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GENETIC RESOURCES FOR THE ADAPTIVE BREEDING OF WHEAT

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At the beginning of the 20th century the problem was being discussed whether it was worth to cultivate wheat in Estonia or not. Was it possible to bake tasty bread from Estonian wheat flour? As a result of extensive studies, Mihkel Pill, the founder of Jõgeva Plant Breeding Institute, gave a positive answer to this question: he found that from the varieties cultivated in Estonia it was really possible to make fairly good bread (Pill, 1935).

Both the introducing of the varieties suitable for Estonia (mainly from the Nordic Countries, Germany and Poland) and the elaborating of the new corn technology resulted in an increased interest to the Estonian wheat grain production. This encouraged the striking increase in the local sown area of wheat. The sown area of winter wheat increased from 6.0 thousands hectares in 1913 to 31.3 thousands in 1939, and the same figures for spring wheat were from 4.5 in 1913 to 43.7 in 1939 (tenfold increase). As early as in 1932 the wheat grain production covered the needs of national market, and in 1939 it was necessary to reduce the output.

In early times most of domestic varieties cultivated in Estonia had been obtained by means of primitive folk's breeding. After the Jõgeva Plant Breeding Station foundation in 1920, the new winter and spring wheat varieties were obtained by means of controlled selection.

The first commercial winter wheat variety 'Parandatud Luunja' was bred from local variety 'Luunja'. Varieties 'Puuk' and 'Universaal' were bred at Jõgeva by crossing of Swedish varieties with local ones. Later also varieties from Finland, Canada and other countries were used as initial material for local selection.

Spring wheat varieties 'Kauka', 'Jõgeva 888' and 'Pikker' were bred by crossing of Swedish, German and American varieties with local material.

Local plant varieties play an important role in adaptive breeding because they determine the adaptive capacity of introduced varieties. This prospect stems from the combining of the efficient alien genes with ecologically suitable genes pool in the breeding process (Küüts et al., 1995).

Nowadays, majority of commercial varieties have been obtained by using traditional intraspecific crosses. The varieties developed in the course of the last years considerably exceeded in yield the previous ones. An extensive spring wheat gene pool, mainly of the best Nordic varieties and lines, was collected in Jõgeva Plant Breeding Institute during the last few years. A collection of winter wheat including 250 cultivars was founded and utilized in the breeding Projects at Jõgeva by Endel Kallas.

Developing of wheat varieties resistant to different pathogens, is one of the key problems of wheat genetics and breeding. Leaf rust (*Puccinia recondita*) and powdery mildew (*Erysiphe graminis*) are the major diseases which occur in the Baltic countries and in almost all temperate regions of the world. To solve the resistance problem, together with traditional approaches, wide hybridization with various species of the subtribe *Triticinae* is actively coming into use.

A great number of alleles for mildew and rust resistance has been designated. However, in commercial varieties only few major resistance genes are currently in use. Since the efficience of the most known resistance genes is more or less exhausted, there is a need for the more effective novel sources of resistance.

Tetraploid wheat species *Triticum timopheevii* and *T. militinae* ($2n = 28$, genome formula AAGG) are well-known for their complex resistance to wheat disease. *Triticum timopheevii* was first found in Western Georgia by academician P. Zhukovsky in 1926. *Triticum militinae* was isolated as a spontaneous mutant from the collection plots of *T. timopheevii* (also by P. Zhukovskaja in 1950) and it differs from the latter by naked grains and black awns. Both species have attracted interest of wheat breeders and geneticists on account of their exceptionally high immunity to diseases. Therefore they have frequently been included as parent components in wide crosses with common wheat. The main obstacle for using these species in wide hybridization is high sterility of hybrids F_1. Cytogeneticists E. Sears, M. Feldman and R. Riley assumed that the poor chromosome pairing in hybrids F_1 is due to the cryptic structural differences between the genomes of hexaploid and tetraploid wheats. However E. Wagenaar suggested that irregularity of meiosis and sterility of hybrids F_1 is due to genetic system with some desynaptic genes, which affects chromosome pairing and chiasma formation.

From the end of 70s on, at the Plant Genetics Department of the Institute of Experimental Biology the crosses have been carried out between susceptible to disease common wheat varieties, on the one hand, and tetraploid wheats as a male parent, on the other hand. Plants of pentaploid hybrids F_1 were completely sterile and were maintained by backcrossings with common wheat parents for three generations. Crossability and fertility of interspecific hybrids and regularity of chromosome pairing at MI of meiosis in F_1 progeny and successive generations were analysed.

In F_4-F_5 progenies disease reaction varied from highly susceptible to entitely resistant free. Using permanent estimation of hybrid plants on the artificial provocative background, about 15 lines of common wheat phenotype and with different degrees of resistance to leaf rust and powdery mildew were identified and stabilized. Five introgressive lines with high resistance to leaf rust and improved agronomical characters were handed over at the World Wheat Collection of the Vavilov's Institute of Plant Industry, St.-Peterburg as the initial material for wheat breeding (Peusha, Enno, 1992).

Genetic analysis of hybrid progeny from crosses between introgressive lines and the susceptible variety Saratovskaya 29 was carried out to determine the number of genes conferring resistance. Analysis of F_2 hybrid population revealed segregation which fitted the 3 resistant to 1 susceptible ratio (table 1). This fact indicated that in seedling stage the plants of introgressive lines had a single dominant gene which governs the resistance to the local population of leaf rust.

Table 1. Segregation of F_2 hybrids from crosses of resistant lines with susceptible cv. Satovskaya 29

Cross combination	No. of plants	Proportion of resistant and susceptible plants		c^{2}
		observed	expected	
CMT 14 × Saratov 29	99	71:28	3:1	0.05
CMT 37 × Saratov 29	97	74:23	3:1	0.09
CMT 27 × Saratov 29	100	71:29	3:1	1.01
CMT 16 × Saratov 29	91	67:24	3:1	0.19
CMT 26 × Saratov 29	100	73:27	3:1	0.21
CMT 11 × Saratov 29	60	48:12	3:1	0.80
CMT 67 × Saratov 29	94	75:19	3:1	1.59
		$P=0.05$		$c^{2}=3.84$

Using isogenic set of variety Thatcher carrying effective resistance genes Lr9, Lr19 and Lr24, inheritance of disease resistance in introgressive lines was studied. The segregation pattern in F_2 hybrid population confirmed that introgressive lines have the resistance genes which are independent of effective genes Lr9, Lr19 and Lr24, and are either identical to gene Lr23 or closely linked with it (table 2) (Enno, Peusha, 1992; Priilinn et al., 1992, 1994).

Table 2. Segregation of F_2 hybrids from crosses of wheat resistant lines with cv. Thatcher tester-lines of known genes for resistance

Cross combination	No. of plants	Proportion of resistant and susceptible plants		c^{2}
		observed	expected	
CMT 5 × Lr 9	100	80:20	13:3	0.11
CMT 5 × Lr 24	119	68:51	9:7	0.03
CMT 11 × Lr 9	110	89:21	13:3	0.13
CMT 11 × Lr 24	100	94:6	15:1	0.14
CMT 14 × Lr 9	100	94:6	15:1	0.14
CMT 14 × Lr 19	100	95:5	15:1	0.29
CMT 14 × Lr 24	110	89:21	13:3	0.06
CMT 16 × Lr 9	120	102:19	13:3	0.30
CMT 16 × Lr 19	120	98:22	13:3	0.01
CMT 16 × Lr 24	87	50:37	9:7	0.43
CMT 26 × Lr 9	100	83:17	13:3	0.19
CMT 26 × Lr 19	100	91:9	15:1	1.28
CMT 28 × Lr 9	100	90:10	15:1	2.18
CMT 28 × Lr 19	80	64:16	13:3	0.07
CMT 67 × Lr 9	100	80:20	13:3	0.06
CMT 67 × Lr 19	100	78:22	13:3	0.57
CMT 67 × Lr 24	100	56:43	9:7	0.03
		P=0.05		c^{2}=3.84

The joint studies on the common wheat genome carried out at the Institute of Experimental Biology and Jõgeva Plant Breeding Institute with the financial support of the Estonian Science Foundation have promoted spring wheat improvement in Estonia. The collection of wheat germplasm has been founded and supplemented by cultivars of diverse origin as well as by different *Triticeae* species.

Wild species of Triticinae offer a rich source of genes for wheat improvement. The identification of the alien chromosomes carrying these genes is an important step in introgression experiments on wheat.

In our search for new resistance genes, a collection of derivatives of *T. timopheevii* and *T. militinae* was screened for the response to a set of differential mildew isolates. The specific interaction between a host plant (resistance) and a pathogen (virulence) were used to identify the resistance genes with the help of a set of varieties-differentiators. Following inoculation with 11 different powdery mildew isolates the response pattern of *T. timopheevii* and *T. militinae* derivatives was very similar to that of the phenotypes which contained resistance genes Pm6 and Pm2+Pm4+Pm6, or Pm2+Pm6 (table 3) (Küüts et al., 1994; Enno et al., 1995; Peusha et al., 1995).

Table 3. Reaction of hexaploid lines of common wheat derived from T. timopheevii and T. militinae inoculated with 11 isolates of E. graminis tritici

Cultivar or line	Resistance gene	Isolates of *E. graminis tritici*										
		2	5	6	9	10	12	13	14	15	16	17

Novosibirskaya 67 × *T. timopheevii* (21n-1)	?	i,r	r	r	r	r	r,i	r	s/r	r	r	r,i
Novosibirskaya 67 × *T. timopheevii* (7n-1)	Pm2+Pm6	s	r	r	r	r	r,i	r	r,i	r	r	i/r
Saratovskaya 29 × *T. timopheevii* (11c-2)	Pm2+Pm6	s	r,i	r	r	r,i	i,s	r	r,i	r,i	r,i	s
Saratovskaya 29 × *T. timopheevii* (3c-2)	Pm2+Pm6	s	r	r	r	r	i/r	r	r	r	r	i,r
Saratovskaya 29 × *T. timopheevii* (11c-1)	Pm2-4+6	s	r	r,i	r	r	r	r	r	r	r	i/r
146-155 × *T. timopheevii*	?	r,i	r,i	r	r	r,i	r	r,i	r	r	r	r
Saratovskaya 29 × *T. timopheevii* × *T. timopheevii* (SMT 34)	?	r	r	r	r	r	r	r	r	r	r	r
Saratovskaya 29 × *T. militinae* × *T. timopheevii* (SMT 45)	?	r,i	i/r	r,i	r	r,i	s	r	s	s	s	s

Notes: r – resistant, s – susceptible, i – intermidate

It is generally assumed that when the homoeologous pairing prevention system is active, genetic transfer between homoeologous chromosomes of wheat and other species of *Triticeae* is extremely limited. The use of the ph1b mutation as a pairing promoting system allows one to increase the level of wheat-alien homoeologous chromosome conjugation and, consequently, the recombination frequency. In our studies varieties of bread wheat including mutant ph1b of cv. Chinese Spring were crossed with wheat species (*T. timopheevii*, *T. militinae, T. persicum*), *Ae. cylindrica* and *Secale cereale*. The analysis of microsporogenesis was carried out and the chromosome pairing at metaphase 1 was evaluated on the average basis. The results of our experiments showed that in all cases in which the mutant ph1b was involved the chromosome pairing at meiosis was higher as compared with the corresponding control hybrids. It was ascertained that effects of the mutant ph1b in wide crosses were higher while the fewer of homoeologous genomes were integrated in F_1 hybrids. The highest rate of homoeologous pairing was observed in wheat-rye F_1 hybrid (Chinese Spring mutant ph1b × self-fertile line of rye) with the mean chromosome associations of 5.7 bivalents and 0.94 multivalents per PMC (table 4). This result showed an important role of rye genotype in the intergeneric hybrid formation and the behaviour at meiosis (Shnaider, Priilinn, 1983, 1984; Shnaider, 1987, 1988).

To overcome incompatibility, the method of embryo cultures have also been applied. Our efforts in this direction has made it possible to increase the number of viable hybrid plants. In all cross combinations between the best 9 Nordic varieties (Bastion, Heta, Laari, Tjalve, Troll, Tähti, Satu, Runar, Luja) and *T. timopheevii* and *T. militinae* viable hybrids were obtained.

The positive results obtained by various in vitro methods and chromosome manipulations can help breeders to overcome many difficulties in wheat improvement. These methods can now be used to induced genetic variability, stabilize cultivated plants and improve economic traits.

Table 4. Meiotic pairing at MI in the different hybrids F_1

Cross combination and genomic formula	No. of cells observed	Bivalents per cell	Univalents per cell	Multivalents per cell	Chiasmata per cell
Chinese Spring (ABD) ×					

T. persicum (AB)	274	13.6	7.5	0.06	25.2
CS mutant ph1 (ABD) × *T. persicum* (AB)	178	13.0	6.0	0.75	25.9
Chinese Spring (ABD) × *T. timopheevii* (ABD)	130	7.2	18.3	0.63	12.0
CS mutant ph1 (ABD) × *T. timopheevii* (ABD)	243	8.3	14.7	1.10	15.4
Chinese Spring (ABD) × *T. militinae* (AG)	32	6.4	21.5	0.25	9.5
CS mutant ph1 (ABD) × *T. militinae* (AG)	313	8.7	14.7	0.85	13.2
Chinese Spring (ABD) × Petkus rye (R)	880	0.3	27.4	0	0.3
CS mutant ph1 (ABD) × Petkus rye (R)	169	4.6	15.3	0.46	7.2
CS mutant ph1 (ABD) × Kc-517/8 rye (R)	217	5.7	13.2	0.94	12.1
Chinese Spring (ABD) × *Ae. cylindrica* (AG)	153	4.8	23.7	0.30	8.4
CS mutant ph1 (ABD) × *Ae. cylindrica* (AG)	86	7.8	16.6	0.80	15.3

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