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SHORT COMMUNICATION: CHANGES OF COMPATIBLE SOLUTES CONTENT IN *TRITICUM AESTIVUM* AND *TRITICUM DICOCCUM* SEEDLINGS IN RESPONSE TO DROUGHT STRESS

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ABSTRACT. The influence of drought stress modelling by polyethene glycol (PEG) on water status, total soluble protein, proline and carbohydrates content in the roots and leaves tissues of three drought resistance wheat genotypes – *Triticum aestivum* L. ('Podolianka', 'Favorytka') and *Triticum dicoccum* Schrank. ('Holikovska') have been studied. Tested varieties of bread wheat and emmer wheat presented the resilience-anisohydric strategy for water balance regulation retaining a high level of relative water content, great variability in organic solutes with osmoprotective properties (total soluble protein, total carbohydrates and free proline) accumulation and retaining the level of low molecular weight proteins in response to drought with noticeable distinctions amid the varieties. However, drought stress increased the efficiency of accumulation of osmoprotectants either protein or carbohydrate nature in all three tested varieties of wheat seedlings.

Introduction

Crops yield is highly susceptible to water stress and constantly balancing between drought survival and mortality. Plants iso/anisohydric strategies of water status regulation might play a role in their ability to tolerate drought conditions. An overall mechanism for plants with an isohydric strategy of water balance regulation arises from the deviation of drought-induced hydraulic insufficiency throughout stomatal closure, resulting in a reduction in net photosynthetic rate (McDowell *et al*., 2008).

By decreasing hydraulic and stomatal conductance to retain an invariable water status, the isohydric plants minimized the exposure of their leaf tissues to water deficit, but also reduced their ability to fix carbon for growth and to ensure productivity in drought conditions. Unlike, the plants with anisohydric strategy conserved a high assimilation rate while leaves tissue water potential decreased, which should resolve higher productivity in the anisohydric plants, but also made those more assailable to damage from extended drought impact (Attia *et al*., 2015). Resilience-anisohydric strategy strikes a balance between plants reaction to water deficit with a rapid closure of stomata to prevent further water wastage via transpiration and ability to keeping a high level of internal osmotic potential by synthesizing various osmoprotectants and osmoregulators.

Therefore, this work aimed to investigate the biochemical adaptive stress response at the level of soluble osmoregulators and low molecular weight protein patterns in leaves of common bread wheat varieties (*Triticum aestivum* L.) and emmer wheat (*Triticum dicoccum* Schrank.), grown under PEG-induced drought stress.

Materials and methods

The objects of the study were three wheat varieties – *Triticum aestivum* ('Podolianka', 'Favorytka') and *Triticum dicoccum* Schrank. ('Holikovska'). Seedlings were grown in a growth chamber at 25 °C with photoperiod 16 h of light, at 200 μmol photons $m^{2\ -1}$ s^{-1} of photon flux density during 7 days. Control variant of seedlings was grown on distilled water. Experimental seedlings were grown on distilled water with polyethene glycol (PEG) 6000 with an osmotic potential of –0.3 MPa.

Total soluble protein content was determined by the method of Bradford (1976) using BSA as standard. Proline colourimetric determination proceeded according to Bates *et al.* (1973). Total soluble carbohydrates content

was measured by phenol-sulfuric acid method Jain *et al.* (2017) using glucose for standard curve plotting.

Electrophoresis was carried out using dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) in vertical slab gel discontinuous buffer system following the method of Laemmli (1970) with minor modifications (Vítámvás *et al*., 2015).

The experiment was conducted with three biological and analytical repeats. The data were subjected to analysis of variance (ANOVA) with subsequent Duncan's multiple range test. Data are expressed as means of replicates + standard deviation and were considered reliable at a significance level of $P < 0.05$.

Results

In response to water imbalance, majority of physiological and biochemical plant functions are disordered. Investigation of the multiple mechanisms by which plants react to drought stress is an invocation to enhancing plant drought tolerance (Soltys-Kalina *et al*., 2016). Parameter relative water content (RWC) shows the balance between water supply to the leaf tissue and transpiration rate. Water deficit (WD) is known as the percentage of water shortage of its total amount in plant tissues when it is fully absorbed. It can occur by the disturbance of the water supply of the plant and cause changes in the progress of physiological and biochemical processes, affecting the productivity of crops (Osmolovskaya *et al*., 2018). These plant water balance traits were previously studied by Smirnov *et al.* (2020). RWC of the leaves tissues of 'Podolianka' and 'Favorytka' varieties were decreased beside 15%, in variety 'Holikovska' – by 7%.

Data analysis revealed that experimental water deficit has increased threefold in varieties 'Podolianka' and 'Favorytka', while in variety 'Holikovska' water deficit has increased by 50%. The authors claim that among tested wheat cultivars large differences in plant water balance were observed as a result, plant architecture parameters differ (Smirnov *et al*., 2020). Thus, increasing of dry matter can be explained so the next step was the comparative analysis of total soluble protein, carbohydrates and free proline contents (Fig. 1).

Comparative analysis of soluble protein content showed a slight decreasing of its level within the limits of 10–15% in roots and leaves tissues of 'Podolianka' and 'Favorytka' varieties under stress condition. In 'Holikovska' variety protein content increased by 30% in roots tissues and remained at the level of the control variant (Fig. 1 A). In response to water deficit in studied varieties, a decrease in protein content usually resulted in a compensatory increase in carbohydrates and/or proline content to maintain a high level of cell turgor (Fig. 1 B, C). There were no significant differences concerning the total soluble protein, carbohydrates and proline contents in the leaves of 'Holikovska' variety.

The alteration of low molecular weight protein synthesis is one of the most basic metabolically stimulated processes that may influence drought tolerance. Analysis of the leaf protein by SDS polyacrylamide gel electrophoresis did not show significant fluctuation levels of polypeptides in stressed leaves (Fig. 2).

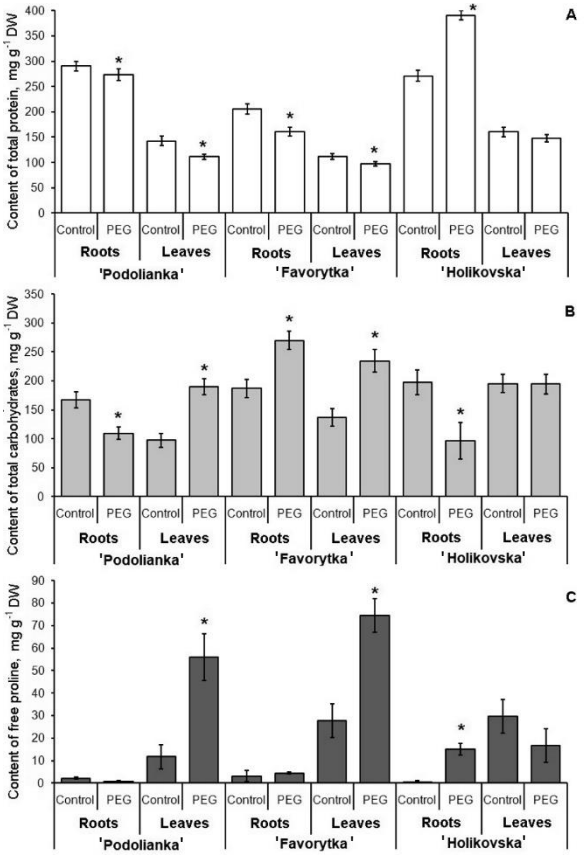

Figure1. Soluble protein (A), carbohydrates (B) and proline (C) content in the roots and leaves tissues of wheat different varieties under the drought stress; n = 15 (PEG – polyethene glycol treatment variant; * – data represent mean values with standard deviation; significant difference at $P < 0.05$ comparing to control level for each variety)

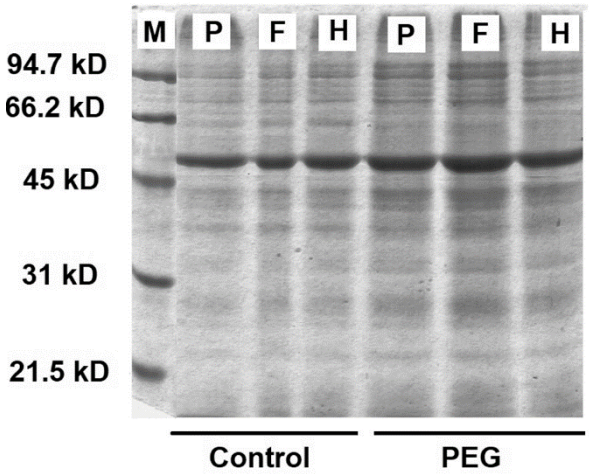

Figure 2. The sodium dodecyl sulphate polyacrylamide gel electrophoresis profile of low molecular weight proteins in the leaves tissues of wheat different varieties under the drought stress; n = 5 (PEG – polyethene glycol treatment variant, M – markers of molecular weight, P – 'Podolianka', F – 'Favorytka', H – 'Holikovska'

Discussion

Osmoregulators accumulation in response to water deficit is important for maintaining cell turgor by reducing water potential (Silva *et al.*, 2009) and certainly, several organic solutes are participating in this process as soluble proteins, sugars, free proline, and others soluble low molecular weight solutes. In this research, it was verified, if there is a variation in the pattern of accumulation of solutes among studied varieties in response to drought stress. Such resilience-anisohydric response suggesting that the synthesis and mobilization of soluble proteins or sugars and proline in wheat leaf and root tissues is a key strategy to cope with prolonged stress and the enhanced risk of osmotic imbalance (Dal Santo *et al*., 2016).

Committed metabolic changes, including the production of compatible soluble organic solutes used to maintain the tissue hydration. Amid the varied mechanisms used by plants to mitigate the negative impacts of drought stress, many plant species accumulate organic compatible solutes, such as osmoregulators and osmoprotectants. This mechanism is known as osmotic adjustment and it is allowed as an important adaptive responsive reaction, which supposes the maintenance of intracellular turgor and favours the water absorption (Medeiros *et al.*, 2012).

Previously studies showed drought stress relative decreasing of levels of soluble proteins whit high molecular weight, while soluble proteins with low molecular weight increases in bread wheat and Egyptian barley genotypes (Moradpour *et al.*, 2014; Hellal *et al.*, 2018). Sustentation of functionally active conformation is especially important for cell survival under stress. Heat shock proteins and late embryogenesis abundant proteins (10–100 kD) are responsible for protein folding; assembling, moving and degrading in many normal cellular processes stabilize protein and membranes and can help in protein refolding under stressful conditions. They can play a crucial role in maintaining the adaptive potential of plants by restoring normal protein conformation and therefore cellular homeostasis (De Britto *et al*., 2011).

Conclusions

Tested varieties of common wheat (*Triticum aestivum* L.) and emmer wheat (*Triticum dicoccum* Schrank.) presented resilience-anisohydric strategy at the level of the biochemical adaptive stress response. Great variability in organic solutes with osmoprotectants properties accumulation and maintaining the level of low molecular weight proteins in response to drought with marked differences among the varieties. However, drought stress increased the efficiency of accumulation of osmoprotectors either protein or carbohydrate nature in all three tested varieties of wheat seedlings. All tested *Triticum* varieties showed diversity in their ability to tolerate chemical dehydration induced by PEG keeping a high level of internal osmotic potential.

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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

OS – author of the idea, guided the research, and is the corresponding author.
AZ, L-AK – performed the biochemical assays and calculation of the results and data statistical analysis.
MK, NT – performed the literature data analysis and discussion of the results.
All authors read and approved the final manuscript.

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