20 mm and 17.60 mm, respectively than the control plate (89.20 mm) on 7th day of incubation at 1500 ppm concentration level. Both fungicides Bavistin (Carbendazim 50% WP) and ACME-COP (Copper oxychloride 50% WP) also checked the growth of pathogen *i.e.* 31.40 mm and 36.20 mm respectively at 1500 ppm concentration compared to the control plate (Table 3).

Table 3. Effect of fungicides incorporated PDA on the growth of *Fusarium verticillioides* Sacc. in different concentration level at 25 °C

Treatments	Mean colony diameter, mm		
	500 ppm	1000 ppm	1500 ppm
SAAF (Carbendazim 12% + Mancozeb 63% WP)	28.00^{e}	20.80^{e}	12.20^{e}
Dithane M-45 (Mancozeb 75% WP)	34.40^{d}	28.60^{d}	17.60^{d}
Bavistin (Carbendazim 50% WP)	58.80^{c}	52.00^{c}	31.40^{c}
ACME-COP (Copper oxychloride 50% WP)	72.80^{b}	66.40^{b}	36.20^{b}
PDA only	89.20^{a}	89.20^{a}	89.20^{a}
Grand mean	56.72	51.40	37.32
P-value	<0.001	<0.001	<0.001
LSD (0.01)	2.11	2.33	1.97
CV,%	2.00	2.50	2.90

Means in column with the same superscript are not significantly differed by LSD ($P \leq 0.01$). WP – wettable powder, PDA – potato dextrose agar

Table 4. Effect of fungicides incorporated PDA on mycelial growth inhibition of *Fusarium verticillioides* Sacc. in different concentration level at 25 °C

Treatments	Mycelial growth inhibition %		
	500 ppm	1000 ppm	1500 ppm
SAAF (Carbendazim 12% + Mancozeb 63% WP)	68.61	76.68	86.32
Dithane M-45 (Mancozeb 75% WP)	61.43	67.94	80.27
Bavistin (Carbendazim 50% WP)	34.08	41.70	64.80
ACME-COP (Copper oxychloride 50% WP)	18.39	25.56	59.42
PDA only			

ppm – parts per million, WP – wettable powder, PDA – potato dextrose agar

The maximum antifungal potential was observed with SAAF which recorded excellent inhibitory activity (95.34%) against *Fusarium verticillioides* Sacc. (86.32%) followed by Dithane M-45 (80.27%), Bavistin (64.80%) and ACME-COP (59.42%) at the concentration of 1500 ppm (Table 4).

Table 5. Effect of *Trichoderma* species on radial colony growth of *Fusarium verticillioides* Sacc. in different incubation periods at 25 °C

Treatments	Mean colony diameter, cm		
	3rd day	5th day	7th day
Trichoderma viride	1.35^{d}	2.45^{d}	3.16^{d}
Trichoderma harzianum	2.15^{c}	3.03^{c}	3.75^{c}
Trichoderma koningii	2.73^{b}	3.75^{b}	4.35^{b}
Control (only *Fusarium monilliforme*)	3.85^{a}	4.45^{a}	8.37^{a}
Grand mean	2.52	3.42	4.91
P-value	<0.001	<0.001	<0.001
LSD (0.01)	0.32	0.29	0.30
CV,%	6.60	4.40	3.10

Means in column with the same superscript are not significantly differed by LSD ($P \leq 0.01$)

All of the *Trichoderma* species exhibited significant inhibition ($P < 0.01$) of the *Fusarium verticillioides* Sacc. as compared to control in different dates of incubation period at 25 °C. Less (3.16 cm) mean colony diameter of the pathogen was measured when the dual culture was done with *Trichoderma viride*. The diameter of the pathogen alone was 8.37 cm on the 7th day of incubation at 25 °C temperature. However, the tested

Trichoderma species differed in their abilities to suppress *Fusarium verticillioides* Sacc. Antagonist *Trichoderma harzianum* (3.75 cm) *and Trichoderma koningii* (4.35 cm) was also checked the growth of *Fusarium verticillioides* Sacc. on the 7th day of incubation at 25 °C temperature (Table 5). Antagonist *Trichoderma viride* completely overgrows *F. verticillioides* and covers the entire medium surface and exhibit more than 60% inhibition on the 7th day of incubation (Table 6).

Table 6. Effect of *Trichoderma* species on mycelial growth inhibition of *Fusarium verticillioides* Sacc. in different incubation periods at 25 °C

Treatments	Mycelial growth inhibition, %		
	3rd day	5th day	7th day
Trichoderma viride	64.94	44.94	62.25
Trichoderma harzianum	44.16	31.91	55.20
Trichoderma koningii	29.09	15.73	48.03
Control (only *Fusarium verticillioides*)			

Discussion

Botanical extracts are recognized as eco-friendly and safe for the management of plant diseases in the quest for better remedies. The results achieved in this study are in agreement with Satish *et al*., (2009) who screened 46 plants belonging to 32 different families against eight species of *Fusarium* and explored aqueous extracts of 12 different Asiatic plant species which showed significant antifungal potentialities against the test pathogen. The result of the experiment is also in line with the findings of many studies conducted with the extracts of *Azadirachta indica, Zingiber officinalis, Curcuma longa, Ocimum sanctum, Acorus calamus, Terminalia chebula, Lantana camera and Catharanthus roseus* which can be exploited in the control of fungicide resistant pathogens (Baligh *et al*., 1999; Bowers and Locke, 2000; Biswas *et al.*, 2002; Pothitirat and Gritsanapan, 2006). The alcoholic extract of *A. calamus* could inhibit many fungi including *Alternaria brassicae* Sacc, *Fusarium oxysporum f.sp. lycopersici, Rhizoctonia solani* Kuhn, *Sclerotinia sclerotiorum* de Bary at the concentration of 0.10% upward (Kungha, 1999). The major antifungal compound found in *A. calamus* is β-asarone which is considered to be the most biologically active compound in the rhizome of *A. calamus* and quantities of β-asarone vary 10–20% in the rhizome of European origin while in Asia, it was found in the range of 70–90% (Karwowska *et al*., 1997). The antifungal bioactive components especially monoterpenes like geranyl, terpinyl and bornyl acetate, bicyclic sesquiterpene ß-caryophyllene and a cyclic monoterpene like limonene extracted from the essential oil of *Lantana camera* restricted the growth of many tested fungi including *Fusarium* spp (Deena and Thoppil, 2000). The aqueous extract of *Acorus calamus* L. was found most effective followed by *Xanthoxylum armatum* and *Lantana camera* when tested against fungi *Exserohilum turcicum* and *Stemphylium botryosum* Walr. both *in vitro* and *in vivo* condition (Subedi *et al*., 2015; Subedi *et al*., 2019).

Bashir *et al*. (2018) studied the effects of fungicides on the mycelial growth of *Fusarium* spp and found a significant mycelial reduction by carbendazim. Studies have shown that carbendazim prevents microtubule formation and inhibits mitosis in fungal cells of the targeted fungi, which prevent mycelial growth (Kling and Jakobsen, 1997; Yang *et al*., 2011). Carbendazim specifically interacts with b-tubulin and stop the mycelial growth of fungi (Zhou *et al*., 2016; Vela-Corcia *et al*., 2018). The growth of *Exserohilum turcicum* causing northern leaf blight of maize was also effectively checked with the application of SAAF (Carbendazim 12% + Mancozeb 63% WP) and Dithane M-45 (Mancozeb 75% WP) under *in vitro* condition (Subedi *et al*., 2019). The bio-control agents *Trichoderma viride* Pers. showed the abundant potentials to suppress the radial colony growth of *F. verticillioides* under laboratory condition. *T. viride* developed more rapidly than *F. verticillioides* in single as well as in dual cultures. The intensive development of *Trichoderma* gives it a significant advantage in competition with pathogens for nutrients and space, besides the production of mycotoxins. Most of the studies showed these bioagents inhibitory activities were most likely due to competition and/or antibiosis (Subedi *et al*., 2019). The antagonism of *T. viride* found in the current study is consistent with the results of other researchers too (Biles and Hill, 1988; Mahamood *et al*., 1995; Ramachandra, 2000). The pathogen control mechanism of *Trichoderma* can involve attacking and binding pathogenic organisms via sugar linkage with the secretion of extracellular protease and lipase (Cal *et al*., 2004). The key reason for the ecological success of *Trichoderma* spp could be a combination of highly active mycoparasitism mechanisms and an efficient defensive strategy induced in the plants (Rosado *et al*., 2007). Trichoderma sp. grows over pathogenic fungal hyphae, coils around them, and degrades the cell walls, limiting the growth and activity of pathogenic fungi, a mechanism known as mycoparasitism, with the release of antibiotics (Harman, 2006).

Conclusions

The aqueous extracts of all the test plants, biocontrol agents and chemical fungicides were subjected to antifungal activity against *F. verticillioides* causing ear rot of maize. The extract of *Acorus calamus* L. at a lower dose also completely check the pathogen growth *in vitro* and found more effective compared to chemical fungicides. Antagonist *Trichoderma viride* suppresses the radial colony growth of *F. verticillioides* and develops more rapidly than the test pathogen. So these botanicals, antagonists and fungicides like SAAF (Carbendazim 12% + Mancozeb 63% WP) could be exploited for ecofriendly management of the diseases caused by the *F. verticillioides* and need to be tested further in field experiments as well as isolation of antifungal bioactive agents.

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Conflict of interest
The authors declare that there is no conflict of interest regarding the publication of this paper.

Author contributions
SS – study conception and design, analysis and interpretation of data, critical revision and approval of the final manuscript; SN – drafting of the manuscript, acquisition of data.

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EFFECT OF SURFACE DRIP IRRIGATION AND CULTIVARS ON PHYSIOLOGICAL STATE AND PRODUCTIVITY OF FABA BEAN CROP

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ABSTRACT. Lack of water is one of the main abiotic factors that affect the change of plant production processes by imposing certain morphological, physiological and biochemical changes. The aim of the research conducted in 2019–2020 was to study the change in the productivity and yield of green beans of different cultivars of *Vicia faba* L. *var. Major* and the formation of a symbiotic system on surface drip irrigation. The results of the biometric analysis showed that the cultivation of faba beans under irrigation contributes to an increasing in plant height by 4.7–12.2%, the number of branches per plant increased by 17.3–30.0%, the leaf area of faba bean crops increased by 21.2–24.9%. The content of total chlorophyll increasing by 16.9–40.5%. Antioxidant enzymes activity decreased depending on the cultivar Catalase activity by 10.6–22.5%, Guaiacol peroxidase – 19.4–25.9%, Superoxide dismutase – 19.3–24.4%. The yield of green faba beans (*Vicia faba* L.) increased by 31.3–39.2%. Growing faba beans on irrigation helped to reduce the protein content by 1.4–2.1 %, but to reduce the dry matter content by 1.3–2.0%, which was significant in both indicators. In general, drip irrigation contributed to the improved development of bean-rhizobial symbiosis of faba bean plants. The mass of the nodules on the drip irrigation increased by 0.3 g plant^{-1} regardless of the cultivar, and their number is 1.5–9.0 pcs plant^{-1}. The presented results give an idea of the functioning of the legume agrocenosis and the impact of irrigation on the main quality indicators of the product. Further research is to study the regimes (rates, timing, and multiplicity) of irrigation.

Introduction

One of the most important problems of agriculture today is the shortage of vegetable protein, equivalent to animal protein. Beans are an important source of biological nitrogen in agriculture, the importance of which has increased especially in a difficult environmental situation with an insufficient supply of mineral nitrogen fertilizers. The share of biological nitrogen in the nitrogen balance is still very small and is about 5%, and when creating favourable conditions for symbiosis, it can increase up to 30% (Stolyarov, 2005).

Given the growing cost of man-made resources and environmental tensions to ensure the sustainable functioning of agroecosystems, alternative approaches to the development of agrotechnologies based on the concept of biologization of agriculture and providing it with resource-saving and sustainable development. Based on this, the selection of cultivars of beans and their cultivation on drip irrigation is an urgent problem of vegetable growing and agricultural production in general (Stolyarov, 2005).

In the modern world, with the high nutritional value of bean seeds, their nutritional value increases. Green beans are rich in B vitamins, which play an important role in preventing ageing and multiple sclerosis. The grains contain 1.0 % of fibre, 0.7 % of ash and 80.0% of water. In terms of calories, beans are 3–4 times higher than potatoes and 6 times higher than corn (Bouchenak, Lamri-Senhadji, 2013; Yamawaki *et al*., 2014; Lizarazo *et al*., 2015; Ali, 2016; Rosa *et al*., 2018; El-Naggar *et al*., 2019).

Currently, beans are not paid attention to, probably due to the presence of vicin and convicine, which limit their consumption. These anti-food factors can cause acute hemolysis in subjects with glucose-6-phosphate

dehydrogenase deficiency (Cappellini, Fiorelli, 2008). At the same time, beans show interesting characteristics due to the presence of some nutraceutical compounds, such as levo-dihydroxy phenylalanine (L-dopa). This compound is used to treat diseases such as Parkinson's, hypertension, renal failure and liver cirrhosis (Korczyn *et al*., 2008).

Today, when growing vegetables, the optimization of the irrigation regime as a factor is of paramount importance. It determines the efficiency of technology and crop quality, total costs, water and energy needs (Molden *et al*., 2010; Gagan *et al*., 2021). Experience of advanced farms and data from research institutions show that good management practices and optimal irrigation regime contribute to the formation of high and stable yields of vegetable crops (Azzeddine *et al*., 2016; 2019). It is well known that irrigation costs and plant productivity vary depending on irrigation methods. Therefore, drip irrigation is promising in the cultivation of vegetable crops (Borodychev, Martynova, 2011; Etemadi *et al*., 2019; Fu *et al*., 2021). Drip irrigation is generally more effective than compared with other types of irrigation in terms of crop yields and water savings.

Beans are usually grown without irrigation, but in unstable climates, drip irrigation becomes a necessity and can significantly improve the efficiency of cultivation technology (Alghamdi *et al*., 2015; Guoju *et al*., 2016). The greatest water demand for beans covers the growth stages from flowering to full harvest (BBCH 60–89), June–July (Dudek *et al*., 2018; Karkanis *et al*., 2018).

Under scarce water in semi-arid areas, irrigation water management aims to provide sufficient water to replenish depleted soil water in time to avoid physiological water stress in growing plants, using modern irrigation technologies such as a drip irrigation system (Saleh *et al*., 2012).

Topak *et al*. (2009) indicate that the highest yield of *Phaseolus vulgaris* was obtained in areas irrigated by drip irrigation with full irrigation (565 mm). The highest level of water efficiency was obtained by drip irrigation.

Lysimetric studies of Tarantino and Rubino (1989), calculated the seasonal water requirement for the maximum yield of beans – 235 L m^{2-1}. While El Noemani *et al*. (2010), recommended using 371 L m^{2-1} for the maximum yield of green bean pods.

Several studies have shown that the effect of the water regime on green beans (Abd El-Mawgowd, 2006; Sezen *et al*., 2008; El-Noemani *et al*., 2010; Abd El-Aal, 2011). But the results of these studies are limited to the area (zone) of research and are mainly conducted using a single variety, therefore these results may give a false idea of the impact of irrigation in other soil and climatic conditions. Sezen *et al*. (2008) found a direct relationship between watering rate and bean yield.

Irrigation reduces pod abortion and is expected to have a significant impact on final yield. The amount and distribution of rainfall during the season greatly affect the rainfed faba bean yield. This is particularly important in water-scarce areas, where the water saved as a result of this practice can be used to irrigate additional land, thus, allowing farmers to achieve higher levels of production (Attila *et al*., 2017).

The purpose of this research is to identify varietal and physiological characteristics of the formation of a high level of the yield of green beans under drip irrigation and the formation of the nodulation apparatus of plants in the Forest-Steppe of Ukraine. For the first time in the conditions of the Forest-Steppe of Ukraine the experimental data connected with the formation of a commodity crop of green beans of a faba bean on drop irrigation are received.

The novelty of the study is a comprehensive study of production processes of faba beans, as previously published data by other authors do not give a complete picture of the impact of irrigation on physiological responses of faba bean plants, changes in nutritional value (the content of protein and dry matter), the concentration of antinutrient composition and formation of nodulation apparatus.

Material and methods

The experiment of the influence of drip irrigation of faba bean (*Vicia faba* L.) cultivars was carried out in 2019–2020 at the Department of Vegetable Growing of Uman National University of Horticulture (Right-Bank Forest Steppe of Ukraine) by national methods (Bondarenko, Yakovenko, 2001). The soil was black, puddle, heavy loam with a well-developed humus horizon (about 2.9% of humus) (Krupsky, Polupan, 2018), (Table 1), in the deep of 40–45 cm. Soil pH was determined in water (soil to water ratio 1:1). The electrical conductivity (ECe) of the soil suspension was measured using the conductivity meter. The P and K were determined by the ammonium bicarbonate-diethylenetriaminepentaacetic acid (ABDTPA) method (Ryan *et al*., 2001).

Table 1. Chemical properties of soil (x ± SD)

Parameter	Value
Organic carbon %	2.2 ± 0.12
Acidity (pH)	6.1 ± 0.10
ECe, µS/cm	24 ± 0.45
Extractable P (ABDTPA), mg kg^{-1}	102 ± 3.33
Extractable K (ABDTPA), mg kg^{-1}	123 ± 2.00
NO_3N, mg kg^{-1}	64 ± 1.53

The scheme of the two-factor experiment included four cultivars of faba beans (factor A), which were grown without irrigation and drip irrigation (factor B), maintaining soil moisture at 80% by applying surface drip irrigation to the technical maturity of beans (El-Noemani *et al*., 2010). Irrigation started from June, 2 to 10, July 2019 and May, 20 to July 25, 2020.

The experiment scheme included the following variants:

Factor A – method of cultivation:

- without irrigation (A_1);
- surface drip irrigation (A_2).

Factor B – cultivars of faba bean:

- 'Karadag' (B_1) – medium-ripe faba bean cultivar of universal purpose with a growing season to technical maturity of 83–90 days. Resistant to disease, drought; suitable for mechanized harvesting. Formed from 22 to 27 beans, each with three to five dark purple seeds at technical maturity;
- 'Biloruski' (B_2) – medium ripe from germination to technical maturity 70–85 days. Pods are straight, smooth, green in technical maturity, with 3–5 large carmine-red seeds, when ripe – red-brown, relatively cold-resistant, unpretentious to growing conditions, characterized by the excellent quality of immature and ripe seeds.
- 'Ukrainski Slobidski' (B_3) – medium–ripe variety of universal purpose with a growing season to technical maturity of 85–90 days. Resistant to disease, drought. Suitable for mechanized harvesting. The plant forms from 24 to 29 beans. Seeds oval, light brown;
- 'Vindzorski' (B_4) – medium-ripe hybrid from germination to technical maturity of 76–89 days. Bush plant. In technical maturity, the beans are characterized by a dark green colour.

Scheme of the variants:

- A_1B_1 – 'Karadag' without irrigation;
- A_1B_2 – 'Biloruski' without irrigation;
- A_1B_3 – 'Ukrainski Slobidski' without irrigation;
- A_1B_4 – 'Vindzorski' without irrigation;
- A_2B_1 – 'Karadag' surface drip irrigation;
- A_2B_2 – 'Biloruski' surface drip irrigation;
- A_2B_3 – 'Ukrainski Slobidski' surface drip irrigation;
- A_2B_4 – 'Vindzorski' surface drip irrigation.

The cultivar 'Karadag' was used as a reference as this cultivar is currently cultivated as a food crop.

The experimental design was a randomized complete block design with four replicates. The area for the sampling – 100 m² (Bondarenko, Yakovenko, 2001). Planting was carried out by the scheme of 60 × 10 cm (166 000 plants ha^{-1}) of April 5, 2019, and April 10, 2020. Early-maturing varieties of faba beans were used for research. Harvesting took place at the technical ripeness of the beans (BBCH 81). The growing period was 82 ± 2 DAP (the day after planting).

The leaf surface area of plants was determined in the phase of technical maturity of beans by the method of "cuttings" (Nichiporovich, 1961). At the experimental site, 10 plants were selected, all leaves were plucked from them and weighed. Then with the help of a cork drill took from these leaves 20 cuts and weighed them. The total leaf surface in the sample was determined by the formula:

$$S = \frac{M \times s \times N}{m}, \quad (1)$$

where S is the total area of leaves in the sample; M – the mass of leaves in the sample, g; s – the area of one cut (1 cm^2); N – number of cuts, pcs.; m – a mass of cuts, g.

By calculating the total leaf area in the sample, we determined the leaf area per plant and, multiplying this indicator by the density of plants per 1 ha, we obtained the area of the leaf apparatus of plants expressed in m^2 ha^{-1} (Grytsaenko *et al*., 2003).

Chlorophylls *a* and *b* content was determined according to the method reported by Albanese *et al*., (2007). Briefly, 2 g of sample was homogenized in 10 mL of acetone/water (80:20 v/v) and then centrifuged at 4000 r. The absorbance of the extract was measured at 646, 663 nm using a spectrophotometer T60U (PG Instruments, UK).

Activity measurements of antioxidant enzymes. Enzyme activities were determined, during the harvest. A one g of plant tissue from control and treated plants was homogenized on ice in 4 ml extraction buffer (50 Mm^{-1} phosphate buffer pH 7.0, containing 1 mM EDTA, 1 mM phenylmethylsulphonyl fluoride and 1% polyvinylpolypyrrolidone). The homogenate was centrifuged for 25 min at 15,000 × g^{-1} and 4 °C. The supernatant was used for enzyme activity assays. The means ± SD were calculated from the data of at least 3 independent measurements. SOD (Superoxide dismutase) activity was determined spectrophotometrically by measuring the ability of the enzyme to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) in the presence of riboflavin in light (Dhindsa *et al*., 1981). One unit (U^{-1}) of SOD was the amount that causes 50% inhibition of NBT reduction in light. The enzyme activity was expressed in terms of specific activity (U mg $protein^{-1}$). CAT (Catalase) activity was determined by the decomposition of H_2O_2 which, in turn, was measured by the decrease in absorbance at 240 nm (Upadhyaya et al., 1985). One U^{-1} equals the amount of H_2O_2 (in μmol^{-1}) decomposed in 1 min^{-1}. POD (Guaiacol peroxidase) activity was determined by monitoring the increase in absorbance at 470 nm during the oxidation of guaiacol (Upadhyaya *et al*., 1985). The amount of enzyme-producing 1 μmol min^{-1} of oxidized guaiacol was defined as 1 U^{-1}.

To calculate the number and risobial formations selected soil monoliths 25 × 25 × 30 cm. After washing the roots of each repetition left 5 plants, separated from the roots of the nodules, counted their average number per plant, dried (Grytsaenko *et al*., 2003).

Green bean weight and yield per plot were weighed in a technical maturity stage.

Dry matter of green beans (%). The average dry matter weight (g) of the green bean after curing were measured by drying 10 randomly sampled beans in an oven with a forced hot air circulation at 70 °C until a constant weight was obtained. The per cent of bean dry matter was calculated by taking the ratio of the dry weight to the fresh weight of the sampled beans and multiplying it by 100.

The crude proteins content (N × 6.25) of the seeds was determined by Kjeldahl nitrogen, according to the AOAC method 955.04 (AOAC, 1991).

Vicine and convicine assays. Analyses of samples from faba bean cultivars were performed by the Natural Resources Institute of Finland, according to the method of Gutierrez *et al*. (2006). Briefly, samples (1 g) were extracted with ultrapure water (30 mL) in a hot-water bath (90 °C) for 3.5 h, with shaking every 30 min. The samples were then cooled in a water bath and centrifuged to remove solids. Concentrated HCl (100 µL) was added to the supernatant (10 mL), followed by an additional centrifugation step (10 min, 2500g) Samples were filtered through a 0.45 µm Acrodisc GHP membrane filter (Pall Corporation, Port Washington, NY, USA) before analysis by HPLC (Agilent 1100 with a diode array detector, HPLC-DAD; Agilent) on an Atlantis T-3 (2.1 ×150 mm, 3 µm) column (Waters Corp., Milford, MA, USA), followed by elution with a gradient of 50 mmol L^{-1} phosphate buffer and methanol at 0.2 mL min^{-1}. Detection of vicine (Sigma-Aldrich, St. Louis, MO, USA) and convicine was conducted at 280 nm, and for identification purposes, the spectrum from 190 nm to 450 nm was recorded. Quantification of convicine was achieved using the calibration curve for vicine.

The weather conditions in the years of research. According to the Uman meteorological station, hydrometeorological conditions in 2019 were characterized by slightly larger precipitation compared to long-term averages. The amount of precipitation for this period in 2020 was much higher than in 2019. Most of them fell in June, which allowed the plants to form better leaf mass The amount of added irrigation in 2020 is higher than in 2019 because the air temperature was higher and the evaporation of moisture was faster (Figure 1, Table 2).

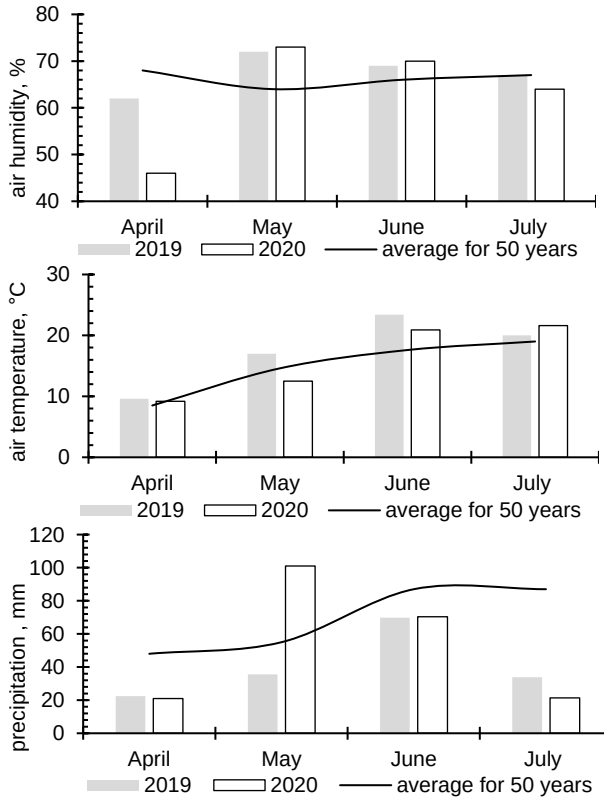

Figure 1. Climate chart for the study period (2019–2020)

Table 2. Moisture content indicators and the total irrigation rates throughout high water needs in faba beans from June to July and dynamics of stock of productive moisture (mm) during the growing season (data of the Meteorological Station "Uman")

Year	Drought level	Absolute precipitation, mm	Evapotrans-piration ratio, mm	Total irrigation rate, mm
Average (1970–2020)	wet	174	81.0	–
2019 (June–July)	mild dry	103.6	92.6	120
2020 (June–July)	mild dry	91.8	97.7	140

Dynamics of stock of productive moisture (mm) in the root zone (in the soil layer 0–30 cm)

Year	Month			
	April	May	June	July
2019	32.40	40.05	19.70	11.50
2020	30.0	71.50	25.50	10.0

The ratio of precipitation to potential evapotranspiration (ET), calculated based on Grabarczyk's (1992) formula:

$$ET = (d + \frac{1}{3} t), \quad (2)$$

where d – mean vapour pressure deficit in hPa; t – mean air temperature in °C.

Irrigation water requirements

The amount of irrigation water for green beans was applied by flowmeter after it was calculated according to the following equation:

$$IW = \left[\frac{ETo \times Kc \times Kr \times I}{Ea} + LR\right] \times 4.2, \quad (3)$$

where IW – irrigation water applied m^3; ET_O – reference evapotranspiration (mm day^{-1}); Kc – crop coefficient; Kr – reduction factor (Keller, Karmeli, 1975); I – irrigation interval, day; Ea = irrigation efficiency, 80%; LR – leaching requirement – 20% of the total water amount delivered to the treatment.

Statistical analysis. Statistical processing of the obtained results was performed with the calculation of the arithmetic mean (x) standard deviation (SD), calculated using Microsoft Excel 2016 and Statistica 10. The obtained data were compared using analysis of variance.

Results

The results of the biometric analysis showed that the cultivation of faba beans under irrigation contributes to an increase in plant height by 4.7–12.2% relative to the options without irrigation. When growing beans under irrigation, the difference increased to 14.8 cm; 13.0 cm and 19.2 cm, in accordance. On average, in two years the number of branches per plant per cultivation under irrigation increased by 17.3–30.0 %, or 0.7–1.0 $plant^{-1}$. The leaf area of faba bean crops increased by the drip irrigation by 21.2–24.9%.

The 'Ukrainski Slobidski' and 'Biloruski' formed a leaf area smaller than the control by 1.0 and 1.7 thousand m^2 ha^{-1} without irrigation (Table 3).

The concentration of total chlorophyll was the highest in the 'Ukrainski Slobidski', which was 8.1% higher than the standard cultivar 'Karadag' without irrigation and 12.9% on irrigation. Drip irrigation on average contributed to an increase in the concentration of total chlorophyll by 16.9–40.5%, in the 'Ukrainski Slobidski' and 'Windzorski' more significantly increases the concentration of chlorophyll *b* (Table 4).

The results of studies of the activity of antioxidant enzymes show that their activity during the cultivation of beans under irrigation is significantly reduced in all cultivars relative to similar options when grown without irrigation Catalase activity decreased depending on the cultivar by 10.6–22.5%, Guaiacol peroxidase – 19.4–25.9%, Superoxide dismutase – 19.3–24.4%. The decrease in the activity of antioxidant enzymes indicates the drought resistance of this culture and the significance of this decrease in the level of drought resistance of the cultivar (Table 5).

Table 3. Effect of irrigation and cultivars on plant growth and leaf area of the faba beans (2019–2020) (x ± SD)

Growing conditions	Cultivar	Plant height, cm	Number of branches	Leaf area, thousand, m^2 ha^{-1}
Without irrigation (natural conditions)*	'Karadag' st	63.46 ± 2.12	3.10 ± 0.14	27.26 ± 1.47
	'Ukrainski Slobidski'	75.20 ± 2.47*	3.20 ± 0.14	26.29 ± 1.75
	'Biloruski'	73.45 ± 2.12*	3.50 ± 0.00*	25.58 ± 1.98
	'Windzorski'	76.30 ± 2.33*	4.05 ± 0.07*	27.53 ± 1.48
Surface drip irrigation (soil moisture 80%)	'Karadag' st	66.46 ± 2.12*	3.85 ± 0.21*	33.38 ± 1.55*
	'Ukrainski Slobidski'	81.25 ± 5.23*	3.90 ± 0.14*	31.88 ± 0.71*
	'Biloruski'	79.45 ± 6.36*	4.55 ± 0.07*	31.43 ± 0.64*
	'Windzorski'	85.65 ± 4.66*	4.75 ± 0.35*	34.38 ± 1.70*
LSD_{05}	A	1.29	0.07	0.50
	B	1.92	0.10	0.79
	AB	2.71	0.17	1.31
CV%		9.8	15.3	11.5

st – standard; values are means (n = 2) ± standard deviation; * shows significance at the P ≤ 0.05 probability levels.

Table 4. Effect of irrigation and cultivars on leaf's chlorophyll content of the faba bean (2019–2020) (x ± SD)

Growing conditions	Cultivar	Chlorophyll a, μg g^{-1} FW	Chlorophyll b, μg g^{-1} FW	Total chlorophyll, μg g^{-1} FW
Without irrigation (natural conditions)*	'Karadag' st	8.16 ± 1.18	4.67 ± 0.18	12.83 ± 1.35
	'Ukrainski Slobidski'	9.31 ± 0.39*	4.55 ± 0.06	13.86 ± 0.46*
	'Biloruski'	7.27 ± 0.60	4.11 ± 0.13	11.38 ± 0.47
	'Windzorski'	8.77 ± 0.46*	4.61 ± 0.01	13.37 ± 0.44
Surface drip irrigation (soil moisture 80%)	'Karadag' st	10.04 ± 1.20*	5.46 ± 0.20	15.50 ± 1.40*
	'Ukrainski Slobidski'	11.23 ± 1.64*	6.26 ± 0.05*	17.49 ± 1.69*
	'Biloruski'	10.47 ± 1.45*	5.51 ± 0.26	15.98 ± 1.71*
	'Windzorski'	10.15 ± 1.35*	5.49 ± 0.18	15.64 ± 1.53*
LSD_{01}	A	0.25	0.10	0.40
	B	0.39	0.16	0.62
	AB	0.56	0.88	0.88
CV%		13.9	13.9	13.7

st – standard; values are means (n = 2) ± standard deviation; * show significance at the P ≤ 0.01 probability levels.

Table 5. Effect of irrigation and cultivars on leaf's antioxidant enzyme activity of the faba bean (2019–2020) (x ± SD)

Growing conditions	Cultivar	Catalase, U mg^{-1} protein	Guaiacol peroxidase, U mg^{-1} protein	Superoxide dismutase, U mg^{-1} protein
Without irrigation (natural conditions)*	'Karadag' st	186.8 ± 16.0	18.3 ± 1.8	18.0 ± 3.0
	'Ukrainski Slobidski'	198.3 ± 18.5*	22.5 ± 2.1*	21.3 ± 2.6*
	'Biloruski'	175.3 ± 19.5	14.4 ± 3.1	15.0 ± 3.0
	'Windzorski'	216.3 ± 25.5*	24.4 ± 1.8*	24.2 ± 3.8*
Surface drip irrigation (soil moisture 80%)	'Karadag' st	144.9 ± 19.0	14.1 ± 2.0	14.0 ± 3.0
	'Ukrainski Slobidski'	177.4 ± 13.5*	16.7 ± 1.3*	16.1 ± 3.9*
	'Biloruski'	139.4 ± 30.5	11.3 ± 2.7	12.1 ± 3.4
	'Windzorski'	192.9 ± 15.0*	19.6 ± 2.4*	18.5 ± 3.5*
LSD_{01}	A	3.54	0.39	0.36
	B	5.60	0.62	0.57
	AB	7.91	0.88	0.81
CV%		17.8	26.7	28.6

st – standard; values are means (n = 2) ± standard deviation; * show significance at the P ≤ 0.01 probability levels.

The number of beans (pods) increased on average in two years on the cultivars with drip irrigation by 47.8% in the 'Biloruski', by 50% – in the 'Karadag' and 'Ukrainski Slobidski', in the 'Windzorski' – by 62.5% compared to the option without irrigation, which was significant. The inter-varietal difference was significant in all variants. Thus, for growing without irrigation, the number of beans in the 'Ukrainski Slobidski' and 'Windzorski' was less by 1 pcs $plant^{-1}$ relative to the standard. In the 'Biloruski', this figure was lower than

the standard by 1.5 pcs. Under the conditions of drip irrigation, the difference between the options increased. Thus, in the 'Ukrainski Slobidski' noted a smaller number of beans compared to the standard by 1.5 pcs plant^{-1}, in the 'Biloruski' – a decrease against the standard cultivar 'Karadag' by 2.5 pcs, the 'Windzorski' formed the 2.5 number (Table 6).

Weight and yield of green beans. Drip irrigation contributed to a significant increase in the mass of green beans on the plant by 35.9–41.9 g.

Crop yield is the most important indicator of the effectiveness of cultivation technology. Under drip irrigation, the commodity yield increased by 3.5–4.2 t ha^{-1}, or 31.3–39.2%.

The results show that the most accumulating protein in the 'Ukrainski Slobidski' (12.3% without irrigation; 10.9% under irrigation) and 'Windzorski' (13.4% without irrigation; 11.8% under irrigation). The protein content under drip irrigation decreased by 1.4–1.5% relative to similar variants without irrigation. As the water intake causes hydrolysis and catabolism of proteins, releasing free amino acids and ammonia, as well as proline.

Growing beans on drip irrigation significantly reduced the dry matter content by 1.2–2.0%. The 'Biloruski' was characterized by a lower dry matter content compared to the 'Karadag' – 0.8 without irrigation and 0.3% higher on irrigation. The 'Ukrainski Slobidski' had a higher dry matter content by 0.3% and 0.5%, in accordance, according to the method of cultivation. The 'Windzorski' was dominated by the 'Karadag' by 0.7% and 1.5%, in accordance, according to the method of cultivation (Table 7).

Table 6. Effect of irrigation and cultivars on the number of pods and seeds pod^{-1} of faba bean (2019–2020) (x ± SD)

Growing conditions	Cultivar		Pods per plant	Seeds per pods
Without irrigation (natural conditions)*	'Karadag' st		13.02 ± 1.41	2.30 ± 0.14
	'Ukrainski Slobidski'		12.05 ± 0.50	2.45 ± 0.21*
	'Biloruski'		11.50 ± 0.71	2.50 ± 0.0*
	'Windzorski'		12.03 ± 0.45	2.30 ± 0.14
Surface drip irrigation (soil moisture 80%)	'Karadag' st		19.50 ± 0.74*	2.80 ± 0.28*
	'Ukrainski slobidski'		18.04 ± 0.55*	3.00 ± 0.0*
	'Biloruski'		17.00 ± 1.46*	3.85 ± 0.21*
	'Windzorski'		19.50 ± 0.87*	3.75 ± 0.35*
		A	0.22	0.06
	LSD_{05}	B	0.35	0.09
		AB	0.50	0.12
		CV%	23.0	21.7

st – standard; values are means (n = 2) ± standard deviation; * show significance at the P≤ 0.05 probability levels.

Table 7. Effect of irrigation and cultivars on weight and yield of green beans, protein and dry matter content in green beans (2019–2020) (x ± SD)

Growing conditions	Cultivar	Weight of green beans, g plant^{-1}	Yield, t ha^{-1}	Protein content %	Dry matter content %
Without irrigation (natural conditions)*	'Karadag' st	92.0 ± 10.00	11.0 ± 1.20	11.6 ± 0.85	13.0 ± 1.17
	'Ukrainski Slobidski'	93.6 ± 9.13	11.2 ± 1.10	12.3 ± 0.71*	13.3 ± 1.13
	'Biloruski'	90.1 ± 5.86	10.8 ± 0.70	10.9 ± 0.92	12.6 ± 0.70
	'Windzorski'	95.4 ± 6.33	11.5 ± 0.76*	13.4 ± 0.23*	13.7 ± 0.44*
Surface drip irrigation (soil moisture 80%)	'Karadag' st	127.9 ± 10.14	14.6 ± 1.16	10.2 ± 0.28	11.0 ± 0.42
	'Ukrainski slobidski'	135.1 ± 11.56*	15.4 ± 1.32*	10.9 ± 0.14*	11.5 ± 0.42*
	'Biloruski'	132.0 ± 12.78*	15.0 ± 1.46	9.4 ± 0.28	11.3 ± 0.42
	'Windzorski'	131.9 ± 7.91*	15.0 ± 0.90	11.8 ± 0.49*	12.5 ± 0.69*
	A	1.80	0.20	0.19	0.16
	LSD_{05} B	2.85	0.33	0.30	0.25
	AB	4.02	0.46	0.42	0.36
	CV%	1.80	16.0	16.5	7.7

st – standard; values are means (n = 2) ± standard deviation; * show significance at the P≤ 0.05 probability levels.

The results of studies have shown that the content of vicin and convicin is significantly reduced under irrigation. The concentration of vicin decreases by 28.6–39.1%, the concentration of convicin – 25.2–46.4% relative to the options without irrigation (Table 8). Mayer *et al*. (2021) report the dependence of these indicators on the genotype.

Studies of the formation of the nodulation apparatus showed that the cultivation of beans under irrigation contributed to a significant increase in the mass of nitrogen-fixing nodules (rhizobia) from 34.2% in the 'Ukrainski Slobidski' to 114.9% in the 'Biloruski'. At the same time, the variability of these traits (CV) was significant (41.6% by weight and 48.8% by the number of nodules), which indicates that the bean-rhizobial system is the most sensitive component of the phytocenosis (Table 9).

For cultivation without irrigation, by weight of tubers, the standard prevailed most significantly to the 'Ukrainski Slobidski' (+34.8%), the difference in the 'Windzorski' (+19.1%) was slightly lower. The 'Biloruski' formed 66.7% fewer rhizobia than the 'Karadag'. The same trend was maintained for growing under irrigation, but the difference between the options was reduced. Thus, the 'Ukrainski Slobidski' and the 'Windzorski' formed rhizobia larger by weight by 21.4% and 13.3% compared to the standard cultivar 'Karadag', the 'Biloruski' had 51.9% less by weight

rhizobia. Drip irrigation contributed to a significant increase in the number of rhizobia on the plant. Thus, the 'Karadag' increased their number relative to the option without irrigation by 5.7%, the 'Ukrainski Slobidski' by 16.4%, the 'Biloruski' by 16.3%, and the 'Windzorski' by 46.7%.

When grown without irrigation, the 'Ukrainski Slobidski' and 'Biloruski' formed more by 28.5 and 8.7 plant^{-1} nodules relative to the 'Karadag' and the cultivar of Windzorski by 13.0% less. During cultivation under irrigation, the 'Ukrainski Slobidski' and 'Biloruski' formed more by 36.0 and 13.0 plant^{-1}. nodules relative to the 'Karadag', and the 'Windzorski' by 8.2%. That is, the reaction of plants of the 'Karadag' was more positive for the growth of rhizobia, which helped to reduce the difference between the options.

Table 8. Effect of irrigation and cultivars on concentracion of antinutrient composition in green beans (2019–2020) (x ± SD)

Growing conditions	Cultivar		Vicine, ug g^{-1}	Convicine, ug g^{-1}
Without irrigation (natural conditions)*	'Karadag' st		5233 ± 8	1422 ± 58
	'Ukrainski Slobidski'		6705 ± 67*	2127 ± 59*
	'Biloruski'		4480 ± 244	971 ± 26
	'Windzorski'		7012 ± 155*	2705 ± 59*
Surface drip irrigation (soil moisture 80%)	'Karadag' st		3361 ± 112	1064 ± 13
	'Ukrainski slobidski'		4086 ± 58*	1207 ± 33*
	'Biloruski'		3105 ± 100	520 ± 8
	'Windzorski'		5008 ± 135*	1588 ± 2*
		A	113.34	22.90
	LSD_{01}	B	179.20	36.21
		AB	253.42	51.21
		CV%	29.3	47.7

st – standard; values are means (n = 2) ± standard deviation; * show significance at the P≤ 0.01 probability levels

Table 9. Effect of irrigation and cultivars on development of the nodulation apparatus of faba beans (2019–2020) (x ± SD)

Growing conditions	Cultivar		Mass of nodules, g plant^{-1}	Number of nodules plant^{-1}
Without irrigation (natural conditions)*	'Karadag' st		0.70 ±0.08	26.5 ±2.12
	'Ukrainski Slobidski'		0.95 ± 0.14*	55.0 ± 2.83*
	'Biloruski'		0.23 ± 0.05	35.2 ± 1.06*
	'Windzorski'		0.84 ± 0.08*	13.5 ± 0.71
Surface drip irrigation (soil moisture 80%)	'Karadag' st		1.05 ± 0.07	28.0 ± 1.4
	'Ukrainski slobidski'		1.27 ± 0.18*	64.0 ± 5.65*
	'Biloruski'		0.50 ± 0.13	41.0 ± 1.41*
	'Windzorski'		1.19 ± 0.16*	19.8 ± 1.70
		A	0.011	0.74
	LSD_{01}	B	0.018	1.17
		AB	0.026	1.66
		CV%	41.6	48.8

st – standard; values are means (n = 2) ± standard deviation; * show significance at the P≤ 0.01 probability levels

Discussion

The importance of the obtained results lies in the comprehensive disclosure of physiological processes, processes of growth and development of plants, crop formation and the relationship between them.

The results are shown in Table 3, Similar results are reported by Al-Suhaibani (2009), bean plants with a higher level of moisture formed more leaves on the plant and, accordingly, a larger area of leaves of the plant. Plants grown in conditions of water deficiency had a smaller leaf area (Alderfasi, Alghamdi, 2010)

The results are shown in Tables 4 and 5 intertwined with the results Erdem *et al*. (2006). Reporting that optimal soil moisture leads to improved various physiological processes, better nutrient uptake, higher rates of photosynthesis, which can affect more leaf area and area and higher yields. Similar results were reported by Abd El-Mawgoud (2006), Siddiqui *et al*. (2015). A correlation analysis showed a strong relationship between the leaf index and yield (r = 0.93; P = 0.0009; R^2 = 0.86). Moreover, there was a good correlation between chlorophyll content and yield (r = 0.96, P = 0.002, R^2 = 0.92) (data not shown).

The results are shown in Table 6, similar to results were obtained by Ashenafi and Mekuria (2015), which reported a significant inter-varietal difference in the number of beans per plant. Accordingly, Mulualem *et al*. (2012), Awol *et al*. (2016), Cerqueira *et al*. (2018) and Souana *et al*. (2020) indicated that the number of beans on a plant-primarily depends on the cultivar. Detected close correlation between the number of grains in the pod and yield (r = 0.83; P = 0.0115; R^2 = 0.68) (data not shown). Therefore, when selecting cultivars or their selection, preference should be given to cultivars with an increased number of grains.

The present study concluded that 80% of ET was quite enough to achieve maximum productivity for green faba beans. The results of our experiment are similar to those previously reported by El-Noemani *et al*. (2010), Liu *et al*. (2021). They noted that increasing the irrigation amount up to 100% of ET prompted the highest growth, although the maximum pod yield was achieved by 80% of ET. Under stress conditions, water deficit had a significant impact on plant growth, leading to a decline in growth, leaf area development, and photosynthetic capacity (Bayuelo-Jimenez *et al*.,

2003). Previous studies revealed that the reduction in bean productivity (number of pods per plant and seed biomass) due to heat stress was associated with reduced leaf water content (Amer *et al*., 2012; Attila *et al*., 2017).

The lowest protein content was obtained by irrigation. These findings may be because protein is considered a good indicator of plant resistance to water deficiency, as the water intake causes hydrolysis and catabolism of proteins, releasing free amino acids and ammonia, as well as proline (Fayed *et al*., 2018; Mayer *et al*., 2021). There are also close correlations between protein content and antinutrient components: protein-vicine – $r = 0.96$; $P = 0.0001$; $R^2 = 0.91$; protein-convicine – $r = 0.96$; $P = 0.0001$; $R^2 = 0.93$ (data not shown). There were also no studies on the influence of elements of technology, particular drip irrigation on the concentration of antinutrient components, there was only one study of varietal characteristics of the accumulation of such substances (Khazaei *et al.*, 2019). Our research has established a positive effect of this factor.

Moreover, from an agronomical point of view, including faba bean in crop rotation systems improves soil, since this crop can fix atmospheric N2 to amounts that may exceed 200 kg N ha^{-1}, and increases soil organic matter. El Idrissi *et al*. (2020), claim that small rhizobia may not accumulate nitrogen at all. In our studies, the mass and number of nodules increased significantly on drip irrigation, which increased the accumulation of biological nitrogen.

Conclusion

Conclusively, at similar experimental conditions, it could be concluded that the irrigation of faba bean plants was effective the proper for enhancing yield and improved green pod quality.

Studies have shown a positive effect of irrigation on reducing the concentration of antinutrient compositions. Are identified genotypes with relatively high accumulation of antinutrient compositions ('Karadag' and 'Biloruski') that need to be included in the selection process. Selection and irrigation will allow the widespread use of faba beans as a valuable high-protein source.

The presented results, based on the data of a field experiment with surface drip irrigation of beans grown in central Ukraine, showed that the yield of green beans due to irrigation increased on average for all cultivars by 34.9%.

Growing beans on drip irrigation contributed to a significant increase in the number of nitrogen-fixing nodules on the plant by 34.2–114.9% and their weight by 5.7–46.7%, which increased the concentration of biological nitrogen in the soil. Growing beans on drip irrigation contribute to a significant improvement in the formation of the bean-rhizobial system, which has a positive effect on the concentration of biological nitrogen in the soil.

Our methodology can be used for assessing the response of different genotypes of faba bean to soil water deficit. The identified tolerant cultivars can be utilized as a source for water stress tolerance in faba bean breeding.

The results presented in this paper are of great importance because they can be used to model the economic consequences as well as to plan the development of irrigation systems in a given area.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author contributions

OU, SP, AY, VY – study conception and design;
VL, VY, OL, VK – acquisition, analysis and interpretation of data;
OU, SP, VL, AY, VY – revision and approval of the final manuscript.

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IN VITRO SHOOT GROWTH PERFORMANCES AND RESPONSES OF POTATO (*Solanum tuberosum* L.) 'MUHZOTO' UNDER DIFFERENT TREATMENTS AND EXPLANT TYPES

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ABSTRACT. Finding optimal shoot growth performances under different treatments and revealing different growth responses of different explant types as main objectives were assessed in the research. Different treatments of 5 000; 8 000 and 11 000 lx in light intensities; 0, 25, 50, 75 and 100 ml l^{-1} in coconut water (CW) concentrations; culture media (CM) of Murashige and Skoog (MS) medium supplemented with 1.5 vitamin strength in the medium in combination with 100 mg l^{-1} myoinositol, 1 mg l^{-1} calcium pantothenate (CaP), and 0.1 mg l^{-1} gibberellic acid-3 (GA_3) (CM-1); 200 mg l^{-1} myoinositol, 1 mg l^{-1} CaP dan 0.1 mg l^{-1} GA3 (CM-2); 1 mg l^{-1} CaP and 100 ml l^{-1} CW (CM-3); and MS medium supplemented with 1.5 total vitamin strength in the medium (CM-4 as control) and shoot tip, first, second, third, fourth and fifth nodes as explant types were gradually tested in the research. Virus-free *Solanum tuberosum* L. 'Muhzoto' explants and MS medium containing 1.5 strength of vitamin were used as explant source and basic medium. Four experiments were arranged in a completely randomized design (CRD) with 6–9 replications. Maximal shoot growth performances indicated by shoot height, stem diameter, internode length, greener leaves per shoot, leaf length and width were established in explants incubated under 11 000 lx light intensity applied continuously. Adding different concentrations of CW could not improve the growth of shoots, but they induced high contamination. Though MS medium containing 1.5 vitamin strength with 200 mg l^{-1} myoinositol, 1 mg l^{-1} CaP and 0.1 mg l^{-1} GA_3 slightly improved shoot growth, there was no significant difference compared to control. Exploring shoot growth responses derived from different types of explants revealed that the shoot tips, 1st and 2nd nodes regenerated high branched shoots with the higher length of internodus; while 3rd, 4th and 5th nodes stimulated low branched shoots with higher stem diameter and the number of leaves per shoot. The branched shoots were a serious problem in preparing high-quality regenerants for 'Muhzoto' explants and significantly overcome by choosing, selecting and applying the right time on subculturing of the 'Muhzoto' explants.

Introduction

Potato (*Solanum tuberosum L.*) is one of the most important dicotyledonous tuber crops worldwide and the position is in ranking fourth in food crop after wheat, rice and maize (Husain *et al.*, 2017). In 2017, total production of potato reached 388 191 000 tons; 19 302 600 ha total harvested area; China, India and Russian Federation as the most important producing countries with an annual production of 99 205 600; 48 605 000 and 29 590 000 tons, respectively (FAOSTAT, 2019; Potatopro, 2019; Thedailyrecords,

2019). In Indonesia, the potato is also the most important vegetable commodity in the third position after hot and chilli pepper (Badan Pusat Statistik, 2019a,b). In 2019, total cultivated areas were 68 683 ha and East Java, Central Java and West Java as the main important producing provinces with an annual production of 1 284 760 tons and productivity of 18.7 tons per ha (Badan Pusat Statistik, 2019a,b,c). The fresh potato products in tuber form are sold from 12 000.00 to 20 000.00 IDR (0.69–1.15 EUR) per kg depending on their types and qualities (Pdppjkotabogor, 2020; Infopangan, 2020; Priangan, 2020; Siskaperbapo, 2020). Though the potato has high economical values, the development and production of high-quality tuber in Indonesia are constrained by the availability of good and qualified planting materials for sustainable production.

Conventionally potato can be propagated generatively using true botanical seeds and vegetatively using tubers, segments of tubers (Hidayat, 2011; Ebad *et al.*, 2015; Mehmood *et al.*, 2016; Husain *et al.*, 2017; Muñoz *et al.*, 2019) and mini-cuttings (Karjadi, 2017; Kasutjianingati *et al.*, 2018). Because potato is highly heterozygous and easily segregates on sexual reproduction, application of the true botanical seeds for commercial purposes is rarely utilized (Ebad *et al.*, 2015). Furthermore, utilizing tuber is the most common asexual propagating technique applied by potato farmers and growers involving in Indonesia. Though it may be frequently attacked by different pathogens and this method is also time-consuming and laborious, the method is the most frequently applied by Indonesian farmers. While mini-cuttings derived from terminal shoots and the single node was now generally used by Indonesian farmers to get planting materials in large quantities with lower prices than tubers (Karjadi, 2017; Kasutjianingati *et al.*, 2018). Furthermore, to reduce immersing low-quality tubers and cuttings due to gradual utilizing them continually, preparing good quality planting materials derived from *in vitro* plantlets significantly addressed.

Preparing good quality planting material for *in vivo* stage was generally initiated by producing high quality *in vitro* materials. Several *in vitro* propagation studies on the potato to establish the purpose were previously reported. The highest number of shoots/explant was obtained for 'Almera' explants cultured on MS medium supplemented with 3.0 mg l^{-1} thidiazuron (TDZ) in combination with 0.1 mg l^{-1} naphthalene acetic acid (NAA) (Khadiga *et al.*, 2009). In the 'Esprit' and 'Meridian' potato variety, maximum numbers of healthy shoots derived from shoot apex and nodal explants with well-expanded leaves per explants were produced on MS medium with 1.0 mg l^{-1} benzylaminopurine (BAP) + 1.0 mg l^{-1} GA3 (Bhuiyan, 2013). Multi shots and roots from nodal explants were established on MS medium supplemented with IBA 1.0 mg l^{-1} + NAA 1.0 mg l^{-1} + kinetin 2.0 mg l^{-1} and 2.0 mg l^{-1} IBA + 2.0 mg l^{-1} kinetin + 2.0 mg l^{-1} NAA + 1.0 mg l^{-1} 2,4-D (Gami *et al.*, 2013). MS medium containing 4.19 μM D-calcium pantothenate, 0.05 μM NAA, 0.29 μM GA_3, 30 g l^{-1} sulphur less sugar and 2 g l^{-1} gelrite stimulated a high number of leaves per shoot, internodal length, number of roots, root length, fresh and dry weight of varied tetraploid potato varieties (Venkatasalam *et al.*, 2013). MS medium with vitamins and solidified by agar without exogenous plant growth regulators and nodal cuttings were successfully produced high shootlet length, number of leaves per shootlet, number of vigorous roots and fresh mass of 'Lady Rosetta' variety (Ebad *et al.*, 2015). Maximum shoot regeneration derived from nodal explants of 'Diamont', '1533' and 'Kufri Badshah' potato varieties were determined on MS medium supplemented with 1 mg l^{-1} BAP (Kaur *et al.*, 2015). MS medium containing 0.12 mg l^{-1} of GA3 and nodal explant for 'Cardinal' variety produced maximum plant height, the higher number of nodes, reduced number of days to root initiation and took less number of days to the transferable height of the plant (Mehmood *et al.*, 2016). From those study they were informed that optimal shoot growth performances were generally established by optimation of MS medium in combination with applying concentration and combination of plant growth regulator, different varieties, solidifying agents, water; however studying and finding optimal shoot growth performances derived from different light intensities, coconut water concentrations, varied-MS media and paying more attention to growth response of different types of explant in *in vitro* culture of Indonesian potato variety, *i.e. S. tuberosum* 'Muhzoto' were not published yet.

The research was aimed to study the effect of different light intensities, coconut water concentrations, and modified-MS media on shoot growth performances of *S. tuberosum* 'Muhzoto' explants and to explore growth responses of different explant types of the variety. From the study, it was expected that optimal light intensity, CW concentration and modified-MS medium for maximal shoot growth performances and different responses of different explant types of *S. tuberosum* 'Muhzoto' were successfully established and explored. Important and unique findings of the research expected could give benefits to others.

Material and methods

Materials and explant preparation

Materials used in the study were plantlets of *S. tuberosum* 'Muhzoto' variety derived from meristem culture and virus free produced by Kalimandi Main Institute for Horticulture Seeds (KMIHS), Banjarnegara District, Central Java province, Indonesia. The plantlets were cultured in jam bottles (7 × 11.6 cm; diameter and height of bottle) containing full strength MS medium hormone-free. The plantlets generally had 5.0 cm in height with 5–6 six leaves after 30 days of culture. Each bottle contained 8–10 plantlets. The cultures were incubated on culture racks under 16 h photoperiod light incubation of cool fluorescent lamps with 5 000 lx and 24 ± 1°C just for ± a week before used as explant sources for the experiments.

Explants used in the study were shoot tip and nodus derived from KMIHS plantlets as explant source.

Explants were prepared by slicing shoot tip with a young leaf, first, second, third, fourth and fifth nodes. Explants were then cultured on a medium or different media based on treatments studied. All experiments used Murashige and Skoog (MS, 1962) medium containing 1.5 vitamin strength as a basic medium.

Shoot growth performances of 'Muhzoto' explants under different light intensities

Explants used in the study were shoot tips and nodes as described previously. While light intensities tested in the study were (1) 5 000 lx derived from 24-watt Tornado Phillip. The lamps were set in 90 cm distance from one lamp to another with 40 cm height position of lamps to rack surface; (2) 8 000 lx derived from 12-watt LED Phillip. The lamps were set in 50 cm distance from one lamp to another with 40 cm height position of lamps to rack surface; and (3) 11 000 lx derived from 19-watt LED Phillip with a similar setting as the previous treatment (no. 2). The experiment was arranged in a completely randomized design (CRD) with 9 replications. Each treatment consisted of 3 bottles. Each bottle contained 10 explants. The continuous light incubation applied in the experiment was due to the incubation as optimal condition established derived from preliminary studies and also generally applied in Indonesian potato tissue culture laboratories.

Shoot growth performances of 'Muhzoto' explants under different concentration of coconut water (CW)

Explants used in the study were shoot tips and nodes. While CW concentrations examined in the study were (1) 0, (2) 25, (3) 50, (4) 75 and (5) 100 ml l^{-1}. Application of different concentration of CW in the media was only carried out by sterilization and no filtration applied for CW. The experiment was arranged in CRD with 5 replications. Each treatment consisted of 3 bottles. Each bottle contained 10 explants. All cultures of explants were incubated in 11 000 lx light intensity derived from 19-watt LED Phillip under continuous incubation as an optimal treatment to support the optimal growth response of shoots.

Shoot growth performances of 'Muhzoto' explants under different culture media

Explants used in the study were shoot tips and nodes. Culture media (CM) of MS medium supplemented with 1.5 vitamin strength in the medium in combination with 100 mg l^{-1} myoinositol, 1 mg l^{-1} CaP, and 0.1 mg l^{-1} GA_3 (CM-1); 200 mg l^{-1} myoinositol, 1 mg l^{-1} CaP dan 0.1 mg l^{-1} GA_3 (CM-2); 1 mg l^{-1} CaP and 100 ml l^{-1} CW (CM-3); and MS medium supplemented with 1.5 total vitamin strength in the medium (CM-4 as control). The experiment was arranged in CRD with 6 replications. Each treatment consisted of 3 bottles. Each bottle contained 10 explants. All cultures of explants were incubated as described in the previous experiment.

Shoot growth response study of 'Muhzoto' explants

Shoot growth response was studied by clustering explants in each group separately. That was mean that the shoot tip was clustered with the shoot tip, the first node with the first node till the fifth node. The different clusters of explants were then used as a treatment in the study *i.e.* (1) shoot tips, (2) 1st nodes, (3) 2nd nodes, (4) 3rd nodes, (5) 4th nodes and (6) 5th nodes. The experiment was arranged in CRD with 4 replications. Each treatment consisted of 3 bottles. Each bottle contained 10 explants. All cultures of explants were incubated as described in the previous experiment.

Variables of experiments

Variables observed in all experiments carried out were (1) height of shoot (cm), (2) stem diameter (mm), (3) internodus length (cm), (4) number of leaves per shoot, (5) leaf length (mm), (6) leaf width (mm), (7) percentage of explant contamination (%), calculated by counting the number of contaminated explants divided by total explant cultured time by 100%, (8) the percentage of air roots (%), calculated by counting the number of nodes with air roots divided by total nodes time by 100% (9) number of air roots per shoot, (10) air root length (cm), (11) leaf length-width ratio, (12) percentage of branched-shoots (%), calculated by counting the number of branched-shoots divided by total shoots cultured time by 100%. The periodical observation was carried out to know the response of explant growth during each experiment conducted. All variables were observed and measured ± 30 days after culture initiation.

Statistical analysis

Data regenerated from all variables observed in each experiment were analyzed by analysis of variance (Anova) using SAS 9.1 program (SAS Institute, Cary, NC). Significant differences between means were assessed by Tukey test, P = 0.05.

Results and discussion

Shoot growth performances of 'Muhzoto' explants under different light intensities

Results of the study reveal that axillary shoots derived from nodus explants were observed 2–3 days after culture. The axillar shoots grew continually to produce new leaf primordia 4–5 days after culture. In further growth, the shoots had the height of shoots from 5.0–7.2 cm with 0.75–1.35 mm in diameter; 0.2–1.4 cm in length of internodus; 5–11 leaves per shoot; 1.0–5.0 mm leaf length and 0.5–3.1 mm leaf width (Fig. 1A). Best performances of shoot growth were exhibited on potato explants incubated under high light intensity.

Three different light intensities tested in the first experiment resulted in different responses and growth performances of potato explants. It was revealed that higher light intensity induced better growth of shoots. The potato explants cultured under 11 000 lx continually induced the best growth performances of plantlets with 6.6 cm in shoot height, 1.25 mm in stem diameter, 0.71 cm in internodus length, 9.4 number of leaves per shoot, 4.4 mm in leaf length and 2.9 mm in leaf width (Table 1; Fig. 1A). The second best treatment was determined on the potato explants incubated under 8 000 lx light intensity. While low light intensity generally stimulated non-optimal shoot growth of explants with thinner stem diameters, pale to light green performances and small leaves.

Table 1. Growth performances of 'Muhzoto' explants cultured under different light intensities

Light intensity, lx	Height of shoots, cm	Stem diameter, mm	Internodus length, cm	Number of leaves per shoot	Length of leaves, mm	Width of leaves, mm
5 000	5.7[a]	0.84[a]	0.63[a]	6.7[a]	1.1[a]	0.6[a]
8 000	6.4[ab]	1.13[b]	0.68[a]	7.9[a]	3.3[b]	2.0[b]
11 000	6.6[b]	1.25[c]	0.71[a]	9.4[b]	4.4[b]	2.9[b]
CV, %	4.90	2.99	19.27	6.45	15.80	19.01

Means followed by the same letter in the same column are not significantly different based on the Tukey test, P = 0.05.

Table 2. 'Muhzoto' shoot growth performances under different concentrations of coconut water added in MS medium containing 1.5 vitamin strength

Coconut water concentration, ml l^{-1}	Height of shoots, cm	Stem diameter, mm	Internodus length, cm	Number of leaves per shoot	Length of leaves, mm	Width of leaves, mm
0	6.3[ab]	1.04[a]	0.82[a]	7.6[a]	3.8[a]	6.0[c]
25	6.1[ab]	1.03[a]	0.72[a]	7.0[ab]	3.3[a]	59.8[b]
50	6.6[a]	1.18[a]	0.77[a]	7.2[ab]	4.2[a]	76.8[a]
75	6.3[ab]	1.12[a]	0.68[a]	7.1[ab]	3.8[a]	82.9[a]
100	5.7[b]	1.05[a]	0.79[a]	6.8[b]	3.7[a]	89.6[a]
CV, %	5.54	9.17	12.42	4.60	11.15	7.57

Means followed by the same letter in the same column are not significantly different based on the Tukey test, P = 0.05

Table 3. Shoot growth performances derived from 'Muhzoto' explants under different culture media

Culture medium, CM	Height of shoots, cm	Stem diameter, mm	Internodus length, cm	Percentage of airy roots per shoot, %	Average number of roots per nodus	Root length, cm	Number of leaves per shoot	Length-width leaf ratio	Percentage of shoots branched, %
CM-1	5.4[a]	0.85[ab]	0.62[a]	74.3[a]	1.5[a]	1.4[a]	6.7[a]	1.40[a]	56.7[a]
CM-2	5.3[a]	1.13[ab]	0.67[a]	61.5[a]	1.6[a]	1.0[a]	7.5[a]	1.42[a]	50.6[a]
CM-3	3.9[a]	0.71[b]	0.53[a]	54.5[a]	1.3[a]	1.1[a]	6.6[a]	1.40[a]	17.9[b]
CM-4	5.3[a]	1.23[a]	0.71[a]	62.7[a]	1.6[a]	1.3[a]	7.3[a]	1.43[a]	45.2[a]
CV, %	15.25	15.56	17.67	12.21	19.94	17.82	11.19	8.95	18.24

Notes: Culture media (CM) of MS medium supplemented with 1.5 vitamin strength in the medium in combination with 100 mg l^{-1} myoinositol, 1 mg l^{-1} calcium pantothenate (CaP), and 0.1 mg l^{-1} gibberellic acid-3 (GA3) (CM-1); 200 mg l^{-1} myoinositol, 1 mg l^{-1} CaP dan 0.1 mg l^{-1} GA3 (CM-2); 1 mg l^{-1} CaP and 100 ml l^{-1} CW (CM-3); and MS medium supplemented with 1.5 total vitamin strength in the medium (CM-4 as control). Means followed by the same letter in the same column are not significantly different based on the Tukey test, P = 0.05.

Shoot growth performances of 'Muhzoto' explants under different concentration of coconut water (CW)

Different concentrations of CW added in MS medium containing 1.5 total vitamin strength in the medium could not give a positive effect on shoot growth performances derived from 'Muhzoto' explants. Adding of different CW concentrations was due to causing high explant contamination. Higher concentration of CW added in the medium, higher explant contamination recorded (Table 2). Shoots derived from the CW treatments were generally having greener and strong stems, darker green leaves and easily grown white friable callus in the bottom position of nodes. Though all CW treatments still successfully induced shoots, all regenerated shoots were generally not suitable to be subcultured in producing qualified planting materials. Optimal shoot growth performances kept noted in 'Muhzoto' explants cultured on MS medium containing 1.5 vitamin strength (Fig. 1B). The treatment stimulated healthy shoots with 6.3 cm in shoot height; 1.04 mm in stem diameter; 0.82 cm in internodus length; 7.6 leaves per shoot; 3.8 mm in leaf length and 6% of contamination.

Shoot growth performances of 'Muhzoto' explants under different culture media

Different culture medium supplemented with adding myo-inositol, CaP, GA3 with 3 in subscript position, and CW in MS medium gave different results. Culture medium stimulating better shoot growth performances was recorded on CM-2 (MS medium containing 200 mg l^{-1} total myoinositol in the medium, 1 mg l^{-1} CaP and 0.1 mg l^{-1} GA_3). The medium induced greener shoots with 1.13 mm stem diameter and the high number of leaves per shoot up to 7.5 leaves (Fig. 1C). Similar results with better stem diameter up to 1.23 mm, longer internodus and a lower percentage of shoots branched of 45.2% were noted on 'Muhzoto' explants cultured on MS medium containing 1.5 vitamin strength as control (CM-4). The lowest results were determined on explants cultured on CM-3 (MS medium added by 100 mg l^{-1} myoinositol, 1 mg l^{-1} CaP, 100 ml l^{-1} CW and 0.5 vitamin strength. Though the treatment had the lowest percentage of shoots branched down to 17.9%, shoots regenerated from the medium generally had shorter and tinner shoots compared to others. Shoots derived from the treatment were also not suitable for producing good planting materials.

Shoot growth response study of 'Muhzoto' explants

Interesting phenomenons were successfully revealed in the fourth experiments when we tried to cluster different explants derived from regenerated 'Muhzoto' shoots and cultured them on a selected medium *i.e.* MS medium supplemented with 1.5 vitamin strength. Shoot tip explants produced shoots with high shoots, 0.82 cm internodus length and percentage of shoots branched up to 24.3% (Table 4; Fig 1D). First nodus explants

stimulated shorter shoots with the highest shoots branched as high as 95.3% (Fig. 1E), followed by the 2nd nodus explant with 71.1% branched shoots (Fig. 1F). Percentage of shoots branched declined on 3rd nodus explant (Fig. 1G) with the lowest percentage of shoots branched down to 6.2% and good quality of shoot performances, homogeneity, vigour and healthy noted on shoots derived from 4th nodes (Fig. 1H). While 5th nodus explants had different responses compared to others. The explants generally induced shoots with varied performances (Fig. 1I). From the experiments, it was revealed that there were different explant responses regenerated from different 'Muhzoto' explants and two different clusters of explants were categorized. The shoot tips, 1st and 2nd nodes generally induced higher results on length of internodus with a high percentage of branched shoots, while 3rd, 4th and 5th stimulated higher stem diameter and the number of leaves per shoot with a low percentage of shoot branched.

Table 4. Shoot growth behaviour of 'Muhzoto' explants cultured on MS medium supplemented with 1.5 vitamin strength

Explant type	Shoot height, cm	Diameter of stems, mm	Length of internodus, cm	Number of leaves per shoot	Percentage of shoots branched, %
Shoot tips	4.8[a]	0.81[c]	0.82[a]	5.1[b]	24.3[c]
1st nodes	3.4[c]	0.82[c]	0.61[a]b	5.0[b]	95.3[a]
2nd nodes	3.6[c]	1.04[b]	0.64[a]b	5.3[b]	71.1[b]
3rd nodes	3.7[c]	1.19[b]	0.46[b]	6.7[a]	9.2[d]
4th nodes	4.1[b]	1.49[a]	0.68[a]b	6.5[a]	6.2[d]
5th nodes	3.7[c]	1.05[b]	0.55[b]	6.6[a]	10.2[d]
CV, %	3.08	5.35	1329	4.25	4.23

Means followed by the same letter in the same column are not significantly different based on the Tukey test, P = 0.05

In a further study, subculturing 3rd and 4th nodes generally induced healthy and vigorous shoots with high homogeneity. Though the 5th nodes had lower results compared to the 3rd and 4th nodes, the nodes were still able to regenerate better shoot growth performances than the shoot tips, 1st, and 2nd nodes. While subculturing the branched shoots derived from the shoot tips, 1st and 2nd nodes continuously till two to four times generally resulted in hairy shoots with thin stems, high airy roots, small and undeveloped leaves (Fig. 1K). The low quality of regenerants usually occurred when the branched shoots did not handle properly during subculture, however, optimal handling of the shoots followed by selecting and applying the right time of shoot subculturing could still maintain the quality of regenerated shoots up to next twice to fourth subcultures (Fig. 1L). From the experiment, it was revealed that the branched shoots were important and significant problems in *in vitro* shoot proliferation of 'Muhzoto' explants vegetatively (Fig. 1J). Therefore, for the 'Muhzoto' variety, producing high quality *in vitro* planting materials could be carried out primarily by subculturing 3rd, 4th and 5th nodes or subculturing the shoot tips, 1st and 2nd nodes with optimal handling. The optimal handling carried out by choosing, selecting and applying the right time of subculture of the 3rd, 4th and 5th nodes were resulted in high quality in vitro planting materials and maintained maximal growth of branched shoots derived from shoot tips, 1st and 2nd nodes till next two to four subcultures.

Different shoot growth performances due to different treatments on 'Muhzoto' explants were successfully revealed from the study. In the experiment, higher light intensity, well and healthy shoots derived from 'Muhzoto' explants were established. High light intensity up to 11 000 lx applied continuously at 24 ± 2 °C stimulated greener and healthy shoots up to 6.6 cm in height, 1.3 mm in stem diameter, 0.71 cm in internodus length, 9.4 number of leaves per shoot, 4.4 mm in leaf length and 2.9 mm in leaf width. In other studies, maintaining culture in culture room at 25 ± 2 °C on 16 h illumination of daylight fluorescent tube lamps with 2 000 lx light intensity induced shoot formation up to 51.2% for 'Daraga' variety using medium protocol C (Al-Sulaiman, 2011). Incubating cultures in a growth chamber at 25 ± 2 °C under a photoperiod of 16/8 h with 3 335 lx using Philips white fluorescent lamps resulted in shoots with 9 cm in height and 9 leaves per shoot (Ebad *et al.*, 2015). Incubating cultures at 25 ± 2 °C under a photoperiod of 16/8 h with 2299 lx using Philips white fluorescent tubes stimulated shoot regeneration up to 96% on 'Diamant' variety (Kaur, 2015). Growth chamber at 25 °C providing 16 h photoperiod of 200 lx light intensity was significantly for producing shoots with 10.3 cm shoot height and 7.4 number of nodes per plant for 'Desiree' variety (Mehmood *et al.*, 2016). Growth chamber with 22-watt LED rod light (± 8 000 lx) in red successfully stimulated the growth of 'Cardinal' shoots with 7.5 cm shoot height, 8.6 number of leaves per shoot, 6.8 number of nodes per shoot and 3.8 roots per shoot (Karmakar *et al.*, 2018).

The utilization of CW to improve the growth of explants was successfully applied in *in vitro* culture of several plants published previously. Adding 10% of CW in half-strength MS medium gave a positive effect in improving the growth of *Dendrobium* plantlets compared to VW, KC and NP medium (Aktar *et al.*, 2008). The treatment induced the height of plantlets up to 1.04 cm with 2.12 leaves per plantlet and 0.31 cm length of leaves.

Maximum shoot length (7.2 ± 0.16), number of shoots (11.5 ± 1.5) and number of nodes (4.6 ± 0.22) were achieved on the MS medium containing 20% (v/v) coconut water with 2.0 mg l^{-1} of BAP for Kiwifruit (*Actinidia deliciosa*) (Nasib *et al.*, 2008). Modified Hyponex media (Hyponex 20N:20P: 20K and 6.5N: 4.5P:19K, 1 g l^{-1}) containing 30 ml l^{-1} CW successfully

induced better fresh dan dry weight of *Calanthe* hybrids 'Bukduseong' × 'Hyesung' plantlets with 9.1 cm shoot height, 1.2 cm leaf width, 7.2 cm number of roots per plantlet and 1.8 cm^2 leaf area; while the similar medium supplemented with 50 ml l^{-1} CW stimulated better growth of *Calanthe* hybrids 'Chunkwang' × 'Hyesung' plantlets (Baque *et al.*, 2011). The best medium for growing and developing seedlings to become fully expanded plantlets was determined on half strength of Murashige and Skoog medium supplemented with 40% (v/v) CW (Hasan *et al.*, 2011). Medium CM5 (one-fourth MS medium containing 0.10 mg l^{-1} NAA, 0.70 mg l^{-1} KIN and 20 % coconut water) was the best in the propagation of *C. rubens* with 70% viability; 2.33 shoots; 47.33 mm shoot length; 36.33 mm root length and 65% callus formation (Gbadamosi, Sulaiman, 2012). Those studies revealed that adding CW on medium had a positive effect on the development and growth of shoots, while in the study, application of different CW concentration caused reducing quality of shoot growth with high contaminations noted. All shoots derived from the treatments were not suitable used for subculturing materials in conjunction to produce qualified potato planting materials for *in vivo* activities.

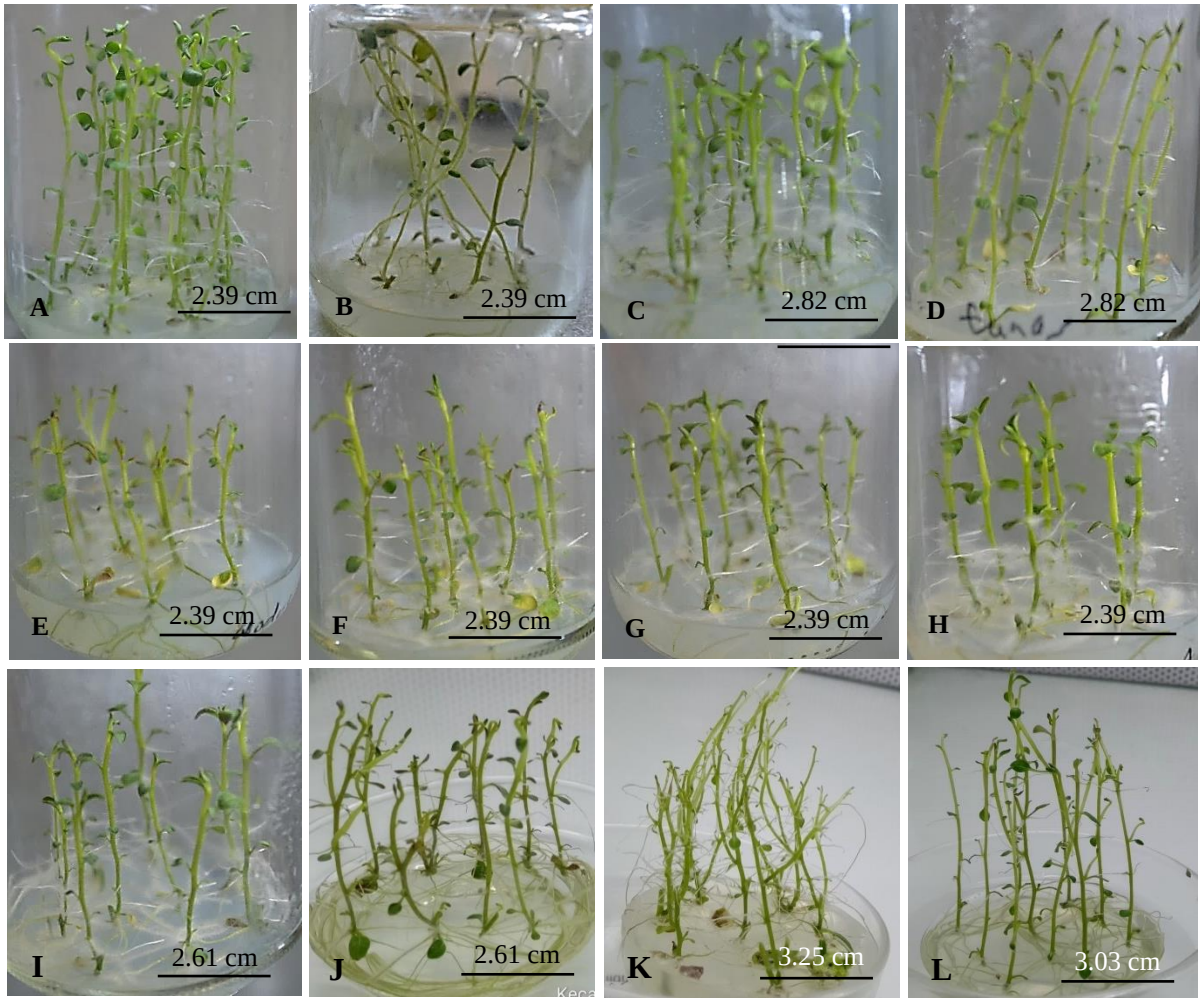

Figure 1. Shoot growth performances derived from 'Muhzoto' explants under different treatments. A. Morphological growth of shoots under 11 000 lx light intensity. B. Shoot growth performances under 0 ml l^{-1} CW in MS medium with 1.5 vitamin strength. C. Shoot performances of 'Muhzoto' explants cultured on MS medium containing 200 mg l^{-1} total myoinositol in the medium, 1 mg l^{-1} CaP dan 0,1 mg l^{-1} GA_3 (CM-2), D. Shoot growth behaviour derived from shoot tip explants, E. Shoot growth behaviour derived from 1st nodus explants, F. Shoot growth behaviour derived from 2nd nodus explants, G. Shoot growth behaviour derived from 3rd nodus explants, H. Shoot growth behaviour derived from 4th nodus explants, and I. Shoot growth behaviour derived from 5th nodus explants. J. The branched shoots derived from 1st nodes in the first culture 40 days after culture. K. The hairy shoots with thin stems, high airy roots, small and undeveloped leaves derived from the branched shoots of 1st nodes after the third subculture. L. Shoot growth performances derived from the branched shoots of 1st node explants after the 3rd subculture under optimal handling dealing with selection and application of the right time of subculture.

In *in vitro* culture, each step needs a suitable medium to obtain the optimal response of explant in different ways of regeneration methods. In the study, optimal shoot growth performances of 'Muhzoto' variety were established using MS medium enriched by 1.5 vitamin strength, 200 mg l^{-1} myoinositol, 1 mg l^{-1} CaP and 0.1 mg l^{-1} GA_3, though there was no significant difference compared to MS medium supplemented with 1.5 vitamin strength. The treatment produced shoots with 5.3 cm in height, 1.13 mm stem diameter, 0.7 cm

internodus length, 7.5 leaves per shoot, 1.42 length-width ratio and 50.6% shoots branched. From other studies it was reported that MS medium containing 0.5 mg l^{-1} BA and IBA was important to produce 2.5 shoots per explant with 5.43 cm shoot length, 100% shoots rooted and 15.8 roots per shoot (Nagib *et al.*, 2003). Ebad *et al.* (2015) successfully induced shoots with 8.3 cm in height and 3 leaves per shoot using MS-0 medium free plant growth regulator with 30 g l^{-1} sucrose and 8 g l^{-1} agar. Shoots with 7.1 cm in height, 7 leaves per shoot, 109.3 mg shoot fresh weight, 13.3 mg shoot dry weight and 12.2 dray matter were significantly regenerated using MS-0 medium hormone-free after 4 weeks of culture under mixo-trophic condition (Khalil *et al.*, 2016). Height of shoots up to 10.3 cm with 7.4 nodes per plant for 'Desiree' variety was strongly induced on MS medium supple-mented with 0.25 mg l^{-1} GA3; while for 'Cardinal' variety, shoots with 10 cm in height and 7.4 nodes per plant were established on MS medium containing 0.12 mg l^{-1} GA3 (Mehmood *et al.*, 2016)

Culture responses expressed on different typical shoot growths were noted in the study. Utilization of shoot tips, 1st and 2nd nodes as explant source resulted in an almost high percentage of shoots branched leading to produce low-quality planting materials, while 3rd, 4th and 5th nodes were suitable explant source utilized to regenerate high-quality planting materials for *in vivo* propagation vegetatively. The branched shoots were an important and serious problem in *in vitro* 'Muhzoto' variety propagation, especially when the shoots were not handling properly under continuous subculture processes. The hairy shoots with thin stems, high airy roots, small and undeveloped leaves were real evidence obtained after 2–4 periodical subcultures. Other studies explored shoot growth performances by culturing node explant on different treatments and determined that good quality of shoots was regenerated by application of sucrose than commercial sugar; ultrapure water than tap water, bacteriological and gelrite than agar (Venkatasalam *et al.*, 2013). Different responses of explant growth also revealed by culturing explants in two different incubation condition and different places of explant on culture media either horizontally, upright or inverted on culture media (Mng'omba *et al.*, 2017). Under different combination treatments, they found different growth responses of explants during *in vitro* culture. Placing explants in inverted position incubated in the dark condition resulted in high shoot length, shoot number, root number and leaf number. The second-best combination treatments were established on explants cultured horizontally and incubated in the dark then transferred to light incubation, while upright position explants cultured in all incubation condition gave low results in all variable observed.

Conclusion

From all treatments applied in the study, it can be concluded that the four different treatments explored gave different and interesting results. Higher light intensity up to 11 000 lx was the optimal treatment in resulting in better shoot growth performances of 'Muhzoto' explants. Adding different concentrations of CW could not improve the quality of shoots, but the treatment was due to leading on reducing shoot growth with high contamination occurred. Varied culture media that were enriched by adding myoinositol, CaP, GA3 and CW, did not increase shoot growth performances significantly, except CM-2 (MS medium supplemented with 1.5 vitamin strength in the medium in combination with 200 mg l^{-1} myoinositol, 1 mg l^{-1} CaP and 0.1 mg l^{-1} GA_3. Though the culture medium slightly improved shoot performances, however, there was no significant difference compared to the MS medium containing 1.5 vitamin strength. Furthermore, exploring shoot growth responses derived from different types of 'Muhzoto' explants successfully revealed that shoot tips, 1st and 2nd nodes generally regenerated high branched shoots with the higher length of internodus; while 3rd, 4th and 5th nodes successfully stimulated higher stem diameter and the number of leaves per shoot with a low percentage of shoot branched. The branched shoots were the serious problem in preparing high-quality regenerants derived from the 'Muhzoto' explants.

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Conflict of interest

We declare that there is no conflict of interest dealing with IAARD that funding this research activity, Kalimandi Main Institute for Horticulture Seeds (KMIHS), Banjarnegara District, Central Java province, Indonesia which supplied the research materials, authors, and Central Java Assessment Institute for Agriculture Technology that facilitated the research activities.

Author contributions

BW – contributed to research planning, executing till finishing, preparing and writing the manuscript until revisions of it.

IGC, RKJ, SCB and BH – were involved in preparing research materials, carrying research and helping data analysis equally. All authors approved the manuscript for publication, take public responsibility for the content.

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AKADEEMILISE PÕLLUMAJANDUSE SELTSI 2020. AASTA TEGEVUSARUANNE

Mittetulundusühingu Akadeemiline Põllumajanduse Selts (APS) tegevuse eesmärk on kaasa aidata Eesti maaelu, põllumajanduse ning põllumajandus- ja keskkonnateaduste arengule. APSi liikmeteks on isikud, kes on tasunud iga-aastase liikmemaksu ning täidavad seltsi põhikirjast tulenevaid kohustusi. Selts asutati 1920. aastal Tartu Ülikooli juures, mil see kandis nime Akadeemiline Põllumajanduslik Selts, seega on APS eelpool nimetatud seltsi tegevuse jätkaja.

Akadeemilise Põllumajanduse Seltsi tööd korraldab eestseisus (juhatus), kuhu kuulub kuni 13 liiget. Eestseisusesse kuuluvad PhD Marko Kass (president), PhD Evelin Loit (asepresident), pm-mag Ingrid Bender, pm-dr Jaan Kuht, MSc Katrin Laikoja, pm-dr Peep Piirsalu, PhD Matti Piirsalu, pm-dr Ülle Tamm, pm-mag Avo Toomsoo, PhD Marina Aunapuu. Seltsi eestseisusesse kuulub ametist tulenevalt ka Agraarteaduse peatoimetaja pm-dr Alo Tänavots ning seltsi sekretär pm-dr Heli Kiiman.

Seltsi ridades oli aruandeperioodi lõpul 212 liiget. APS-i koosolekutel ja sündmustel osaleb aktiivselt ligi kolmkümmend liiget. Seltsi võeti 2020. aastal vastu üks liige (Piret Peiker, Tallinna Ülikoolist esitas avalduse 12.06). Seltsil on 31 auliiget, sh kolm aupresidenti.

Traditsiooni kohaselt annab seltsi president aasta lõpus üle presidendi rändkarika koos aunimetuse Aasta Tegija 2020, mille pälvis Alo Tänavots olulise panuse eest seltsi juubeliürituste korraldamisse ja ajakirja edendamisse.

Toimus viis eestseisuse koosolekut (29.01, e-koosolekud toimusid 03.06, 15.06, 01.10, 11.11) ning kaks ettekandekoosolekut (neli ettekannet). Eestseisuse koosolekutel oli peamisteks aruteluteemadeks juubeliga seotud ürituste korraldamine, seltsi ajakirja väljaandmine (ka toimetuse nõuandvasse kogusse uute liikmete värbamine ning tegevtoimetaja leidmine), liikmete tunnustamine ja toimkondade loomine. Eestseisuse korralisel koosolekul 29.01 loodi seltsi juurde ajalootoimkond, mille juhtimist veab eestseisuse liige Jaan Kuht. Pidevalt otsitakse võimalusi täiendavate rahaliste vahendite kaasamiseks nii seltsi tegevuste kui teadusajakirja välja andmise jaoks. Palvet seltsi toetada saadeti nii teaduste akadeemia juhatusele kui maaülikooli rektorile. C.R. Jakobsoni nimeline Torma kool eesotsas direktor Heiki Sildnikuga palus aasta alguses (04.02) esitada seltsi esindaja Jakobsoni kõnevõistluse žüriisse. Eestseisus otsustas aprillis loobuda üldkoosoleku kokku kutsumisest seoses koroonaviirusega levikuga. Lisaks oli kevadkuul päevakorral võimalik liitumine Põllumajandus-Kaubanduskojaga, ent liikmemaksu summa on seltsi eelarvet arvestades liialt suur ja otsuse vastuvõtmine lükati järgnevale aastale. Seoses seltsi asutamise sajanda tähtpäevaga koostab toimkond koosseisus Jaan Kuht, Alo Tänavots, Marko Kass ja Katrin Laikoja juubeliraamatut "Sada aastat. Sada lugu".

Seltsi eestseisuse juures tegutsevad seltsi 100. juubeliaasta tähistamiseks kaks töörühma: esimene korraldas visioonikonverentsi ning teine koostab seltsi ajaloost näitust ja juubeliraamatu. Samuti osalevad APSi liikmed Eesti Teaduste Akadeemia ja teiste (teadus)-seltside poolt korraldatud sündmustel.

8. jaanuaril toimus seltsi möödunud aastat kokkuvõttev koosviibimine Tartus. Külastati esmalt Tähtvere tegutsevat Õllemuuseumit, misjärel uudistati A. Le Coq tootmistsehhis jookide valmistamist, hiljem tutvuti tehase ajalooga ja prooviti kodumaiseid karastusjooke. Pidulikul õhtusöögil Lauluväljaku kohvikus kõlasid seltsi presidendi uusaastatervitus ja seltsiliikmete sõnavõtud. Sealsamas anti pm-mag Sirje Tammele üle "Aasta Tegija 2019" tiitel koos seltsi presidendi rändkarikaga. Osales 33 liiget.

30. jaanuaril toimus seltsi ettekandekoosolek maaülikooli Tehnikainstituudis, kus ettekande tegid taimekasvatuse instituudi vanemteadur Kristiina Laanemets teemal "Toidutaimede geneetiline muundamine: ohtlikum või ohutum?" ning seltsikaaslane, toiduhügieeni professor Mati Roasto kõneles teemal "Toiduhügieen ja -ohutus koduköögis". Osales 23 liiget.

19. veebruaril toimus K.E. von Baeri majas (Veski 4, Tartu) Eesti Vabariigi 102. aastapäevale pühendatud aktus. Pärast seltsi aupresidendi tervitust pidas akadeemilise kõne Põllumajanduse- ja keskkonnainstituudi direktor pm-mag Aret Vooremäe emakeelse põllumajanduskõrghariduse ajaloost. Ettekandele järgnes noorte muusikute Kairiin Kuke (klaver) ja Hermine Aintsi (laul) etteaste. Hiljem toimus keskustelu koos kihiseva joogi ja suupistetega. Osales 16 liiget ja 7 kutsutud külalist.

7. aprillil kirjutas seltsi president liikmetele seoses eelseisva suure juubeliga. Kiri oli järgmine: "*Head seltsi liikmed! Loodetavasti olete terved ja positiivsed, tundes rõõmu päikeselisest kevadest! Seltsi eestseisus arutas tekkinud olukorra valguses edasist tegevuskava, jõudes ühisele otsusele, et enne järgmist õppeaastat seltsi üritusi ei toimu. Kui olukord on muutnud märkimisväärselt paremaks, tuleb kõne alla suvelõpu väljasõit. Samas oli eestseisus positiivselt meelestatud juubeliaastaga seotud ürituste korraldusliku poole osas. Seltsi juurde loodud ajalookomisjon, eesotsas eestseisuse liikme Jaan Kuhtiga, sai ülesandeks aidata eestseisusel koostada seltsi juubeliraamatut. Ühtlasi on kõigil seltsi liikmetel võimalus anda oma panus selle õnnestumiseks. Ootame seltsi liikmete kirjatöid ja mälestusi seltsi tegevuste ja ürituste kohta või lihtsalt meenutusi möödunud aegadest. Raamatu juhtmõtteks on sada aastat – sada lugu. Aitäh teile ette ja olge terved!*".

19. maist kutsuti seltsi liikmeid külastama Viljandi Muuseumis avatud näitust "100 aastat aiandust Pollis", mis kajastas Polli aianduse ajalugu 20. sajandi algusest kuni tänapäevani.

21. mail teatas "Agraarteaduse" peatoimetaja liikmeid uudisest, et seltsi ajakiri leidis rahvusvahelist tunnustamist. Tollest hetkest peale on ajakiri lülitatud Poola teaduse ja kõrghariduse ministeeriumi teadusajakirjade nimekirja (MSHE), mis soovitab teadlastele,

kus oma artikleid avaldada. Nendes ajakirjades artikleid avaldanud Poola teadlastele omistatakse 20 punkti artikli kohta, mida kasutatakse teadlaste ja ülikoolide hindamisel. Iga teadlane peaks nelja-aastase hindamisperioodi jooksul esitama vähemalt neli artiklit, mis avaldatakse MSHE-indekseeritud teadusajakirjades. Agraarteaduse info on nähtav *ICI World of Journals* lehel. *ICI World of Journal* veebisaiti külastab igas kuus hinnanguliselt 70 000 kasutajat 180 riigist.

22. juunil andis seltsi eestseisus liikmetele teada, et seltsi üldkoosolekut seoses eriolukorraga ei toimu. Aasta kokkuvõtet seltsi tegevustest on võimalik lugeda ajakirja jooksva aasta esimesest numbrist.

28. oktoobril toimus video vahendusel seltsi ettekandekoosolek, kus ettekande tegid Aberystwyth Ülikooli teadur Merko Vaga, teemal "Putukad põllumajandusloomadena, söödaks ja toiduks" ning Kristi Zilenski (Bugimine OÜ) teemal "Putukasõnniku mõju tomati istiku kasvule ja keemilisele koostisele". Osales 27 liiget.

29. oktoobril tähistati Eesti Taimekasvatuse Instituudi 100. juubelit, mille puhul toimus veebikonverents "Eesti Taimekasvatuse Instituut – 100 aastat põllumehe teenistuses". Konverentsi avas instituudi direktor Andre Veskioja.

Seltsi teadusajakirja "Agraarteadus" toimetus üllitas 2020. aastal kaks põhinumbrit, mis ilmusid vastavalt juunis ja detsembris. Esimene number pühendati ajakirja 30. juubelile, kus toimetajaveerus meenutasid peatoimetaja ja seltsi president möödunud aegu, milleks oli peaasjalikult emakeelse põllumajandusteaduste saavutuste viimine siinsete põllumeesteni. Samuti on ajakirjal oluline roll valdkonna eestikeelse terminoloogia hoidmisel ja uuendamisel. Teine number oli kümnendi kõige mahukam, avaldati 17 teadusartiklit. Toimetus alustas taas ajakirja saatmist naaberriikide põllumajandusülikoolide raamatukogudele. Ajakirjas avaldatud teadusartiklid indekseeritakse SCOPUS® andmebaasis ja on ETISe andmebaasis tähistatud klassifikaatoriga 1.1.

5. detsembril toimus Tartus seltsi 100. juubelile pühendatud visioonikonverents. Konverentsi avas seltsi presidendi ettekanne "Visioon põllumajandusteadustele". Teiste seas said sõna teaduste akadeemia president Tarmo Soomere ettekannetega "Kombates ümmarguse planeedi piire", maaülikooli teadusprorektor Ülle Jaakma "Ringmajandus ja keskkond", Helsingi Ülikooli dotsent Priit Tammeorg "Kestlikuma põllumajanduse suunas", keskkonna asjatundjaid MSc Sirli Pehme "Põllumajandussektori kliimamõju" ja PhD Liisa Parts Oxfordi Ülikoolist Suurbritanniast "Toidujulgeolek resistentsuse küüsis: vaade tuleviku strateegiatele pestitsiidiresistentsuse vältimiseks". Konverents toimus videosilla vahendusel. Muusikaga astus ülesse Tartu ülikooli professor David Beecher viiulil. Tervitusi ja õnnitlusi jagasid lisaks teaduste akadeemiale Eesti Taimekasvatuse Instituudi direktor ning sõsarseltsid Soomest, Leedust ja Lätist. Päeva kõnejuht oli PhD Evelin Loit.

Selts kasutab oma uudiste ja informatsiooni edastamiseks meililisti ja sotsiaalmeediat. Ka jagatakse APSi sündmuste kohta teavet Eesti Teaduste Akadeemiaga assotsieerunud ühingute ümarlaua abil. Seltsi sissetulekuallikateks on liikmemaksud ja annetused. Lisaks toetab rahaliselt seltsi tegevust Eesti Teaduste Akadeemia. Ka on ühekordseid sissetulekuid seoses mittevaraliste lepinguliste tegevustega. Ebaregulaarselt on seltsi ajakirja väljaandmist rahastanud Eesti Maaülikooli õppetoolid. Seltsil palgalisi ametikohti ei ole. Võlgnevusi pole.

Selts osales teaduskoostööprojektis LIFE Agri Adapt partnerina.

Seoses riigis kehtinud eriolukorraga toimus 2021. a aastakoosolek tavapärasest hiljem, juunis, kus seltsi üldkogu kinnitas möödunud aasta tegevusaruande.

Marko Kass, president
Heli Kiiman, sekretär

ERKKI-GENNADI HANNOLAINEN – 80

Erkki-Gennadi on ise kirjeldanud oma elu esimesi aastaid alljärgnevalt. Ta sündis 13. juulil 1941. aastal Ingerimaal Vellankontu külas, mis paiknes Leningradi oblasti Lomonossovi rajoonis. Teises maailmasõjas, alates septembrist 1941, hõivasid Vellankontu küla Saksa okupatsioonivõimud. Sõjaolukorra tõttu nälgis 1941/42. aasta sügistalvel kogu küla elanikkond, kellest enamus kuulus "Trud i Sila" kolhoosiperede hulka. Kuna Vellankontu küla oli võetud nn rindekülade nimistusse, siis seoses rindeolukorra teravnemisega evakueeriti 1943. aasta septembris kogu küla elanikkond saksa sõjaväevõimude poolt rindejoonest kaugemale – sakslaste okupeeritud Eesti Vabariigi piiridesse. Ajutisteks peatuspaikadeks Eestis olid kogumis- ja karantiinilaagrid Tapal ja Kloogal ning samuti Paldiski sadam. Oktoobrikuu lõpupäevil viidi Soome laevaga "Virgo" evakueeritud Paldiskist Soome Vabariiki Turku kogumislaagrisse. Sealt järgnes evakueeritute teekond Nastola karantiinilaagri kaudu Savonlinna, kus Soome ametivõimude poolt toimus meie pere (minu ning minu ema, vanaema ja tädi) määramine uude elukohta – Säämimki valla Liistonsaare Jatkola tallu, kus elati kuni 1944. aasta detsembrikuuni. Minu isa oli juba sõja alguspäevadel mobiliseeritud Nõukogude Armeesse. Sõja lõppemisel tuli vastavalt Soome Vabariigi ja Nõukogude Liidu vaherahuleppele, Nõukogude Järelvalvekomisjoni nõudel, pöörduda "vabatahtlikult" tagasi Nõukogude Liitu. Meid lubati viia koju tagasi, kuid tegelikult toimus rongisõit tundmatusse. Koduküla asemel viidi meid Pihkva oblasti Porhovi rajooni Osteritsa külla, sealsesse Pobeda nimelisse kolhoosi. 1946. aasta juunis õnnestus meil salaja tulla Eestisse ja asuda elama Kuremaale.

Eesti keeleruumi integreerumist sai Erkki-Gennadi alustada 1947. aastal Kuremaa lasteaias. Mooritsa Algkooli lõpetas ta 1953. a ning seejärel 1956. a Laiuse 7-klassilise kooli. 1957. a asus Erkki-Gennadi õppima Kuremaa Loomakasvatuse Tehnikumi, mille lõpetas agronoom-zootehniku kutsega 1961. a suvel. Tööle suunati ta Jõgeva rajooni Ausi sovhoosi keskuse osakonda traktori-põllundusbrigaadi brigadiriks. Peale aastast töötamist sooritas ta sisseastumiseksamid EPA-sse ja asus õppima agronoomiateaduskonnas. EPA lõpetas ta 1967. a õpetatud agronoomi kutsega, mille järel suunati tööle Sakku, Eesti Maaviljeluse ja Maaparanduse Teadusliku Uurimise Instituudi mullauurimise osakonda.

Osakonnajuhataja Rein Kask soovitas tal teadusteemana hakata uurima mulla orgaanilise aine sisaldust, sh esmajoones huumuse fraktsioonilist koostist. Peale aastast töötamist, mis piirdus peamiselt metsamuldade mullaproovide võtmisega, toimus teema küsimuses diskussioon mullauurimise osakonna juhataja Rein Kase ja põllunduse osakonna juhataja Arnold Piho vahel. Selle tulemusena leiti ühiselt, et ainult huumuse alaste uuringutega ei ole perspektiivi kandidaadi dissertatsioonini jõudmiseks. Piho tegi omalt poolt ettepaneku viia uurimisteema rohkem maaviljelusele sobivamaks. Konkreetselt pakkus ta välja idee, et tulevase kandidaadiväitekirja andmete kogumiseks tuleks lisaks muule hakata uurima Kuusiku katsepõldudel automorfsete muldade lämmastikurežiimi. Sellega nõustus ka Rein Kask, kui tulevase kandidaadi väitekirja juhendaja ning venekeelne dissertatsioon "Eesti automorfsete muldade lämmastiku sisaldus ja selle kasutamine odra poolt", (maht 155 lk) valmis 1980. aasta lõpuks. Väitekirja edukas kaitsmine toimus 1981. aastal Jelgavas, tolleaegses VASHNIL-i Lääne osakonna teadusnõukogus. Pärast VAK-ist 1982. a tulnud agrokeemia erialase teaduskraadi kinnitust vormistati instituudis Erkki-Gennadi Hannolaineni üleviimine vanemteaduri ametikohale. Uurimistöö Eesti muldade taksonoomiliste ühikute geneetilise ja agronoomilise iseloomustamise raames jätkus kuni 1985. a kevadeni.

Seoses instituudi direktori Ilmar Aamisepa poolt läbiviidud kaadrialaste ümberpaigutamistega edutati juubilar maaviljeluse osakonna juhatajaks, kus töötas aastatel 1985–1988. Seejärel, pärast järjekordset instituudi reorganiseerimist, moodustati uurimiskeskused. Aastatel 1992–1994 töötas ta maaviljeluse uurimiskeskuse juhatajana, ühtlasi instituudi asedirektorina. Kooskõlas 1994. a toimunud EMMTUI jaotumisega iseseisvateks instituutideks, jätkas Erkki-Gennadi Hannolainen tööd Eesti Maaviljeluse Instituudis asedirektorina ja samaaegselt ka agroökoloogia osakonna juhatajana kuni pensionile jäämiseni 2007. aastal. Tema teaduslik uurimistöö hõlmab mulla ja väetistega antud lämmastiku dünaamikat, mulla toitainete sisaldust ja väljauhtumist drenaaživetega, samuti erinevate kultuuride lämmastiku omastamist, mitmesuguseid mullaharimise võtteid ja harimisriistadega muldade tallamise ulatust. Ta on avaldanud üle 50 publikatsiooni teadustööde kogumikes ja perioodikas. Tänu soome keele valdamisele on ta olnud üks Eesti-Soome põllumajanduse koostöö algatajaid ja selles töös osalejaid. Oma töös on ta alati olnud kohusetundlik, sõbralik, abivalmis ning omandanud kaastöötajate hulgas hea juhi autoriteedi. Ta on ka pensionipõlves tundnud jätkuvalt professionaalset huvi põllumajanduse, eriti aga põllumajandusteaduse arengu vastu. Teda hinnatakse kui heatahtlikku ja asjatundlikku nõuandjat noorematele instituudi töötajatele.

Aastate jooksul on juubilar jõudumööda tegelenud hobispordiga (orienteerumine, suusatamine, kalastamine) ning püüdnud anda omapoolse panuse Eesti Keskkonnaanomaaliate uurimisel.

Kolleegide nimel Arvo Sirendi.

JAANUS SIIM – 80

Jaanus Siim sündis 23. veebruaril 1941. a Harjumaal Padisel riigiteenistuja perekonnas. Alghariduse omandas Hageri 7-klassilises koolis ja keskhariduse Rapla Keskkoolis, 1960. a asus õppima Eesti Põllumajanduse Akadeemia Mehhaniseerimise teaduskonda. Insener-mehaaniku diplomi sai J. Siim 1965. a.

EPA lõpetamise järel töötas J. Siim 1966–1967. a Maaviljeluse kateedris, kus uuris Elmar Halleri juhendamisel külvieelse mullaharimise minimeerimise tehnilisi võimalusi.

1968. a kutsuti J. Siim tööle Eesti Maaviljeluse ja Maaparanduse Teadusliku Uurimise Instituuti (EMMTUI), kus ta töötas mehhaniseerimise osakonnas tehnoloogi, teaduri ja vanemteadurina. Ta osales kultuuride viljelemise ratsionaalse tehnoloogia ja masinate süsteemi väljatöötamises ning põllutöömasinate Eesti oludele sobivuse hindamises. Alates 1971. a keskendus J. Siim kartulikasvatuse tehnoloogiate ja masinate uurimisele. Läbiviidud põldkatsete tulemusena valmis kandidaaditöö "Pealsete koristuseelse eemaldamise mõju Eesti NSV-s enamlevinud kartulisortide mugulate saagile ja vigastatavusele", mille eest omistati talle 1979. a põllumajanduskandidaadi teaduslik kraad. J. Siim konstrueeris seadmeid kartulimugulate vigastatavuse võrdlemiseks, juhendas mitmete kartulikasvatusmasinate konstrueerimist, töötas välja kartulisorteerlate tehnoloogilisi lahendusi, uuris kartulihoidlaid ja säilitusrežiime ning kartulikasvatuse (sh nn "šoti tehnoloogia") ja kartulitärklise tootmise tasuvust ning seemnemugulate puhtimise tehnilisi lahendusi. 1978. a oli ta Tallinnfilmi kaheosalise filmi "Teine leib" konsultant. Pikka aega oli ta kartulikasvatuse mehhaniseerimise ainevallas kompetentseim spetsialist vabariigis. Põllumajandusministeeriumi eritellimusel viis ta läbi suhkrupeedi kasvatamise tasuvusarvutused. Tolleaegsetes majandamistingimustes osutusid tasuvusarvutuste tulemusena nii kartulitärklise tootmine kui ka suhkrupeedi kasvatamine suhkru tootmiseks majanduslikult mittetasuvateks, seega hoiti ära riskantsed investeeringud.

EMMTUI reorganiseerimise käigus moodustati 1993. a Arvi Kallase initsiatiivil mehhaniseerimise osakonna baasil Eesti Põllumajanduse Mehhaniseerimise Instituut (EPMI), kus J. Siim asus tööle instituudi katsetööde osakonna juhataja ja asedirektorina. 2002. aastal liideti EPMI Eesti Maaviljeluse Instituudiga (EMVI), kus J. Siim jätkas töötamist asedirektorina. Aastail 2002–2003 oli ta EMVI teadusnõukogu esimees. Alates 2013. a töötas juubilar Eesti Taimekasvatuse Instituudi agroökoloogia osakonna vaneminsenerina kuni pensionile jäämiseni 2020. a.

J. Siim osales traktorite tüübikinnituse süsteemi väljatöötamisel, korraldas mõõteaparatuuri uuendamist ja tänapäevaste mõõtevahendite ning -meetodite esmakordset kasutuselevõttu Eestis agrotehnilistes uuringutes (sh digitaalsed mõõtmistulemuste salvestid, elektrooniline mullaniiskuse mõõtur, elektronmugul jne). Ta oli 1997–1998 PHARE finantseeritud Eesti-Soome ühisprojekti "Introducing a Quality System for Enhancing the Level of Agricultural Machinery in Estonia" Eestipoolne juht ja täitja, projekt valiti 30 eduprojekti hulka. 2001–2004 oli ta PHARE projektide 99/A/113 ja 01/A/283 "Establishment of type approval system and technical requirements for wheeled agricultural and forestry tractors, their equipment and parts in Estonia according to EU tractor directive" juht ja täitja.

Alates 2000. aastast keskendus J. Siim taimekaitsepritside tehnilise kontrolli (TTK) metoodika/juhendite (sh vastavate standardite tõlkimisele/ülevõtmisele) ja seadusandluse väljatöötamisele ning temast sai selle käivitamise põhitegija – ta korraldas TTK tegijate väljaõppeks ja täiendkoolitusteks kokku 15 õppepäeva.

Oli 1995–2008 Euroopa Kartuli Uurimise Assotsiatsiooni (EAPR), 2002–2007 Inseneride Kutsekoja ja 2002–2006 Katseasjanduse Nõukogu liige. J. Siim on enda teadmisi täiendanud Soomes, Taanis, Saksamaal, Tšehhis, Austrias, Hollandis, Rumeenias ja Inglismaal. 1997–2005 oli ta Vilniuse rahvusvahelise messi "Agrobalt" eksponaatide hindamiskomisjoni tehnikaekspert. Esines ettekandega 16-l rahvusvahelisel teadusüritusel.

J. Siim on autasustatud EMMTUI medaliga "Külvaja", põllumajandusministeeriumi teenetemedaliga "Kalevipoeg kündmas" ja 5 aukirjaga.

Bibliograafia: 211 publikatsiooni (sh 7 masinakataloogi ja), olulisemad: "Kartulisorteerlad" (1990), "Kartuli mahapanek ja hooldamine" (1992), "Kartulikasvatus" (2002, autoreid), "Kartulikasvatuse šoti tehnoloogia" (2004), "Uuenduslikud võimalused teraviljakasvatuses" (2006, autoreid), "Väetamisest majandusliku surutise tingimustes" (2009, autoreid), "Vedelsõnnik ja mullaharimine" (2012 autoreid), "Laua- ja tärklisekartuli mahetootmise kalkulatiivne tasuvus" (2013), õppefilmi "Taimekaitseseadmed. Pritsimise ABC" stsenaarium (2013), "Väetiste käitlustehnoloogiad" (2013 autoreid), "Taimekaitse. Pritsimiskaod. Põhjuseid ja vähendamisvõimalusi" (2015, kordustrükina "Taimekaitseseadmete ohutu kasutamine" 2017). J. Siim on toimetanud või koostanud 14 trükist, pidanud üle 300 loengu ja ettekande ning olnud õppe-, tehnoloogia- ja masinademopäevade põhi- või kaaskorraldaja.

Kalvi Tamm

JAAK SAMARÜTEL – *in memoriam*

11.05.1961–†17.05.2021

Käesolev kirjatükk oleks pidanud olema juubelitervitus armsale kolleegile, kes on jõudnud oma elu parimate aastate künnisele. Too pajatus oleks suuresti vaadelnud ajajärku, kui teadlase loomesulg on veel hoogne ja aina rohkem sünnitab rõõmu peale kasvava põlvkonna uljus, et juubilari poolt alustatut edasi viia. Paraku tahtis saatus teisiti. Ühel kaunil lehekuu päeval kutsuti Jaak siitilmast ära. Ühekohtuselt liiga vara. Hää hulk asju jäi pooleli, mõõtmatult palju jäi alustamata…

Kolleegi elule ja tööle tagasi vaadates tundub selle kokkuvõtmine ühel leheküljel kuidagi ebaõiglasena, aga paberi kohale tõstetud sulg ei luba järgnevaid ridu kirjutamata jätta. Mingi sisemine sundus pani mälusoppidest otsima hetki, leidmaks käesoleva ülestähenduse tarvis mälestuskilde. Ehk on järgnev kuigivõrd fragmentaarne, ent siinkirjutaja ei seadnudki eesmärgiks täielikku läbilõiget möödanikust. Seevastu, üle paarikümne aasta teadusele elatud elu väärib pilguheitu olnule, kasvõi viivuks.

Laenates klassikult, võiksin nentida, et kui mina omadega doktorantuuri jõudsin, oli Jaagul väitekiri juba kaante vahel. See tuli vaid edukalt kaitsta. Millega ta kahtlusteta suurepäraselt ka hakkama sai. Milline oli ilm 2009. aasta juunis, mäletavad ehk vähesed, ent fakti, et mõned aastad varem oli rakendunud doktoritöö kaitsmise uus kord, mäletavad nähtavasti paljud. Nimelt tähendas nimetatud muudatus peaasjalikult seda, et väitekiri pidi baseeruma vähemalt kolmel eelretsenseeritud ja rahvusvahelise levikuga ajakirjas avaldatud artiklitel. Viimane sundis Jaaku ja paljusid saatusekaaslasi kohanema uute oludega, tehes ennastsalgavaid ponnistusi sihile jõudmiseks. Jaagu doktoritöö koosneski neljast teadusartiklist, millest kaks oli avaldatud toona paljudele kättesaamatuna näivates ajakirjades nagu "Journal of Dairy Research" ja "Reproduction in Domestic Animals". Tõik, et tema doktoritöö koosnes neljast artiklist, on loomakasvatuse ja veterinaaria-alastes doktoritöödes üsna haruldane tänini. Seega, Jaak seadis lati üsna kõrgele tulevastele kraadinõudlejatele, kaasa arvatud siinkirjutajale.

Peale selle, ei saa märkimata jätta asjaolu, et Jaagu eestvedamisel juurutati siinmail California ülikooli teadlaste Edmonsoni jt (1989) poolt loodud skeem, hindamaks holsteini tõugu veiste toitumust. Tänaseks on lüpsilehmade toitumuse hindamine teada-tuntud igale veisefarmi juhile Virust Võruni. Lood oleks veelgi sümpaatsemad kui ka siinsete loomapidajate keelepruugist saaks välja rehitsetud inglisekeelne laen "kehakonditsioon" ning maksvusele pääseks emakeelne "toitumus". Aga sellele vaatamata, elab Jaagu poolt üle kahe aastakümne tagasi alustatud uurimissuund täna läbi oma helgemaid aegu.

Teadurileib on pikk ja mine tea, võimalik, et ka peenike. See-eest too amet on õppimine läbi elu, osundades lakkamatule tarvidusele edasi pürgida. Viimane tähendab suuresti, et kui filosoofiadoktorikraad taskus, ootab ees järel-doktorantuur. Siingi oli Jaak üks esimesi vapraid, kes südikalt Berni ülikooli juures sealse riikliku grandi toel järel-doktorantuuri läbi tegi. Julgus jätta kodu ja pere aastaks oma käele toimetama, mõistavad ehk vähesed – ainult need, kes selle kadalipu ise läbi teinud. Ent too ohverdus ei tulnud pelgalt teadmiste janust, vaid pidi eelseisval kümnendil tooma kõrgemat lendu siinsele teadusrühmale, seeläbi kogu loomakasvatusteadusele. Siinkirjutaja saab vaid alandlikult tänulik olla, kuna Jaagu poolt kahe väikeriigi vahel toona avatud uksed pole tänini sulgunud. Kui viisin mai keskel kurva uudise šveitslasteni, vastas tema sealne kolleeg, vanemteadur Josef Gross longuspäi, et hindas Jaagus kõrgelt tema sõbralikku ja toetavat hoiakut. Täiendades eelnevat tõdemusega, et Jaagu sealne teadustöö panustas märkimisväärselt kahe riigi teadlaste viljakale koostööle.

Josefi öeldut kinnitas ka Jaagu õde Kai, kelle sõnul oli ta lähedaste jaoks tagasihoidlik, abivalmis, sõbralik, äärmiselt põhjalik, küllap ka perfektsionist. Tema suhtumist näitas ilmekalt asjaolu, et enne Šveitsi siirdumist leidis ta eneses tahtejõudu alustada omal käel prantsuse keele omandamist. Hiljem, kodumaale naastes, võttis nõuks keeleoskust täiustada vastavatel kursustel.

Kahe Bernis veedetud perioodi vahele (jaanuarist märtsini 2012; oktoobrist 2013 septembrini 2014) mahtus Jaagu juhatatud rahvusvaheline doktorikursus "Biomarkers in Dairy Science" 2013. aasta varasügisel. Tema poolt ohjatud neljapäevane kursus tõi Tartusse viie riigi noorteadlased ning heade mõtete linna külastasid loomakasvatusteaduse suurkujud – professorid Klaus Lønne Ingvartsen ja Lotte Bach Larsen Taanist, Rupert Bruckmaier Šveitsist ja Pekka Huhtanen Soomest. Jaak oli lahti teinud järjekordse ukse suurde maailma. Ja siinjuures tuleb sedastada, et kursus sai jätku 2019. aasta septembris, mil teiste suurnimede seas külastas ülikoolilinna professor Bruckmaieri õpilane ja Jaagu hea kolleeg Berni-päevilt Josef Gross isiklikult.

Noid avatud uksi leiab hea kolleegi varasalvest teisigi. Tema väitekirja oponendiks olnud professor Kjell Holtenius Uppsalast jäigi pärast Emajõe Ateena külastamist siinse töörühma sõprade ringi. Tänu Jaagu lahti tehtud uksele on siinkirjutajal olnud tänuväärt võimalus külastada mitmel korral professor Holteniuse laborit, paluda tema abi käsikirjade retsenseerimisel ja teha ühiselt uurimistööd.

Elul on hakkajale palju pakkuda, jättes vähe ruumi juhusele. Vaadates läinud ajale kerkib pinnale ikkagi küsimus, miks n.ö arstide perest ja laia silmaringiga tarmukas abiturient valis oma kutsumuseks veterinaaria.

Ehk peitub vastus selles, et nii humaanmeditsiin kui veterinaaria on valdkonnad, mis ametikandjalt nõuavad püsivust ja rutiinitalumist. Temal oli seda kaunikesti ohtralt, teha asju tasa ning targu, kindlal meelel. Vahest kallutas loomaarsti ameti poole andunud anestesioloogist ja õppejõust isa pühendumus, mis pani noore mehe arvama, et ehk on too professioon liialt keeruline. Teab, kas tasubki proovida, jääb ehk kättesaamatuks, võis ta toona valikut langetades enesega heidelda. Aga mis ühele erialale kaotus, see teisele mäekõrgune võit. Toda n.ö pealejäämist iseloomustab "maailm samme täis", mille Jaak meie teaduspõllule oma kindlal kõnnil vajutas.

Hüva mehe oskuste repertuaarist ei puudunud hulk hobisid nagu matkamine või kokkamine. Tema virtuooslikku köögikunsti mul paraku nautida ei õnnestunudki, ent sisukaid vestlusi ja heatahtlikke õpetussõnu pikkadel autosõitudel aga küll, mida vürtsitasid kõnelused ajalooks saanud konverentsidest, taevakanalitelt nähtud loodusdokumentaalide kirjeldused ja meenutused uperpalle täis 90-ndatest.

Ja nüüd kui armas kolleeg on taevastel teedel, jääb meie kohuseks hoida lahti Jaagu poolt avatud uksi. Sama sihikindlalt ja pieteeditundeliselt kui tegi seda tema.

Marko Kass

MART JAAGUS – 100

26. aprillil 2021 möödus 100 aastat põllumajandusteadlase, heintaimede sordiaretaja, Mart Jaaguse sünnist. Ta lapsepõlv möödus Pärnumaal omaaegses Tammiste vallas Pärivere (tollal Soo) külas Lõo talus. Oli nelja poisslapsega peres vanim. Hariduse omandamine algas Are 6-kl koolis 1930. aastal. Omakäelises elulookirjelduses kirjutab ta, et õppimine edenes tal algkoolis vaevaliselt nii kesiste võimete kui laiskuse tõttu. Sellele räägib vastu tegelikkus: Mart viidi kolmandast klassist kohe viiendasse ning 1935. aastal saadud lõputunnistusel olid tal ainult neljad-viied. Niisugune hinneterida võimaldas aastatel 1935–1939 jätkata õpinguid Pärnu Kaubanduskeskkoolis. Selles koolis omandas ta hea masinkirja oskuse, saavutas kõrgeid kohti (ja auhindu) nii koolisisestel kui -välistel võistlustel. Vanima poja õigusega pidi Mart pärima talu, mistõttu oli plaan edasi õppida üks aasta põllumajanduskeskkoolis. Sissesaamisel oli aga nõutud gümnaasiumi lõputunnistus, kaubanduskeskkooli tunnistusest ei piisanud. Kaugõppe teel Tallinnast gümnaasiumi hariduse omandamise segas ära poliitiline muutuste rida ja peatselt alanud sõda. Esimesel okupatsiooniaastal võeti Mart Jaagus kutsealusena küll Pärnus arvele ja kohustati õppima vene keelt, kuid 1941. aasta juulis ta mobilisatsioonist pääses. Jäi talusse tööle, veebruaris 1944 mobiliseeriti aga Saksa sõjaväkke. Kutsealuste kogunemine oli Pärnus Kaubanduskeskkooli ruumes. Kui otsiti masinkirja oskusega inimest, meenus kooli direktorile masinakirja võistlustel edukalt esinenud Mart Jaagus. Nii sattus Jaagus sõjas mitte eesliinile vaid staapi, kus pidas igapäevast laskemoona arvestust – palju mürske välja tulistatud, palju veel alles. Sõda lõppes Mart Jaagusele Permikülas 18. septembril kui rinne lagunes ja algas kaootiline taganemine. Mart Jaagusel õnnestus kolme päevaga kodutallu jõuda, riided vahetada ja pikkamööda ka isikuttõendav pitsatiga paber hankida.

Novembris võttis taastunud nõukogude võim Pärnu sõjakomissariaadis ta jälle arvele ja suunas filterlaagrisse remonditöödele – esialgu Pärnus, kuid detsembrist juba Tallinnas. Peamine töö oli Estonia teatri taastamine. Hea masinakirja oskus ja oskus arvelauda käsitleda tuli siingi kasuks. Vajati arveametnikku, kelleks Mart Jaagus täpselt sobis. Uus amet võimaldas tal filterlaagri territooriumilt vabamat välja pääsemist. Poole õppeaasta pealt andis dokumendid sisse õhtukeskkooli, mille 1946. aasta kevadel eksternina edukalt lõpetas. Eksternina pidi kõigis õppeainetes, mille hinne läks tunnistusele, sooritama eksami. Samal kevadel õnnestus tal saada pass ja tunnistus filterlaagrist vabanemise kohta. Juulis sõitis Tartusse, otsustas õppida ülikoolis põllumajandust. Sisseastumiseksamid sooritas hiilgavalt – kõik viitele, kuid mandaatkomisjon leidis, et niisuguse taustaga inimestel ülikoolis kohta ei ole. Asjasse sekkus Mart Jaaguse ema vend professor Julius Tehver, kes oma autoriteediga saavutas ülemustelt loa võtta Mart Jaagus siiski üliõpilaseks, seda tema vastutusel.

Õppimine ülikoolis kulges Mart Jaagusel väga edukalt, sai alates teisest semestrist kogu aeg kõrgendatud stipendiumi. Toidutalonge maalt pärit üliõpilastele ei antud, elas kodust saadud toiduainetega professor Tehveri peres. Teisel kursusel juhtus intsident koduloomade anatoomia ja füsioloogia eksamil. Tavaliselt luges seda ainet professor Tehver, kuid Mart Jaaguse kursusele millegipärast professor Vau. Professorid omavahel ei klappinud ja Vau hindas kiusu pärast Mart Jaaguse vastused nelja vääriliseks. Dekaan Osvald Hallik, kes professorite omavahelist nägelemist teadis, andis Mardile võimaluse eksam komisjoni ees uuesti sooritada. See õnnestus ja diplomiga kaasaskäiv hinneteleht jäi rikkumata. Põllumajandusteaduskonna riigieksamid toimusid 1950. aastal lõpetanutele septembris (varem alati kevadel). Enne eksamitele lubamist toimus jälle mandaatkomisjon, mida juhtis ministrite nõukogu tookordne esimees Arnold Veimer. Komisjonile tuli esitada põhjalik elulookirjeldus ja täita pikk üksikasjalik ankeet. Mart Jaagusele ähvardas kõik see saada saatuslikuks: kuulumine ja osavõtt omakaitse ettevõtmistest aastatel 1941–1944, osalemine 1944. a suvel sõjas saksa poolel, lisaks kodutalu kulaklikuks kuulutamine. Komisjoni ette suunati üliõpilasi tähestiku järjekorras. Dekaan August Eenlaid, teades Mart Jaaguse kriitilist olukorda, sokutas ta komisjoni ette väljaspool järjekorda nii, et komisjoni liikmetel ei olnud eelnevalt aega tema materjalidega tutvuda. Komisjoni liikmeid vaigistava Arnold Veimeri käeviipega lubati Mart Jaagus riigieksamitele ja määrati lõpetajana põllumajandusministeeriumi käsutusse. Mart Jaagus lõpetas ülikooli kiitusega. Valitses teadmine, et kiitusega lõpetaja saab põllumajanduse ministeeriumi raames ise töökoha valikul kaasa rääkida. Käis ministeeriumis kaadrite valitsuse juhataja jutul. Ei lastud aga isegi istuda. Püstijalu anti teada, et tuleb asuda tööle määratud kohal. 1. novembrist 1950 asus Mart Jaagus Vasalemma MTJ-is tööle jaoskonna agronoomina, sealt omakorda määrati 17. detsembrist 1953 kolhoos "Kalev" teenindavaks agronoomiks. Agronoomi töö kõrvalt puutus Mart Jaagus kokku probleemidega, mis seotud tööde mehhaniseerimisega, eriti uute masinate kasutuselevõtuga. Omandatud oskuste tõttu saavutas vilumuse ja tuntuse, mistõttu kutsuti (sõidutati) teda masinaid töösse juurutama ka väljapoole teeninduspiirkonda. Oskuste süvenedes otsustas omandada teise kõrghariduse põllumajanduse mehhaniseerimise teaduskonnas. Varasema kõrghariduse baasil võeti ta 1953. aasta jaanuaris ilma sisseastumiseksamiteta teisele kursusele nii, et ei pidanud ankeeti täitma ja mandaatkomisjoni ees käima. Edukalt kulgenud õpe katkes siiski neljandal kursusel, pärast seda kui Mart Jaagus asus 1955. aasta juulis teadurina tööle Jõgeval. Edasine teadustöö nõudis kogu tähelepanu. Tööle kutsujaks ja ministeeriumiga asjaajajaks oli tuntud rohumaateadlane ning kultuurkarjamaade spetsialist Aleksander Adojaan. Tööülesandeks oli esialgu kultuurkarjamaade rajamis-

ja hooldamistööde mehhaniseerimisvõimaluste leidmine. Töö tulemina ehitas ja juurutas tootmisse karjamaaäkke, mille tööpõhimõtet kasutatakse tänapäevani. Lisaks lahendas Mart Jaagus kõrreliste heintaimede laiarealiselt rajatud seemnepõldude, rühvelkultuuride ja kartuli reavaheltharimise küsimuse traktori jõul, konstrueeris sellekohased riistad. Kõiki neid töid tehti varemgi, kuid algeliste riistadega hobujõul.

1961. aastal pühendus Mart Jaagus liblikõieliste heintaimede karjamaasortide aretamisele. Esialgu piirdus töö valge ristikuga, hiljem lisandusid vegetatiivse levikuvõimega lutsernivormid. Valge ristiku temaatikat käsitles Mart Jaaguse 1971. aastal edukalt kaitstud kandidaadiväitekirjas teemal: "Valge ristiku sortide ja kohalike päritolude rohumaaviljeluslikke omadusi". Töö jätkuna aretas koos kolleegide V. Smoljakova ja E. Raudsepaga sordi 'Tooma', mis on leidnud tootmises kasutamist lisaks Eestile ka Kanadas. On läbi ajaloo teine Eestis aretatud taimesort, mida on Ameerika mandril tootmises kasutatud (talirukki 'Sangaste' kõrval). Lutsernivormidest on jõudnud sordina söödatootmisse võsunditega vegetatiivselt leviv 'Karlu' ja juurevõrsetega leviv 'Juurlu'. Mõlemad nimetatud sordid on populaarsed lähinaabritegi juures, sort 'Karlu' ka Saksamaal.

Mart Jaagus oli 1959. aastast abielus tuntud köögiviljade sordiaretaja Valvega (neiuna Aamisepp). Lapsi neil ei olnud. Pärast abikaasa surma (1995) ja pensionile jäämist (1999) veetis Mart Jaagus vanaduspäevi Pärnumaal kodutalus Lõol. Talu peab Mardi venna Jaani tütar Epp. Tegi seal jõukohaseid talutöid, korrastas maaparandussüsteemi, ehitas elektritootmiseks tuulegeneraatori, hooldas mesilat jne.

Mart Jaagus suri nädal enne 88-aastaseks saamist 20. aprillil 2009 ja on maetud Tartus Raadi kalmistule Aamiseppade hauaplatsile abikaasa kõrvale.

Mart Jaagus õppis ülikoolis viimasel kursusel, mille agronoomia eriala lõpetajad said Tartu Riikliku Ülikooli diplomi. 1950. aastal lõpetas 70 agronoomi. Koos Mart Jaagusega said diplomi kiitusega veel Ülo Oll, Heino Kärblane ja Rudolf Pant. Kursusel õppinute seast kasvas välja rida taimekasvatuse eriala teadlasi: Arnold Piho, Vilmar Kruus, Jaan Liiv, Ervin Talpsepp, Tiit Nõges, Ella Kukk – kõik doktori- või kandidaadikraadiga teadlased. Samal kursusel õppis ka hilisem mitmekordne põllumajandus- ja aiandusminister Harald Vambola Männik.

Ants Bender