Acta Phytopathologica et Entomologica Hungarica 38 (3–4), pp. 267–273 (2003)

Development of Alloplasmic Lines of Spring Soft Wheat and Study of Their Resistance to Leaf Rust

N. A. KHAILENKO and A. I. SEDLOVSKIY

Institute of Plant Physiology, Genetics and Bioengineering Ministry of Education and Science, Republic of Kazakhstan, 480090 Almaty, Kazakhstan
e-mail: Khailenko@yandex.ru and gen_sai@mail.ru

Genetic and resistance studies were carried out on alloplasmic and backcross lines of spring soft wheat cultivars Leningradka and Saratovskaya-29. As a result of interspecific crossings some lines and hybrids exhibited cytoplasmic male sterility (CMS). Restorers of fertility and resistance to infections were also determined. The importance of use of alloplasmic lines for practical wheat breeding and for genetic studies is discussed.

Keywords: Wheat spp., alloplasmic line, backcrossing, leaf rust.

Creation of high-producing, disease resistant wheat cultivars that can also tolerate stress factors of environment has primary importance in many countries, including Kazakhstan where wheat is a steple crop. Most of the disease resistant cultivars and hybrids express resistant genes originated from wild forms of wheat. Thus, it seems important to preserve old cultivars and wild forms in gene banks.

The classical method of creating spring wheat cultivars resistant to fungal pathogens is still to perform crossings between species and genus of the tribe *Triticeae Dum.* and transfer the required genes to cultivated crops. In the past crossings have been made between wheat and rye as well as wheat and couch-grass to obtain rust resistant spring and winter wheat cultivars used for forage and grain production.

Remote hybridization was also applied to create alloplasmic lines. Alloplasmic hybrids F_7–F_n and lines BC_6–BC_8–BC_n have been analyzed for resistance to several phytopathogens. It is possible to analyse traits, determinated by only nuclear genes or plasmogenes, as well as traits controlled by two systems, including such traits as resistance to races of *Puccinia* (Palilova, 1986, 1990).

The use of interspecific and intergeneric hybrids of soft wheat with *Triticum boeoticum, T. timopheevii, T. durum, Aegilops squarrosa, Ae. speltoides* (F_3–F_5) in breeding hexaploid winter wheat for resistance in Poland resulted in selection of 237 forms with high multiresistance to leaf rust, powdery mildew an other infections, including 90 forms with complete resistance to *Septoria tritici* and *Fusarium graminearum* (Pilch et al., 1993). It was claimed that these forms could be used for improving resistance of hexaploid wheat to infections.

Georgieva et al. (1994) in Bulgaria transferred resistance genes to pathogenic races of *Puccinia* from *Aegilops* into a soft wheat genome. In this case the genome D of cultivar Avrora was substituted by genome U from *Ae. umbellulata* and *Ae. uniaristata.* Progenies of backcrosses were used for increasing resistance of cultivated wheat cultivars.

Recently, the idea to transfer resistance genes of barley to the soft wheat genome was also raised and the strategies for breeding rust resistant was discussed on the basis of genetic researches (Sawhney and Joshi, 1996).

Analysis of former publications (Doropheev, 1978) shows that the following species carry resistance genes to the pathogenic races of *Puccinia recondita Rob.* et *Desm.* f. sp. *tritici: Triticum boeoticum, T. sinskayi, T. turgidum, T. compactum, T. polonicum, T. aethiopicum.* Other Triticum species exhibited resistance to *P. graminis* f. sp. *tritici,* at least against the majority of races of stem rust. *T. timopheevii* and *T. kiharae* had complex resistance to many stem rust races. According to Guljaev (1975) *Triticum polonicum,* in spite of its excellent grain production, is very susceptible to several fungal infections. Furthermore, *T. macha* and *T. spelta,* which are typical mountain wheats with drought resistance and fertility restorer genes, exhibit resistance to several infections, including leaf and yellow rusts. Recently, a series of resistance genes have been identified. About 30–38 leaf rust resistance genes were located (Wilcoxon, 1971; Parleviet, 1988).

Our aim was to produce alloplasmic lines of wheat by crossing different species and genus of the tribe Triticeae and study their resistance to leaf rust *(Puccinia recondite).* Furthermore, our aim was also to produce high-producing new spring soft wheat cultivars.

Materials and Methods

The following species, cultivars and hybrids *(Table 1)* as well as alloplasmic and backcross lines of wheat were applied in our experiments: *Triticum timopheevii* Zhuk., *T. macha* Dek. et Men., *T. spelta vagelesdinked* v. *album* L., *T. aethiopicum* Jakubz., *T. dicoccoides* v. *vavilovii, T. kiharae* Dorof. et Miguch., *T. polonicum* v. *villosum, T. boeoticum* v. *thaodar, T. araraticum* v. *araxiacum thumanianis, T. dicoccum* v. *atratum, T. turgidum livitella* Razza v. *nigrobarbatum, T. compactum* Host., *T. sinskajae* A. Filat. et Kurk., *T. aestivum* L. (cultivars Leningradka, Saratovskaya-29, Kazakhstanskaya-126, Hostianum-88, Grecum-476); alloplasmic lines with the nuclear genome from cultivar Leningradka BC_5–BC_{18} on cytoplasms: *T. dicoccum* Schuebl. ex Vernal., *T. dicoccoides* Korn. v. *spontaneonigrum* Flaksb., *T. dicoccoides* Korn. v. *nudiglumis, T. polonicum* v. *chrisospermum, T. timopheevii* Zhuk., *Aegilops squarrosa* z. v. *typica, Ae. comosa* Sibtb. et Sm., *Ae. cylindrica* Host. v. *typica, Ae. ventricosa Tausch.* v. *vulgaris, Ae. variabilis* Eig. v. *intermedia* Eig. et Fein., *Ae. juvenalis;* hybrids F_1–F_9 and alloplasmic lines with the nuclear genome from cultivar Saratovskaya-29 on cytoplasms: *T. kiharae* Dorof. et Migusch., *T. spelta* L., *T. macha* Dek. et Men., *T. aethiopicum* Jakubz., *T. dicoccum* Schuebl. ex Vernal., *T. timopheevii* Zhuk. v. *typica* and etc.

The crossings of all species, cultivars, alloplasmic lines, hybrids, sterile analogs were conducted using the method of Udolskaya (1961) with modifications (Khailenko,

Table 1

Setting of seeds at interspecific hybrids of wheat

No.	Combination of crossings, genomes, origin of species and cultivars	Year	Quantity of		Percent of success	F_1
			flowers	seeds		
1.	*Triticum timopheevii* Zhuk. (A^bG, VIR) x Saratovskaya-42 (A^uBD, Saratov)	1988	60	19	31.66	sterile
2.	*T. timopheevii* Zhuk. (A^bG, VIR) x Kazakhstanskaya-126 (A^uBD, KazRIA)	1988	28	1	3.57	sterile
3.	*T. timopheevii* Zhuk. (A^bG, KazRIA) x Saratovskaya-29 (A^uBD, Saratov)	1988	162	62	38.27	sterile
4.	*T. timopheevii* Zhuk. (A^bG, KazRIA) x Saratovskaya-29 (A^uBD, Saratov)	1989	234	170	72.65	sterile
5.	*T. timopheevii* Zhuk. (A^bG, VIR) x Saratovskaya-29 (A^uBD, Saratov)	1989	302	178	56.62	sterile
6.	*T. timopheevii* Zhuk. (A^bG) x Saratovskaya-29 ((A^uBD, Saratov)	1989	136	59	43.38	sterile
7.	*T. timopheevii* Zhuk. (A^bG, VIR) x Siete Cerros (A^uBD, Bulgaria)	1989	32	11	34.37	sterile
8.	*T. timopheevii* Zhuk. (A^bG, VIR) x Kazakhstanskaya-126 (A^uBD, KazRIA)	1989	306	87	28.43	sterile
9.	*T.dicoccoides* v. *vavilovii* Koêrn. (A^uB, VIR) x Gostianum-88 (A^uBD, IPPGB)	1989	126	82	65.08	sterile
10.	*T. aethiopicum* Jakubz. (A^uB, VIR) x Gostianum-88 (A^uBD, IPPGB)	1989	316	129	40.82	sterile
11.	*T. aethiopicum* Jakubz. (A^uB, VIR) x Grecum-476 (A^uBD, IPPGB)	1989	122	2	1.63	ruin
12.	Kazakhstanskaya-3 (A^uBD, KazRIA) x *T. timopheevii* Zhuk. (A^bG, VIR)	1989	90	22	24.44	sterile
13.	Saratovskaya-29 ((A^uBD, Saratov) x *T. timopheevii* Zhuk. (A^bG, RIPC)	1989	237	22	9.28	sterile
14.	*T. timopheevii* Zhuk. (A^bG, VIR) x T. Zhukovskyi Men. et Er. (A^bA^bG, KazRIA)	1989	110	4	3.64	sterile
15.	*T. timopheevii* Zhuk. (A^bG, VIR) x Saratovskaya-29 (A^uBD, Saratov)	1990	64	16	25.00	sterile
16.	*T. timopheevii* Zhuk. (A^bG, VIR) x Leningradka (A^uBD, Sankt-Peterburg)	1990	104	36	36.62	sterile
17.	*T. macha* Dek. et. Men. (A^uBD, 6-4, VIR) x Saratovskaya-29 (A^uBD, Saratov)	1990	96	28	29.17	h/sterile
18.	*T. spelta L.* (A^uBD, VIR) x Saratovskaya-29 (A^uBD, Saratov)	1990	272	174	63.97	h/fertile
19.	*T. aethiopicum* Jakubz. (A^uB, VIR) x Saratovskaya-29 (A^uBD, Saratov)	1990	22	9	40.90	ruin
20.	*T. aethiopicum* Jakubz. (A^uB, VIR) x Gostianum-88 (A^uBD, IPPGB)	1990	100	15	15.00	sterile
21.	*T. aethiopicum* Jakubz. (A^uB, VIR) x Grecum-476 (A^uBD, IPPGB)	1990	106	62	58.49	sterile
22.	*T.dicoccoides* v. *vavilovii* Koêrn. (A^uB, VIR) x Grecum-476 (A^uBD, IPPGB)	1990	54	5	9.26	sterile

Table 1 (cont.)

No.	Combination of crossings, genomes, origin of species and cultivars	Year	Quantity of		Percent of success	F_1
			flowers	seeds		
23.	*T. kiharae* (A^{b}GD, VIR) x Gostianum-88 (A^{u}BD, IPPGB)	1992	76	56	73.68	sterile
24.	*T. kiharae* (A^{b}GD, VIR) x Grecum-476 (A^{u}BD, IPPGB)	1992	90	31	43.45	sterile
25.	*T. kiharae* (A^{b}GD, VIR x Kazakhstanskaya-126 (A^{u}BD, KazRIA)	1992	24	8	33.33	sterile
26.	*T. kiharae* (A^{b}GD, VIR) x Saratovskaya-29 (A^{u}BD, Saratov)	1992	234	127	54.27	sterile
27.	Saratovskaya-29 ((A^{u}BD, Saratov) x *T. kiharae* (A^{b}GD, VIR)	1992	102	22	21.57	fertile
28.	*T. araraticum* Jakubz. (A^{b}G, VIR) x Saratovskaya-29 (A^{u}BD, Saratov)	1995	70	32	45.71	sterile
29.	*T. boeoticum* Boiss. (A^b, VIR) x Saratovskaya-29 (A^{u}BD, Saratov)	1995	56	40	71.43	ruin
30.	*T. sinskajae* A. Filat. et. Kurk. (A, VIR) x Saratovskaya-29 (A^{u}BD, Saratov)	1995	92	3	3.26	sterile
31.	*T. aethiopicum* Jakubz. (A^{u}B, VIR) x Saratovskaya-29 (A^{u}BD, Saratov)	1995	76	22	28.95	sterile
32.	*T. aethiopicum* Jakubz. (A^{u}B, VIR) x Gostianum-88 (A^{u}BD, IPPGB)	1995	122	100	81.97	sterile
33.	*T. dicoccum Schuebl.* (A^{u}B, VIR) x Saratovskaya-29 (A^{u}BD, Saratov)	1995	62	0	0	–
34.	*T. turgidum* L. (A^{u}B, VIR) x Saratovskaya-29 (A^{u}BD, Saratov)	1995	78	34	43.59	fertile
35.	*T. boeoticum* Boiss. (Ab, VIR) x Saratovskaya-29 (A^{u}BD, Saratov)	1996	34	25	73.53	sterile
36.	*T. sinskajae* A. Filat. et. Kurk. (A, VIR) x Gostianum-88 (A^{u}BD, IPPGB)	1996	50	0	0	–
37.	*T. sinskajae* A. Filat. et. Kurk. (A, VIR) x Saratovskaya-29 (A^{u}BD, Saratov)	1996	29	4	13.79	sterile
38.	*T. turgidum* L. (A^{u}B, VIR) x Saratovskaya-29 (A^{u}BD, Saratov)	1996	74	23	31.08	fertile
39.	*T. aethiopicum* Jakubz. (A^{u}B, VIR) x Saratovskaya-29 (A^{u}BD, Saratov)	1996	22	13	59.09	fertile
40.	*T. compactum* Host. (A^{u}BD, VIR) x Saratovskaya-29 (A^{u}BD, Saratov)	1996	92	33	35.87	fertile
41.	*T. compactum* Host. (A^{u}BD, VIR) x Gostianum-88 (A^{u}BD, IPPGB)	1996	122	67	54.92	fertile
42.	*T. polonicum* L. (A^{u}B, VIR) x Saratovskaya-29 (A^{u}BD, Saratov)	1996	98	56	57.14	sterile
43.	*T. polonicum* L. (A^{u}B, VIR) x Grecum-476 (A^{u}BD, IPPGB)	1996	80	38	47.50	sterile

1972, 1995). Five–fifteen ears were emasculated and pollinated for each combinations. Ears of sterile analogs of cultivars Leningradka, Saratovskaya-29, Kazakhstanskaya-126, Hostianum-88, Grecum-476 and sterile hybrid plants of all generations were pollinated without emasculation. For this purpose individual parchment insulators were dressed on large part of ears. Ears of mother cultivars were pollinated with the help of the twel-method, with paring or without paring of scales. Pollinations were conducted 2–3 times within 10–15 days at the maturing of stigmatas of flowers. Fertility of pollen in flowers of sterile plants and hybrids was checked before pollination.

Resistance of alloplasmic lines and hybrids of spring soft wheat to phytopathogens was checked in model experiments on the fields KazRIA (The Kazak Research Institute of Agriculture) and SRAI (Scientific Research Agriculture Institute) during 1996–1999. An evaluation of plants for resistance to leaf rust was conducted by conventional methods (Parleviet, 1988).

Selection and analysis of promising forms and lines of wheat, presumable resistant to leaf rust, were conducted annually. New hybrid forms, bearing genes of resistance to various races of *Puccinia recondita* and *Septoria tritici* were created. Wheat species from the tribe Triticeae, having positive characters (such as precocity, resistance to phytopathogens and unfavorable environmental factors) were applied in crossings *(Table 1).*

The alloplasmic lines of cultivars Leningradka and Saratovskaya-29 on 18 cytoplasms of the species *Triticum* and *Aegilops* were selected and sown in experimental plot of IPPGB (Institute of Plant Physiology, Genetics and Bioengineering), and also in KazRIA and SRAI in 1996–1999. About 500 lines were investigated in total. The phytopathological evaluation were conducted by the expert groups of KazRIA and SRAI.

For studying the segregation of resistance to leaf rust of the hybrid material, collections of alloplasmic and backcross lines were divided into equal parts and were sown in KazRIA and SRAI on the field in rust nursery and on control isolated plots with natural background in IPPGB. After harvest, all the date were analysed statistically.

Results and Discussion

Collection of alloplasmic lines of spring soft wheat have been made in Kazakhstan since 60 years and more than 7000 lines are registered in the IPPGB. The majority of these lines exhibited high resistance to leaf rust in long-term experiments under natural conditions.

Collection of alloplasmic lines exhibited resistance to all races of *Puccinia recondita* as well as to *Septoria tritici* under natural conditions in plots of IPPGB. In rust nursery under artificial inoculations up to 90 per cent of plants exhibited resistance to all of the races of *Puccinia* and *Septoria.* These experiments were conducted during 1996. In 1997 selected families of alloplasmic lines of wheat, which showed resistance to leaf rust in rust nursery of KazRIA, were marked. Ten–twelve plants were marked per each combination.

It was established that both moderately rust resistant and moderately susceptible plants occurred among the tested plants of cultivars Saratovskaya-29 and Leningradka.

The alloplasmic lines of *T. aestivum* on cytoplasms *T. dicoccum, T. timopheevii, T. kiharae* and *Ae. cylindrica* were also characterized by moderately resistant plants, while on cytoplasm from *T. polonicum, T. kiharae, T. timopheevii* plants exhibited moderately susceptible reaction. Only one plant from the combination of *T. kiharae* × Saratovskaya-29 BC_3 (F_6) exhibited full resistance to rust. The remaining plants were scored as 2/5–2/10. It was concluded that plants from all of the combinations were rather susceptible except *T. kiharae* × Saratovskaya-29 and CT *T. polonicum*/Leningradka × Saratovskaya-29. One can suppose that in this material genes or gene modifiers coding unspecific resistance to leaf rust are present.

On the basis of our model experiments it seems possible to use small samples and individual plants for genetic and breeding plans associated with diseases resistance. In breeding for disease resistance one can create new rust resistant spring wheat cultivars by using the first hybrid generations and backcrosses, which in regular work are rejected. The statistical analysis of the element of the yield structure has shown that the number of grains in fertile ears of alloplasmic lines did not exceed 50 per cent in the rust nursery, however this number reaches 85–90 per cent in ears of fertile plant from plots where local epidemics did not occur. In plants from these letter plots grains were larger than in plants from the rust nursery and they had smooth glassed endosperm and normal embryo. However, grains from the rust nursery had puny and pursy endosperm and poorly developed embryo. During the 4-year-experiments in the rust nursery rust resistance of some lines increased, particularly in the second and third years. One can suppose that, as a result of infection, some plants of the alloplasmic lines increased their resistance.

Some alloplasmic lines from the combination *T. kiharae* × Saratovskaya-29 exhibited complex resistance to several diseases. It has large glassy grains and has the phenomenon of CMS. Some plants of these combinations have restorers of fertility.

In experiments conducted in 1998 and 1999 cultivars Leningradka and Saratovskaya-29 turned to be more susceptible to leaf rust in the nursery. This could be explained by the appearance of new races of the fungus in 1999. However, under natural conditions both cultivars were free from infection of leaf rust.

In the combination CP. *Ae. cylindrica*/Leningradka × Saratovskaya-29, BC_7F_{10} all plants were susceptible in 1999 in the rust nursery, however in 1997 and 1998 plants of this combination exhibited resistance.

In combinations *T. kiharae* × Saratovskaya-29 BC_6F_9 and *T. kiharae* × Saratovskaya-29 (BC_3 + free pollination)F_9 we observed segregation to resistant, moderate resistant, moderate susceptible and susceptible forms. This makes it possible to select genes for resistance even under this conditions.

As regards the combination CP. *T. timopheevii*/Leningradka × Leningradka BC_{18} (BC_7F_{11}) and CP. *T. timopheevii*/Leningradka × Saratovskaya-29 plants exhibit mainly susceptibility, however, we found some resistant plants. Their resistance did not segregate during the 4-year-experiment in the nursery. Under non-infectious circumstances in the plot of IPPGB these hybrid combinations turned to be resistant to leaf rust infection.

Similar results were obtained with following hybrid combinations: CP. *T. dicoccum*/Leningradka × Saratovskaya-29 (BC_7F_{10}), CP. *T. polonicum*/Leningradka × Saratovskaya-29 (BC_7F_{10}).

In conclusion, under mass infection by the leaf rust one can select resistant plants which subsequently segregate to resistant and susceptible forms. The degree of resistance of the alloplasmic lines depends mostly on nuclear genes, however only insignificantly on the cytoplasm in which the nucleus of the cultivar exists. Analysis of alloplasmic lines and cultivars has shown that under natural conditions in plots (in IPPGB) there was no infection by the leaf rust in spite of the fact that these plants were grown in the rust nursery during 1996–1999. Ability for tillering and grain development seemed normal. Productive tillering and normal grain production were characteristic. During several generations the «rugosity of endosperm» of the seeds was seen in the combination *T. kiharae* × Saratovskaya-29. On the basis of these results one can conclude that in the alloplasmic lines there was no interaction between nuclear and cytoplasmic factors determining leaf rust resistance of wheat.

Literature

Doropheev, V. F. (1978): Wheat of the World. “Ear”, 438 p. (in Russian)

Georgieva, V., Todorova, M. and Ganeva, G. (1994): Studying the possibilities for introgression of genes for resistance to *P. recondita* from cultivars of *Aegilops* species in *T. aestivum* L. genome. Rep. 4 Nat. Symp. on Immunitet of Plants. Kim bolesti and neprijateli, Dobrich, 7–11. (in Bulgarian)

Guljaev, G. V. (1975): Private breeding of field cultivars. “Ear”, 436 p. (in Russian)

Khailenko, N. A. (1972): Creation and study of sterile analogs at wheat. Abstract of Ph.D. thesis. Alma-Ata, 23 p. (in Russian)

Khailenko, N. A. (1995): Remote hybridization of plants: study of alloplasmatical lines collection of wheat. Genebanks of plants and its use in breeding. Proceeding of International Meeting, Almaty, 74–82. (in Russian)

Palilova, A. N. (1986): Genetics Systems of Plants and Their Interaction. Minsk: Science and Engineering, 230 p. (in Russian)

Palilova, A. N. (1990): Genetics and Crop. Minsk: Harvest, 184 p. (in Russian)

Parleviet, J. E. (1988): Strategies for the utilization of partial resistance for the control of cereal rusts. In: N. W. Simmonds and S. Rajaran (eds): Breeding Strategies for Resistance to the Rust of Wheat. CIMMYT, Mexico, pp. 48–62.

Pilch, J., Kubara-Szpunar, L. and Glowaer, E. (1993): Przydatnosc niektorych miedzygatunkowych I meidzyrodzajewych mieszancow *T. aestivum* L. w hodowli odpornosciowej heksaploidalnejpszenicy ozimej. Biuletyn Inst.Hodowli i Aklimatyzacji Roslin. N 187, 7–12. (in Polish)

Sawhney, R. N. and Joshi, B. C. (1996): Genetic research as the valid base of strategies for breeding rust resistant wheat. Genetica 97, 243–254.

Udolskaya, N. L. (1961): Selection of Spring Wheat. Alma-Ata: Kazgosizdat, 164 p. (in Russian)

Wilcoxon, R. D. (1971): Genetics of slow rusting in cereals. Phytopathology 61, 989–993.