

ITEMS FROM ROMANIA

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Assessment of winter wheat cultivars for resistance to Fusarium head blight.

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Fusarium head blight or scab is a fungus disease that has become of increasing concern in most of wheat-growing areas. Yield losses from FHB are due to sterility of the florets and formation of shriveled kernels with light test-weight. FHB-infected grains may be downgraded by the market. The disease not only causes yield and quality losses, but also may be associated with mycotoxins that are dangerous for human or animals health.

Disease incidence is related to wet and warm weather at flowering. Loose smut is seedborne and, therefore, widely distributed. Seed treatment with fungicide and certification of subsequent seed generations should ensure low incidence of this disease. However, FHB occurs frequently in humid wheat-growing regions such as Transylvania in Romania. Damaging levels of disease occur about one year in ten. Although, a certain degree of control can be achieved by cultural practice and using fungicides, growing resistant cultivars is the most practicable and effective means of reducing disease incidence. Evidence suggests that the current cultivars available to growers are more or less susceptible. FHB resistance is a trait that is subject to a large 'genotype x environment' interaction. Screening data based on naturally infected nurseries is notoriously unreliable because of spatial and temporal heterogeneity leading to substantial variation in the relative resistance status of genotypes.

We wanted to assess the FHB-resistance status of adapted cultivars currently grown in the Transylvanian region (Table 1). These cultivars were tested at ARDS–Turda under artificial inoculation in a field nursery over two growing season (2001 and 2003). The cultivars were included in the Fusarium-screening nursery, where each entry consists of 2 rows, 1-m rows with 25 cm between rows. Inoculation was made with local ecotypes of *F. graminearum*. Isolates were obtained by culturing naturally infected wheat grains. Following purification and assessment for aggressiveness, highly aggressive isolates were increased on liquid Czapek-Dox medium. The isolates were cultured for 7 days under continuous aeration from an aquarium pump through a sterile filter. After 7 days, the liquid media containing a mixture of conidia and mycelia was homogenized by a mixer and adjusted with distilled water to approximately 50,000 spores/ml. For point inoculation, ten heads/plot (cultivar) were chosen at anthesis and 0.5 ml of inoculum was injected by a medical syringe directly through the glumes into a central floret of two spikelets at the middle of the spike (one on each side). The inoculated spikes were marked with colored clips. Disease progresses up and down from the central infected spikelets and may infect up to all spikelets in a spike when weather conditions are favorable and the genotype is susceptible to FHB.

Disease severity was assessed in the point-inoculation treatment (marked heads) by counting the number of visually diseased spikelets 10 and 20 days after inoculation in relation to the total number of spikelets of the respective head. The result was percent infected spikelets. The results of observations were used to compute AUDPC for each cultivar in each of the 2 years. AUDPC was calculated according to the function:

$$\text{AUDPC} = \sum_i^{n-1} [(y_i + y_{i+1})/2](t_{i+1} - t_i);$$

in which n is the number of assessment times (minimum 2), y is the disease measurement, and t is the time (days) from inoculation.

The level of disease recorded in this experiment as measured by FHB severity and AUDPC for the eight currently-grown adapted cultivars showed a moderate to highly susceptible status being conferred on them (Table 1).

FHB severity was recorded 20 days post inoculation and represents the mean scoring of ten inoculated heads/cultivar in 2001 and 2003. Despite the fact that no highly resistant cultivars were found, there are large differences in reaction to FHB of the studied cultivars. Three of the analyzed cultivars (Ariesan, Apullum, and Ardeal) reached the highest level of severity (100 %). However, Turda 95 and especially Dumbrava (see Ann Wheat Newslet 50:102-103) scored lower than the mean FHB severity (86 % and 67 %, respectively).

Table 1. Mean *Fusarium* head blight severity and AUDPC of recommended winter wheat cultivars for Transylvania region in inoculated treatments at ARDS Turda (2001 and 2003).

Cultivar	Mean severity (%) 20 days after inoculation	AUDPC		
		Mean	Range	
			Minimum	Maximum
Ariesan	100	1,301	1,291	1,310
Fundulea 4	97	1,234	995	1,472
Turda 2000	99	1,226	1,145	1,306
Transylvania	92	1,150	1,045	1,255
Apullum	100	1,097	980	1,214
Ardeal	100	1,095	1,090	1,099
Turda 95	86	884	695	1,073
Dumbrava	67	430	405	454

Mean AUDPC, which reflects both severity and duration of disease, showed the same situation in the response to FHB inoculation of the studied genotypes. In this respect, all of recommended cultivars seem to be highly susceptible to FHB, except Turda 95 and Dumbrava, with lower means for AUDPC values (884 and 430, respectively). The mean level of disease expression shown by a genotype can be considered as a reflection of the effectiveness of the resistance genes carried by that genotype. From the standpoint of wheat breeders or wheat producers, cultivars with lower mean level of disease are of greatest interest, because they possess great capacity to limit disease expression.

The stability of a cultivar’s response to FHB inoculation also is important. Therefore, in the search for recommended cultivars approximately equal importance is given to the magnitude and the stability of reaction to the disease. In this study, the stability of performance response to FHB was measured by ranking the cultivar on the basis of the distance between the minimum and maximum limits of AUDPC (Table 1). According to this criterion, the most stable cultivars in their reaction to FHB appear to be Ardeal, Ariesan, and Dumbrava, which had a smaller distance between the range limits of AUDPC. Ariesan and Ardeal are stable in their susceptibility; Dumbrava is stable in that it is consistently moderately resistant to FHB. The other analyzed cultivars are more or less variable in their reaction. We thought it interesting that the level of disease infection and the stability of Turda 95, which has a reduced mean of AUDPC, was highly variable in its reaction. However, the maximum range value of AUDPC is slightly smaller than the minimum range value of Ardeal, which was the most stable.

The fact that no current commercial wheat grown in Transylvania is resistant and only few (Dumbrava and Turda 95) have been found to have acceptable tolerance reaction to FHB is a compelling reason to intensify the breeding search for wheat resistance to this disease. Thus, a *Fusarium*-screening nursery has become an integral part of our wheat-breeding program, in which a lot of other cultivars and advanced breeding lines are annually tested for FHB resistance in the same way. Some of the previously tested genotypes with low disease expression have been identified as sources of resistant genes to FHB. These have potential as parental material in a crossing scheme aimed at the introgression of resistance genes into agronomically acceptable and highly productive winter wheat genotypes, allowing opportunity for recombinants with even higher FBH resistance than that of currently grown cultivars.

ITEMS FROM THE RUSSIAN FEDERATION**AGRICULTURAL RESEARCH INSTITUTE OF THE CENTRAL REGION OF NON-CHENOZEM ZONE****143026, Moscow region, Nemchinovka, Kalinina 1, Russian Federation.*****Previously unknown genes of soft wheat.***

V.G. Kyzlasov.

The author created new lines of soft spring wheat possessing unique features and properties (multipistillate and stamenless flowers and xenia-type caryopsis coloration) that have no analogs in world collections.

Plants with multipistillate flowers were selected (Kyzlasov 1996) from hybrid populations of a *T. aestivum*/*Ag. glaucum* cross. From two to five caryopses are formed in every flower of such wheat. The more caryopses formed in a flower, the smaller their size. The weight of the caryopsis in the lines with multipistillate flowers varies from 10 to 90 mg. The share of caryopses formed in a flower decreases as the number of pistils increases. The selected lines had multipistillate flowers more often in years of drought. The progeny of these lines consistently formed three stamens in a flower. The multipistillate feature is a result of duplicate action of three recessive genes in homozygote: $AA BB CC$ (monopistillate line) / $aa bb cc$ (multipistillate line) $\times F_1 Aa Bb Cc$ monopistillate line. Segregation in the F_2 is 63 monopistillate plants : 1 multipistillate plant. In subsequent generations, the segregation is monohybrid (3:1), dihybrid (15:1), and trihybrid (63:1).

Stamenless plants were found (Kyzlasov 1998) in matromorphic populations obtained by pollination of soft spring wheat with pollen of spring barley. No barley-type plants were found among the progeny examined. The spikes that form pistils instead of stamens are characterized by high degree of sterility of the pistillate sphere. Therefore, solitary caryopses are formed in these spikes after free windblown pollination. The absence of stamens in the flowers is maintained in reproduction at the heterozygous level and is inherited as a recessive trait: $aa bb cc$ (stamenless plant) / $AA BB CC$ (staminate plant) $\times F_1 Aa Bb Cc$ (staminate plant). Segregation in the F_2 is 63 staminate plants : 1 stamenless plant. The absence of stamens in the flowers is caused by the action of three recessive genes.

Among the progeny of stamenless plants, lines with fertile pollen and dark coloration of caryopses were selected (Kyzlasov 2001). Such coloration is inherited in the dark-caryopsis / light-caryopsis hybrids as a xenia-type feature. The peculiarity of this segregation is the fact that caryopses of different colors are formed within one spike of an F_1 -hybrid plant. Such caryopsis formation has never been observed previously in any known wheat. The xenia-type inheritance, which was observed in caryopsis coloration, also was found in 1,000-kernel weight. A separate sampling of differently colored caryopses from the same spike (Kyzlasov 2003) showed that the colored caryopses were heavier by 10–12 % than the colorless ones. After pollination by a dark caryopsis wheat, dark-colored caryopses formed in the flowers of maternal light-caryopsis lines. The weight of these hybrid caryopses also is higher than that of the light caryopsis, self-pollinated maternal plants. Among the F_1 plants, segregation by caryopsis color is 9 dark-colored caryopses : 7 light-colored. Dark coloration of caryopses appears in the phenotype as a result of complementary interaction of two hypostatic genes. In populations of the second and subsequent generations, dark-colored caryopses is inherited as monohybrid (3:1) or dihybrid (9:7). The dark-colored caryopses differ from normal by enhanced resistance to germination in the spike under damp conditions.

This research has revealed expression of three recessive genes of multipistillate flowers, three recessive genes of stamenless flowers, and two hypostatic genes of xenia-type caryopsis coloration in the phenotype of soft wheat.

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Apomictic development of seed in embryos of rye, soft wheat, and triticale.

V.G. Kyzlasov.

A single specimen of R-1 winter rye was found when planting the soft winter wheat W-1. Five hundred flowers of this plant were emasculated and pollinated by the pollen of soft winter wheat. Seed set was 38 (7.6 % of the number of pollinated flowers). The F₁ seed was sown in the autumn. All the plants overwintered successfully. No hybrid plants were found in the progeny. All the plants were matromorphic diploids (2n = 14) of rye. No depression, like that observed in rye inbreeding, was detected in the plants. Upon flowering, 1,000 flowers were emasculated and again pollinated with winter wheat. Sixty-nine caryopses formed in the pollinated flowers (6.9 % of the number of pollinated flowers).

The seed obtained after the second pollination by winter wheat were sown in a greenhouse. At flowering, all the plants appeared to be matromorphic progeny of rye. The results of two previous years of field experiments were exactly the same. The matromorphic plants of R-1 winter rye may be reproduced by means of pollination of their flowers by wheat pollen. No caryopses form in R-1 winter rye when emasculated and kept from pollination. In case of inbreeding, the percentage of seed set is 0–5 %. Thus, the genes of self-incompatibility in matromorphic progeny of R-1 rye function properly.

The diploid (2n = 14), matromorphic plants of R-1 winter rye created by the method described above differ by culm length (70–160 cm), 1,000-kernel weight (23–33 g), flowering period (1–20 days), and seed shape. The obtained progeny indicate that the original R-1 rye plant was heterozygous. The mechanism of origin and embryonic development of matromorphic progenies still needs to be investigated. Embryos were formed without pollination; possibly originating from haploid cells of embryo sac after their fusion with one another or after diploidization. In the case of apomictic development of seed embryos, the caryopsis endosperm may be of hybrid origin (2n = 14 chromosomes of rye + 1n = 21 chromosomes of wheat). Apparently, somatogamy is manifested in this case.

Matromorphic plants also were discovered after pollinating of R-1 rye with triticale pollen. The fact that matromorphic plants arise after pollinating of soft wheat and triticale by R-1 rye pollen is of interest for breeding. We next emasculated 520 flowers of F₁ soft wheat hybrids and pollinated them with R-1 rye pollen. Forty-one caryopses were obtained (7.9 % of the number of pollinated flowers). All the plants grown from these seeds were diploid (2n = 42). The F₂ hybrid families reproduced after pollinating of F₁ hybrids by R-1 rye pollen did not segregate. This fact indicates that they may be dihaploids. We need to investigate the fertilization and embryogenesis processes of soft wheat and triticale after pollinating of their flowers by R-1 winter rye pollen.

Cytological analysis of pollen grains of several R-1 rye plants showed that they have adequate nutrient reserves. However, vegetative nuclei and sperm are missing in most. Only some plants possess one, or occasionally two, sperm. Viable pollen grains (a vegetative nucleus + two sperms) are nearly absent. In further investigations, more detailed studying of the anatomic peculiarities of R-1 rye pollen grains is planned.

Apomictic development of embryos in soft wheat and triticale is caused by pollination of the castrated flowers with R-1 rye pollen. Matromorphic R-1 rye plants appear after pollination of the flowers by soft wheat or triticale pollen. Investigations aimed at enhancing the technology for reproducing soft wheat, rye, and triticale matromorphic plants will continue. Identifying the genetic factors determining formation of embryos without pollination is still a problem. The regularities of embryo and endosperm formation during the process of embryonic development of the matromorphic plants is needed. Our process for obtaining matromorphic plants may eventually be applied for creating soft wheat and triticale lines that are homozygous for all the genes. In rye breeding, this technology may be applied for selection of lines with a high combining ability.

Induction of doubled haploids in common wheat and its hybrids.

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We continued our long-term effort to development genetic stable homozygote material for wheat improvement in 2002–04. Our objective is to produce dihaploids of spring and winter wheat and its hybrids with *Ae. speltooides* and *Agropyron erectus*. The 49 accessions include 30 F₁ of spring wheat, 15 F₁ of winter wheat, three hybrids with *Ae. speltooides*, and a hybrid with *Agropyron*. We obtained DH lines by two methods. We started to use the ‘wheat/maize’ DH system in 2002. Emasculated wheat and hybrid heads were hand-pollinated with mature maize pollen. Embryos were excised 12–14 days after pollination and cultured in tubes containing nutrient agar medium B5. The resulting seedlings were immersed in a 0.1 % colchicine solution, rinsed, and transplanted into pots with soil in greenhouse. This process was repeated in 2003–04 with eight accessories. We also use anther and microspore culture technique for DH production. Wheat and hybrid anthers were isolated and immersed in a PII nutrient-agar medium for 20–30 days. Embryo-like structures were transplanted onto 192 medium with 0.5 mg/l kinetin. The resulting plantlets were then immersed in a 0.1 % colchicine solution, rinsed, and transplanted in pots in the greenhouse. These experiences were repeated in 2003–04. In total, we obtained four dihaploid lines by *in vitro* culture and 102 DH lines using the ‘wheat/maize’ DH system. We are now testing all our dihaploid lines in field plots.

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High-yielding biotype of spring durum wheat cultivar Valentina.

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Protein markers are a simple and reliable way to identify crop plant genotypes that are some times difficult to distinguish by morphological or other characteristics. In wheat-breeding programs, the analysis of gliadin proteins make it possible theoretically to distinguish up to 20 millions genotypes. Moreover, the quest for new protein markers linked to useful traits is of great interest, because these proteins may be used in improving cultivars for desirable properties, especially in early generation screening. We have analyzed the gliadin electrophoretic spectra of the spring durum wheat cultivar Valentina, which was released for use in 1998. This cultivar was selected from the cross ‘Saratovskaya 59/Leukurum 1897 (S₃F₆)/D-1973/Saratovskaya zolotistaya’. Valentina inherited the best characteristics of Saratov spring durum wheat genotypes; increased resistance to loose smut and barley yellow dwarf virus, lodging and drought resistance, early maturity, high grain quality, and high yield capacity (3.1–3.4 t/ha).

Gliadin proteins were extracted with 70 % ethanol from distal half of the seed and fractionated by PAGE using aluminium lactate buffer (pH 3.1). Although Valentina is homogenous for morphological characters, it is polymorphic for biotypes (designated Valentina I and Valentina II) occurring in a ratio of approximately 1:1. These biotypes differ from one another in gliadin components of α - and ω -zones that are controlled by genes on chromosomes 6A and 1B, respectively. The embryo half of the seeds were used for propagation and seed production during the 2 years. Both biotypes were estimated for morphological and useful traits for 3 years, using plots 2.4 m² (4x multiplication). Plants of both biotypes did not differ for morphological characters but were considerably different in head-producing capacity (Table 1, p. 102).

The data revealed that the increase in all Valentina II indices influenced head productivity compared with biotype I. As a result, grain yield in biotype II considerably exceeded that of Valentina I and the parental cultivar in all years tested (Table 2, p.102). Biotype II has been revealed to have stronger gluten (according to higher index of SDS-sedimentation) that of biotype I (Table 2, p. 102).

Table 1. Characterization of the spike productivity in Valentina biotypes. Data are average values from 2001–03.

Biotype	Spike length (cm)	No. of spikelets/spike	Grain weight/spike (g)	No. of grains/spike	No. of grains/spikelet	1,000-kernel weight, (g)
Valentina I	16.8	15.1	1.80	36.6	2.42	49.1
Valentina II	19.9	16.1	2.17	39.1	2.43	55.5
LSD _{5%}	0.8	0.9	0.20	1.9	0.30	2.5

Table 2. Yield capacity and micro-SDS test of Valentina biotypes.

Biotype	Yield (t/ha)				Micro-SDS test (ml)			
	2001	2002	2003	Average	2001	2002	2003	Average
Valentina	3.9	3.5	3.9	3.8	51	50	55	52.0
Valentina I	3.2	3.2	3.2	3.2	41	41	45	42.3
Valentina II	4.1	3.7	4.2	4.0	50	50	57	52.3
LSD _{5%}				0.18				2.2

In durum wheat-breeding programs, gliadin electrophoresis is used not only for cultivar identification, but as a small-scale breeding tool to predict cooking quality. The gliadin protein, designated γ -45 is well known to be associated with strong gluten and, hence, good pasta-cooking quality, whereas another protein, γ -42, is correlated to weak gluten and poor pasta quality. These two components are alleles of a gene on chromosome 1B. The analysis of gliadin proteins has shown the presence of γ -

45 in both Valentina biotypes. This fact agrees with SDS-sedimentation values; the average value of this parameter in Valentina I is greater than 40 ml. This data indicates strong durum wheat gluten. The higher SDS-sedimentation values in the Valentina II biotype are probably linked to increased expression of the gene controlling synthesis of proteins coupled with cystron γ -45. On the other hand, we now know that genes for γ -45 and γ -42 are linked tightly to different blocks of ω -gliadin. Thus, quantitative differences in ω -gliadin composition of two Valentina biotypes might be the cause of differences in gluten properties.

Biotechnological approaches in developing wheat-rye hybrids.

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Rye was used in bread wheat selection and triticale genome reconstruction by *in vitro* techniques. To increase the effectiveness and acceleration of breeding, different methods of organ and tissue culture are used. Our principle objective was to obtain qualitatively new wheats and triticales that were ecologically adapted (drought- and frost resistant).

Using embryo rescue followed by colchicine treatment, primary hexaploid and octoploid triticales, genomic constitution AABBRR and AABBRRDD, respectively, were developed using Saratov winter bread wheat cultivars Atkara, Gubernia, Saratovskaya ostistaya, and Saratovskaya 90 and the rye cultivars Saratovskaya 5, Saratovskaya 6, Saratovskaya 7, Krasnokoloska, and Marusenka. Secondary triticales were created on the base of intra- and intergenomic recombinations via crossing of the primary hexaploid and octoploid triticales with each other and with the best cultivars. Combining traditional and biotechnological methods allows us to create hexaploid triticale with substitution of some rye chromosomes with wheat D-genome chromosomes.

Doubled haploid plants were obtained on the base of the primary hexaploid and octoploid triticales through anther culture. Maximum embryogenesis was with 300 mg/l glutamine added to the inductive nutrient medium. Protein markers were used in the genetic analysis of the developed triticales, allowing us to study chromosomal fragments in amphidiploids and control substitutions in the breeding process.

Genetic and cytogenetic research of new spring bread wheat–*Ae. speltoides* lines.

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The gene pool of *Ae. speltoides* is very useful for bread wheat breeding for resistance to leaf rust. At present, the following *Lr*-genes have been identified: *Lr28* (T4AS·4AL-7S#2S), *Lr35* (2B), *Lr36* (6BS), *Lr47* (Ti7AS-7S#1S-7AS·7AL, T7AS-7S#1S·7S#1L, and Ti7AS·7AL-7S#1L-7AL), and *Lr51* (T1AS·1AL-1S#F7-12L-1AL and T1BS·1BL-1S#F7L-1BL).

At ARISER, the bread wheat–*Ae. speltoides* lines were kindly provided by Dr. Odintsova (N.I. Vavilov Research Institute of Plant Industry, St. Petersburg). The *Lr* gene(s) of bread wheat–*Ae. speltoides* lines are highly effective to the Saratov population of *P. triticina*, IT = 0, 0;. These lines have a strong 'cuckoo' effect. We crossed these lines were with Saratov-bred cultivars and lines of bread wheat. After crosses with cultivars L503 and Dobrynya, the cuckoo effect significantly decreased. In segregating F₂ populations of these hybrids for resistance to leaf rust, susceptible plants were observed. Thus, the cultivars L503 and Dobrynya have gene(s) for suppression of gametocidal activity. The C-banding pattern of these lines showed the translocation T2D-2S. We confirmed the presence of the translocation by meiotic analyses of the F₁ hybrids between bread wheat–*Ae. speltoides* lines and the leaf rust-susceptible cultivar Saratovskaya 29. In the majority of PMCs, we detected 21 bivalents but observed univalents, trivalents, and quadrivalents in a few PMCs. The identification of *Lr* gene(s) in bread wheat–*Ae. speltoides* lines will be provided in 2005.

The effects of alien *Lr*-gene combinations.

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The effects of alien *Lr*-gene combinations on a set of NILs was studied in 2003–04. These NILs have the combinations alien translocations *Lr9+Lr19*, *Lr19+Lr25*, and *Lr19+Lr26* in the genetic background of cultivars L503, Dobrynya, and line L2032. These combinations of *Lr* genes are highly effective against the local population of leaf rust (infection type 0; in Dobrynya, L503, and line L2032 the IT = –3). In 2003 and 2004, the majority of leaf rust-resistant isolines had no difference in grain yield from the check cultivars and lines, except for pairs containing *Lr19+Lr26* in L503 and line L2032 backgrounds (in the 2004 the grain yield was higher) and *Lr19+Lr9* in line L2032 background (grain yield lower) (Table 3). For

grain test weight, differences between cultivars Dobrynya, line L2032, and their NILs with *Lr9+Lr19* were observed. The NILs had significantly lower parameters. For grain protein content, differences were observed only in 2004. A possible cause of these results is moderate epidemics of leaf rust during the grain-filling period. The significant increase in grain-protein content was detected for combinations of *Lr19+Lr25* (in Dobrynya) and

Table 3. Agronomic performance of cultivars and isolines containing various leaf rust gene combinations in 2003 and 2004 at the Agricultural Research Institute for the South-East Regions, Saratov, Russian Federation.

Cultivar/NIL	Grain yield (t/ha)		Test weight (g/l)		Protein content (%)		SDS volume (ml)	
	2003	2004	2003	2004	2003	2004	2003	2004
Dobrynya <i>Lr19</i>	4.8	3.4	799	782	15.12	15.77	92	91
Dobrynya <i>Lr19+9</i>	4.7	3.3	787	775	14.90	16.40	95	95
Dobrynya <i>Lr19+25</i>	4.8	3.3	800	785	15.90	16.70	95	95
L503 <i>Lr19</i>	4.7	2.7	791	781	15.70	15.20	92	87
L503 <i>Lr19+26</i>	4.4	3.0	775	777	14.90	16.50	77	72
L2032 <i>Lr19</i>	4.8	2.8	792	771	14.25	15.80	88	90
L2032 <i>Lr19+26</i>	4.8	3.3	798	780	15.73	16.45	81	83
L2032 <i>Lr19+9</i>	4.0	2.3	782	757	14.88	16.68	93	94
F*	5.06	15.01	6.41	9.4	1.67	3.91	2.78	7.34
LSD	0.40	0.28	10.03	6.65	NS	0.76	8.02	6.29

Lr19+Lr26 (in L503). For grain quality, the differences for SDS volume was obtained only between cultivars L503 and L2032 and NILs with of *Lr19+Lr26*.

Intraracial structure and variability in virulence to Ustilago tritici.

A.E. Druzhin, V.A. Krupnov, T.D. Golubeva, and T.V. Kalintseva.

The severity of susceptibility to loose smut in bread wheat cultivars depends on many factors, including the pathogenicity of the inoculum. We inoculated a set of bread wheat cultivars and lines with race 23 of *Ustilago tritici* for a period of 5 years and noted that the pathogenic reaction varied in highly susceptible cultivars, despite of optimum conditions for pathogen development.

To study the pathotype structure of *U. tritici* race 23, we selected the pathogen from spikes of cultivar L505. Inoculation of L505 was repeated two times to stabilize the race. This inoculum was used to infect differentials of four cultivars, L505, Saratovskaya 66, Saratovskaya 36, and Saratovskaya 60, and line L164 of bread wheat. After stabilizing the inoculum on these cultivars, we selected L505 isolate A, Saratovskaya 66 isolate B, L164 isolate D, Saratovskaya 36 isolate C, and Saratovskaya 60 isolate F. These isolates then were used to inoculate smut-resistant cultivars differing in *Ut* genes (Druzhin et al. 2004) and Russian and Canadian differential cultivar sets (Table 4). The isolates differed in their virulence. In the Canadian cultivars, isolates A and F were detected as race 18, but isolates C, B, and D obviously are new races that could not be identified (the Russian set of test cultivars has not proven useful for detecting virulence in isolates). Using of the Canadian set of test cultivars (number of differentials is 19), has allowed us to identify three new races, whereas the Russian set (consisting of nine cultivars) did not elucidate any new races.

Table 4. Level of susceptibility (%) to loose smut in bread wheat cultivars and lines after inoculation by isolates of race 23 of *Ustilago tritici*. Isolate A was selected from L505, B from Saratovskaya 66, C from Saratovskaya 36, D from L164, and F from Saratovskaya 60.

Cultivar/line	Race 23	Isolates				
		A	B	C	D	F
Zhiguljovskaya	7.7	13.3	4.8	41.7	51.7	0.0
Bezentshuokskaya 98	0.0	0.0	0.0	0.0	0.0	0.0
Saratovskaya 29	27.8	23.3	35.7	65.7	59.3	46.9
Saratovskaya 36	23.5	21.7	30.9	54.2	40.0	33.6
Saratovskaya 70	7.7	10.0	8.33	68.2	31.0	0.0
Saratovskaya 60	7.5	19.8	12.9	26.9	4.0	42.8
Belynka (L400)	32.0	32.0	21.1	55.4	31.0	32.0
L2040	7.8	14.3	10.0	24.0	0.0	6.5
L400/S60	14.0	14.5	8.6	24.6	15.5	14.0
L2772	13.3	4.8	0.0	35.6	35.9	12.7
L2358	0.0	0.0	0.0	4.8	0.0	0.0

Further stabilization (homozygous for virulence) of race 23 has led to a reduction in pathogen aggressiveness in cultivar L505 from which it was selected. The severity of infection in L505 after inoculation with race 23 is 92.8 %, with isolate A (a selection of race 23) 80.9 %, with isolate A1 (a selection from isolate A) 64.7 %, with A2 isolate (a selection from isolate A1) 35.7 %, and with isolate A3 (a selection from isolate A2) 25.6 %.

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Effects of *Lr* genes on resistance to preharvest sprouting.

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A significant reduction in the bread-making quality of spring and winter bread wheats in the Volga Region was produced by preharvest sprouting. In the last few years in this area, cultivars of spring bread wheat carry *Lr* genes from *T. turgidum* subsp. *durum*, *T. turgidum* subsp. *dicoccum*, and *Ag. elongatum*, but the *Lr* genes in winter wheat are from *S. cereale*. In 2003 and 2004 we studied the influence of genes *Lr9*, *Lr14*, *Lr23*, *Lr19*, *Lr25*, and *Lr26* on resistance to preharvest sprouting. This research was done on the spring bread wheats Belynka, Dobrynya, L 503. and L 2032 and their NILs differing for *Lr* genes. Spike sampling for germination tests began with a plant whose peduncle had turned yellow and with no green color in the glumes. Seeds were harvested and hand-threshed, dried, and stored under refrigeration at -20°C . Germination tests were made in a growth chamber at $20\pm 1^{\circ}\text{C}$ in the dark on filter paper in Petri plates containing 6 ml water. Results from 2 years of research did not reveal any significant difference for resistance to preharvest sprouting between the controls and the lines containing *Lr9*, *Lr14*, *Lr23*, *Lr19*, *Lr25*, and *Lr26*. The SDS-sedimentation volume tends to decrease in lines with the *Lr25* translocation; the decrease is significant decrease in lines with the *Lr26* translocation. In other lines, the differences trait were not significant.

Black point: influence on bread-making quality.

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In the Volga Region, black point of the embryo end of grain of spring bread wheat is observed annually. The symptoms are especially strong during seasons with rainy weather, high humidity, and sharp fluctuations of temperature during the period from spike formation to maturation, i.e., in 2003. However, in 2004, under warm and dry conditions during the same period, severe black point infections were observed. We examined the effect of black point on bread-making quality of four popular cultivars, L 503 and Dobrynya (red grains and resistance to preharvest sprouting), Saratovskaya 55 (white grains and moderately resistant), and Belynka (susceptible to preharvest sprouting). Seed was lightly infected with *Alternaria* spp. The possible cause of black point was the physiological influence of the vegetative conditions. The grains of each cultivar were divided into two groups, with (A) and without (B) black point (control). To analyze a grain, we studied the following parameters: 1,000-kernel weight, grain-protein content, SDS sedimentation, wet and dry gluten content, evaluation of strong gluten (index of gluten deformation), activity of α -amylase (falling number).

In 2004, the average grains yield of all bread wheat cultivars was smaller than in 2003 (Table 5). The mean of 1,000-kernel weight for seed infected with black point in 2003 and 2004 were significantly higher than the control. For grain-protein content, wet and dry gluten content of grain infected with black point in both years not differ from healthy grains. However, activity of

Table 5. Influence of black point grains on bread-making quality. Number with the same letter within a column are not significantly different at the 0.05 % probability level as determined by Duncan's Multiple Range test.

Cultivar		Yield (kg/ha)		SDS volume		α -amylase activity	
		2003	2004	2003	2004	2003	2004
Saratovskaya 55	Control	44.47	27.28	83 e*	91	361 cf	307 e
	Black point			82 de	83	282 b	246 cd
Belynka	Control	45.15	36.27	72 abc	79	290 bc	261 d
	Black point			69 a	74	170 a	151 a
L 503	Control	47.53	26.82	70 ab	77	368 f	279 dc
	Black point			70 ab	71	351 ef	264 d
Dobrynya	Control	47.89	32.58	75 bc	85	313 c	223 bc
	Black point			77 cd	87	271 b	191 b
Mean		46.26	30.73	75	80	301	240

α -amylase in the black-pointed grains was significantly higher than the control, especial in the Saratovskaya 55 and Belynka. A similar tendency was seen after evaluation of SDS volume.

New, spring bread wheat cultivars for the Volga River region of the Russian Federation.

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Wheat is a major cereal crop in Volga River region of the Russian Federation. Crop capacity fluctuates from 0.3 to 4.0 t/ha. The main stress factors are water deficiency, extremely high temperatures, fungal plant diseases, and pests. The breeding programs of ARISER aim to reduce abiotic and biotic stress influence on plant growth and increase yield capacity and grain quality. Plant breeders have been working these problems for the past 95 years.

Two new spring bread wheat cultivars have been released for use in 2004, **Saratovskaya 68** and **Saratovskaya 70**. Saratovskaya 68 has increased yield capacity compared with Lutescens 62, first cultivar developed. Yield gain is 0.72 t/ha

(Table 6). The yield gain is 3 times higher than the increase caused by environmental factors. Both Saratovskaya 68 and Saratovskaya 70 are characterized by higher grain yield values when compared with that of the check cultivar Lutescens 62, especially under extreme drought conditions. Yield gain is 60 and 35 % for Saratovskaya 68 and Saratovskaya 70, respectively) (Table 7). The higher yield capacity of Saratovskaya 68 mainly is due to increased grain yield from unit of cultivated area and number of seeds/head. This cultivar is tolerant to leaf rust and powdery mildew, and resistant to lodging and loose smut. Saratovskaya 70 has high values for 1,000-kernel weight, seed-volume weight, and fullness. This cultivar is resistant to loose smut and leaf rust. Both new spring bread wheat cultivars are recommended for growing in arid regions with frequent summer droughts.

Table 6. The role of plant breeding and environmental factors in yield increasing of spring bread wheat. Saratovskaya 68 is a new spring bread wheat cultivar for the Volga River Region of the Russian Federation released in 2005.

Cultivar	Years tested	Grain yield (t/ha)	Yield increase (t/ha)	Yield gain due to	
				Environmental factors	Plant breeding
Lutescens 62	1912–1926	1.24	—	—	—
Lutescens 62	1995–2004	1.46	0.22	23.0	—
Saratovskaya 68	1995–2004	2.18	0.72	—	77.0

Table 7. Grain yield (t/ha) of new spring bread wheat cultivars from the Agricultural Research Institute for South-East Regions of the Russian Federation in extremely dry and in favorable years.

Cultivar	Extremely dry years (1998,1999)	Favorable years (2000–04)	Average (1998–2004)
Lutescens 62	1.078	1.863	1.471
Saratovskaya 68	1.730	2.637	2.184
Saratovskaya 70	1.460	2.638	2.049

New, spring durum wheat cultivars for Volga River region of Russia.

N.S. Vassiltchouk, S.N. Gaponov, V.M. Popova, and G.I. Shutareva.

During 20 years of durum wheat breeding, ten cultivars had been produced that showed high values for semolina quality factors (on the world standard level). The descriptions of two newest cultivars are presented here.

Nik. Early and resistant to loose smut, Nik was produced for the conditions of the South-East Region of the Russian Federation. Nik is characterized by high gluten strength and a yellow pigment content equal to that of Saratovskaya

Table 8. Yield, 1,000-kernel weight, falling number, test weight, protein content, SDS-sedimentation test, and carotenoid pigment content of Nik and Zolotaya volna, new cultivars for the South-East Region of the Russian Federation, and some check cultivars. All data is the mean from plants grown at Saratov in 2002–04.

Cultivar	Yield (t/ha)	1,000-kernel weight (g)	Falling number (sec)	Test weight (g/l)	Protein content (%)	Micro-SDS-sedimentation test (mm)	Carotenoid pigment content (mg/kg)
Krasnokoutka 10	2.27	42.2	448	820	13.4	28	5.3
Saratovskaya zolotistaya	2.20	43.6	451	800	13.4	47	8.1
Nik	2.50	39.6	452	762	13.2	47	7.5
Zolotaya volna	2.57	36.2	446	775	13.5	51	7.4
LSD (5 %)	0.3	3.1	67	34	0.3	7	1.5

zolotistaya (Table 8). This cultivar has higher values for grain yield and falling number than the check cultivars Krasnokoutka 10 and Saratovskaya zolotistaya. Nik is resistant to lodging, true loose smut, and BYDV.

Zolotaya volna. The early, strong gluten cultivar Zolotaya volna is recommended for dry conditions of Russia Volga River Region. This cultivar was derived from crosses of local lines with Siberian cultivars. The protein and yellow pigment content of Zolotaya volna are equal to that of Saratovskaya zolotistaya (Table 8). The yield and micro-SDS-sedimentation tests Zolotaya volna higher than that of cultivars Krasnokoutka 10 and Saratovskaya zolotistaya. Falling number is near the check. Zolotaya volna is resistant to lodging because of strong straw. Zolotaya volna is resistant to true loose smut and highly tolerant to BYDV and leaf spot.

The breeding of winter bread wheat in arid Zavolzhje.

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At present, many plant breeders attempt to create universal cereal crop cultivars adapted to different growth conditions. Scientific practice, however, shows that a greater economical effect may be obtained when cultivars are developed for concrete climatic zones.

Zavolzhje (the left bank of Volga River, the most droughty agricultural area in the Saratov region) is remarkable for a sharp continental climate with cold, not snowy winters. The vegetative period is characterized by irregular soil moisture. The temperature total fluctuates during the growing season from 2,500°C to 3,100°C, whereas winter wheat needs 1,390°C–1,430°C. A considerable part of the thermal energy remains unused, its consumption by plants with moisture during the vegetative period.

Plant breeding experiments at the Ershov laboratory are done under both extremely dry and irrigated conditions using inorganic fertilizers. This scheme reveals lines with high-yielding power and resistance to lodging and the main diseases (leaf rust and powdery mildew). Lines are tested under dry conditions for resistance to natural abiotic and biotic stresses. This technology allows us to predict cultivar behavior under more favorable climatic conditions. The data from cultivar tests in 1996–2004 indicates that the new varieties to have the advantage over standard cultivars (Table 9).

Table 9. Average grain yield (hkg/ha) of winter bread wheat in the ecological test in 1996–2004 at the Ershov Experimental Station of Irrigation Farming, ARISER, Ershov, Russian Federation.

Cultivar	Irrigation farming	Dry farming
Donskaya bezostaya (Check)	61.0	32.3
Mironovskaya 808 (Check)	52.5	32.8
Ershovskaya 10 (regionalized)	65.1	34.5
Ershovskaya 11 (regionalized)	61.7	34.2
Levoberezhnaya 1 (regionalized)	65.2	36.9
Levoberezhnaya 2	63.6	37.8
Levoberezhnaya 3	66.3	37.9

The analysis of cultivar and line productivity in 1989–2000 shows that increases in yield capacity is, to great extent, because of increased productivity, and to smaller degree, to 1,000-kernel weight and larger spikes. The new cultivars Levoberezhnaya 1, Levoberezhnaya 2, and Levoberezhnaya 3 meet these requirements. Levoberezhnaya 2 and 3 are submitted to the state cultivar tests.

White-grained, winter bread wheats also are bred at the Ershov laboratory. This program is because of the great demand for these wheats. The market for Russian, white-grained wheats is for spring types. In 2003–04, two cultivars (Albidum cultivar)

Jangal and **Obereg** were submitted to the state variety tests (Table 10). Table 8 shows that white-grained cultivars are quite competitive with red-grained cultivars in the market-place.

Table 10. Average grain-yield capacity and quality values of the new white-grained winter bread wheat cultivars Jangal and Obereg, and Ershovskaya 10 (check) in 2002–04 from Ershov Experimental Station of Irrigation Farming, ARISER, Ershov, Russian Federation.

Cultivar	Grain yield (hkg/h)	Protein content (%)	Total hardness (%)	Wet gluten (%)	Flour strength (u.a.)	Loaf volume (cm ³)	Porosity marks
Jangal	59.8	15.6	80.0	32.6	285.0	773.0	4.7
Obereg	51.1	17.1	85.0	36.6	366.0	817.0	5.0
Ershovskaya 10	50.5	16.2	76.0	38.0	266.0	847.0	4.9

Ershov spring bread wheat cultivars.

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The zone of spring bread wheat cultivation in the southeast part of the Russian Federation is characterized by variability in weather, high temperatures during the growing period, and frequent hot winds. In these conditions, wheat cultivars are required to be drought- and heat-resistant, and tolerant to disease (especially to leaf rust), but have a high yield capacity (5–6 t/ha) and good, stable grain quality when favorable conditions exist.

Plant breeders at the Ershov Experimental Station, which is situated in the most droughty part of Saratov region, simulate agroecological conditions using different rates of watering and mineral fertilizers that allow selecting and evaluating spring wheat genotypes with yield capacity from 0.1 to 6.0 t/ha. The spring bread wheat cultivars created at the Ershov Station show high competitiveness and are widely distributed.

Prokhorovka. Tests have shown this cultivar to be lodging- and leaf rust-resistant and to have a multispiked and multiseeded head. Prokhorovka is capable of the highest grain yields in favorable conditions. The top grain yield (6.1 t/ha) exceeds that one of standard cultivar Saratovskaya 58 by 1.0–1.5 t/ha. Under drought and heat stress, the grain yield of Prokhorovka (1.0 t/ha) is equal to that of the best, drought-resistant cultivar Saratovskaya 55. Prokhorovka is recommended for use in seven regions of the Russian Federation, from Kuban to Volga River Region. The length of the growth period is 94–97 days. The cultivar has good grain quality and a protein content of 13–14 %.

Yugo-Vostochnaya 2. This cultivar is recommended for regions with black humus soil. The grain yield capacity is high, up to 6.0 t/ha. Yugo-Vostochnaya 2 is resistant to drought, heat, lodging and to main diseases. The cultivar has good grain filling with high volume weight. The red grain is of high quality (protein content is 13–14 %) and ripens in 96 days.

Yugo-Vostochnaya 4. Yugo-Vostochnaya 4 is recommended for dry conditions of Volga River region. This cultivar is drought and heat resistant with a top grain yield of 6.2 t/ha. This cultivar is tolerant to leaf rust and to loose smut and has white, coarse grain. Bread-making quality is high and protein content is 13–14 %.

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Selection problems of high quality soft spring wheat cultivars in far-eastern Russian Federation.

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One of the most important problems when breeding *T. aestivum* subsp. *aestivum* cultivars for the far-eastern Russian Federation is good technological and bread-baking qualities. Wheat breeders from the far-eastern region have developed 50 cultivars, including 20 that were entered in the Register of Selection Achievements of the Russian Federation. However, no cultivar that meets the parameters of strong wheat also is resistant to biotic and abiotic factors (drought, high humidity, and fungal diseases).

One of the reasons why we cannot develop a strong wheat cultivar is that there is not an integral wheat-quality index. We have to select a cultivar according to a great number of indicators to define technological properties of a cultivar (Pumpyanskey 1971). There are 20 indicators under the State Russian Standard (GOST).

Second, forming high-quality grain in the far-eastern region of the Russian Federation is limited by heavy precipitation, high temperatures, and high humidity (90–100 %) during the ripening stage. These negative factors increase fermentative process that leads to the decay of starch and protein and to further colonization of spikes and seed by saprophytic and semiparasitic fungus, because the secretion of soluble carbohydrates is a good nutrient medium. As a result, seed carbohydrate and protein is reduced, which leads to the loss of organic substances and deterioration of technological and baking qualities. This phenomenon is called enzyme and mycosis exhaustion in seeds (Shindin et al. 2004).

Despite these difficulties, wheat breeders of the far-eastern region have bred spring wheat cultivars that meet bread baking requirements (Table 1). **Lyra 98** is the best among four newest cultivars (the authors were part of the breeding team for this cultivar), but it does not meet the GOST's strong wheat requirements for three indexes (vitreous-

Table 1. Seed quality in spring *Triticum aestivum* cultivars bred for far-eastern Russian Federation by the Far Eastern Research Institute of Agriculture, Khabarovsk.

Characteristic	Russian standard for strong wheat, not less than:	Cultivar			
		Amurskaya 1495	DALGAU-1	Primorskaya 40	Lyra 98
1,000-kernel weight (g)	30	38.6	28.5	34.7	30.7
Grain unit (g/l)	740	792	690	745	790
Vitreousness (%)	60	50	53	53	51
Protein (%)	14	13.6	14.0	14.8	14.7
Flour output (%)	65	67	70	70	73
Gluten content in flour (%)	32	28.0	28.8	31.2	32.6
Dough elasticity (alveograph, mm)	80	59	84	63	80
Elasticity and stretching ratio (units)	0.8-2	0.5	1.0	1.0	0.8
Flour strength (alveograph units)	280	206	242	147	285
Water absorption (%)	75	62	64	64	64
Dough resistant to dilution (min)	7	7	5	3	8
Dough dilution (pharinograph units)	<60	70	100	140	50
Valorimetric mark (valorimetric units)	70	70	58	52	70
Bread output from 100 g of flour (cm ³)	1,200	1,070	1,030	970	1,130
Baking quality (mark)	4.5	4.3	4.0	3.5	4.5

ness, water absorption, and bread output). Thus, Lyra 98 needs further genetic improvement in these indexes. Other cultivars (**Amurskaya 1495**, **Dalgau-1**, and **Primorskaya 40**) are highly productive (5–5.5 t/ha) and resistant to lodging and sprouting, are used in selection as donors of some value technological and agronomic characteristics. Research on improving the technological and bread-making characteristics of soft wheat in the far-eastern region of the Russian Federation is on-going.

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Impact of long-term mineral fertilization on the spring wheat harvest on Pribaikal'ye Gray Forest land.

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The interaction of mineral fertilizers (as anthropogenic factor) on all the components of the agroecosystem are obvious. In the long term, soil fertility and harvests are affected. Early investigations (Zhitov et al. 2004) revealed that without mineral fertilizers soil fertility is gradually degraded, most pronounced in the reduction of the humus content of the soil. Long-term mineral fertilization (NPK) in the complex proportions optimal for a given soil type stabilizes this and other parameters of soil fertility. The consolidated results of 20 years investigations on the productivity of spring wheat on the typical Pribaikal'ye (Eastern Siberia) gray forest cultivated land depending on the mineral fertilization are summarized here.

Materials and methods. The studies were made in a five field-crop rotation regime: pure fallow, wheat, barley, side-rate fallow, wheat, and 8-variant scheme as follows: control (no fertilizer); N_{60} ; P_{40} ; K_{60} ; $P_{40}K_{60}$; $N_{60}K_{60}$; and $N_{60}P_{40}K_{60}$. Tests were repeated four times. Potassium chloride, double super-phosphate, and ammonium nitrate were used as fertilizers. The area under crops equaled 480 m² and the registered area was 240 m². Test sites were located according to the field tests using the spring wheat Tulunskaya 14 and the spring barley Odessky 115. Oily radish (*Raphanus sativus* L., var. *oleifera*) was used as a side-rate plant. Productivity in wheat and barley were expressed in metric centner/ha (mc/ha).

Results. Spring wheat productivity in the last 20 years with various predecessors in the crop rotation (Tables 1 and 2, p. 111) confirms that potential soil productivity depends significantly on the climatic conditions. Productivity fluctuated in the control variant throughout the years. With pure fallow before wheat, productivity ranges from 7.1 to 32.4 mc/ha; with side-rate fallow, from 7.3 to 33.2 mc/ha; and with a crop predecessor, from 7.5 to 26 mc/ha.

The most stable harvests were obtained with pure fallow as a predecessor in the variant with PK introduction, the harvest values ranged between 11.0 and 34.6 mc/ha. This variant demonstrated the highest average productivity in 15 years, 24 mc/ha, which corresponded to the NPK variant, and exceeded the control variant by 3.8 mc/ha. This variant also had the maximum harvests in 2002. With side-rate fallow as a predecessor, the most stable harvests were in the variant with complete mineral fertilization, fluctuating from 11.2 to 37.2 mc/ha in the last 20 years.

When spring barley follows wheat, fairly stable harvests throughout the years were observed in variants with the introduction of nitrogen in combination with phosphorus or potassium and complex.

Considering the efficiency of different forms of mineral fertilizers when crops precede, in the Pribaikal'ye Forest-steppe Zone, introduction of nitrogen fertilizers, particularly with their one-sided use for pure fallow, is inefficient

(see multiyear data in Table 1). Significant increase in efficiency follows with the introduction of phosphorus and potassium fertilizers and their combination. With side-rate fallow depending on the weather conditions, variants with PK and NPK proved to be the most efficient.

Without nitrogen applied to the fore crop under the weather conditions of our region, we do not expect good results. The highest efficiency is with complete fertilizer (NPK) in moderate doses. In 2002, the barley harvest was doubled compared to control due to N₆₀P₄₀K₆₀ use (Table 3).

Analyzing the change in productivity with time shows that by the efficiency of increasing the complexity of mineral fertilization grows compared with the control.

The above results may be summarized as follows: the soil type under investigation possesses a medium potential productivity and fairly stable agrochemical parameters and, therefore, may ensure a relatively high, long-term productivity of crops using established crop-rotation conditions and introducing moderate doses of mineral fertilizers.

Table 1. Long-term impact of mineral fertilization on spring wheat productivity (pure fallow as a predecessor, metric center (mc)/ha). Data is for the Pribaikal'ye Gray Forest cultivated land of Eastern Siberia.

Treatment	Average productivity				2002 harvest (mc/ha)	Harvest value, fluctuation (mc/ha)
	1981–85	1986–90	1991–95	For 15 years		
Control (no fertilizer)	19.7	23.2	17.6	20.2	19.7	7.1–32.0
N ₆₀	24.1	22.8	16.3	20.8	19.0	5.6–30.0
P ₄₀	23.7	24.5	20.3	22.8	22.0	8.9–32.8
K ₆₀	24.8	24.3	17.7	22.1	22.0	8.4–34.6
P ₄₀ K ₆₀	25.8	25.0	21.5	24.0	22.5	11.0–34.6
N ₆₀ P ₄₀	23.7	23.1	19.2	21.9	22.7	8.0–31.4
N ₆₀ K ₆₀	22.5	24.1	18.4	21.6	21.4	8.2–32.6
N ₆₀ P ₄₀ K ₆₀	25.3	25.1	21.2	24.0	22.0	7.5–35.9

Table 2. Long-term mineral fertilization impact on spring wheat productivity (side rate fallow as a predecessor, metric center (mc)/ha). Data is for the Pribaikal'ye Gray Forest cultivated land of Eastern Siberia.

Treatment	Average productivity				2002 harvest (mc/ha)	Harvest value, fluctuation (mc/ha)
	1981–85	1986–90	1991–95	For 15 years		
Control (no fertilizer)	20.0	22.7	14.7	19.4	21.5	7.3–33.2
N ₆₀	21.3	24.1	16.7	21.0	17.6	5.3–36.3
P ₄₀	20.8	24.6	16.7	21.0	21.8	8.8–35.2
K ₆₀	21.1	24.3	15.8	20.7	26.2	8.8–34.8
P ₄₀ K ₆₀	21.6	25.2	17.5	21.7	23.4	9.5–37.8
N ₆₀ P ₄₀	23.1	23.6	17.1	21.6	23.0	9.9–35.3
N ₆₀ K ₆₀	24.0	24.3	16.6	22.0	21.9	6.5–36.7
N ₆₀ P ₄₀ K ₆₀	24.2	25.0	18.1	22.8	22.2	11.2–37.2

Table 3. Long-term mineral fertilization impact on spring barley productivity (side rate fallow as a predecessor, metric center (mc)/ha). Data is for the Pribaikal'ye Gray Forest cultivated land of Eastern Siberia.

Treatment	Average productivity				2002 harvest (mc/ha)	Harvest value, fluctuation (mc/ha)
	1981–85	1986–90	1991–95	For 15 years		
Control (no fertilizer)	10.7	9.4	15.8	11.7	16.4	7.5–26.0
N ₆₀	14.7	16.3	20.8	17.0	24.8	6.7–27.0
P ₄₀	12.8	11.0	17.5	13.5	14.6	8.5–24.0
K ₆₀	11.8	9.8	16.2	12.3	17.4	5.4–20.0
P ₄₀ K ₆₀	12.8	11.0	18.7	13.9	17.5	6.2–22.7
N ₆₀ P ₄₀	17.3	18.2	23.9	19.5	28.1	11.0–26.9
N ₆₀ K ₆₀	18.5	18.3	23.3	19.8	27.1	11.7–28.5
N ₆₀ P ₄₀ K ₆₀	19.3	19.7	26.0	21.3	32.6	11.1–28.9

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The analysis of Puccinia graminis f.sp. tritici populations in the Russian Federation and the Ukraine in 2001.

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In Russia, stem rust develops on wheat crops at different frequencies during different seasons. In 2001, disease development was noted on many wheat cultivars in separate regions of the Russian Federation and the Ukraine. We analyzed 75 monouredinal clones of *P graminis f.sp. tritici* that were isolated from samples of infected wheat plants in the Northern Caucasus (the Rostov area), in the central Russia (Moscow and the Moscow area), and in Ukraine (the Kiev area). Twenty-one races of the pathogen were determined based on the standard Pgt scale (Roelfs and Martens 1988) with the addition of *Sr* lines 9a, 9d, 10, and Tmp (Table 1).

Twelve races from the Northern Caucasus were identified. Race TKNT dominated with a frequency of 27.3 %. Seven races were identified in the Ukraine and central Russian Federation, and race TKNT also prevailed over all others (45.8 % (the Ukraine) and 28.6 % (central Russian Federation)) (Table 2, p. 114).

The Shannon diversity index (H), which commonly is used to characterize diversity (Magurran 1983) was calculated for evaluating the races

Table 1. Races of *Puccinia graminis f.sp. tritici* in different regions of the Russian Federation and the Ukraine (2001). An * indicates isolates used for RAPD analysis. Table 1 is continued on p. 113.

Isolate No.	Region	Wheat cultivar	Race
1.1	Northern Caucasus	Albidum 31	TKNT
1.2	Northern Caucasus	Albidum 31	TKNT
1.3	Northern Caucasus	Albidum 31	TKNT
2.1	Northern Caucasus	Albidum 28	TKNS
2.2	Northern Caucasus	Albidum 28	TKNS
2.3	Northern Caucasus	Albidum 28	TKNS
3.1	Northern Caucasus	Persianovka	MKLS
3.2	Northern Caucasus	Persianovka	MKLS
3.3	Northern Caucasus	Persianovka	MKLS
4.1	Northern Caucasus	Ekodob	RTDS*
4.2	Northern Caucasus	Ekodob	RTDS
4.3	Northern Caucasus	Ekodob	RTDS*
5.1	Northern Caucasus	Saratovskaya 42	TKNT
5.2	Northern Caucasus	Saratovskaya 42	TKNT
5.3	Northern Caucasus	Saratovskaya 42	TKNT
6.1	Northern Caucasus	Orenburgskaya	PKBS*
6.2	Northern Caucasus	Orenburgskaya	CKCS*
6.3	Northern Caucasus	Orenburgskaya	MKCS*
7.1	Northern Caucasus	Volgodonskaya	MTNS*
7.2	Northern Caucasus	Volgodonskaya	RTNS
7.3	Northern Caucasus	Volgodonskaya	RTNS
8.1	Northern Caucasus	Bitá	MKNS*
8.2	Northern Caucasus	Bitá	MKNS*
8.3	Northern Caucasus	Bitá	MKNS*
9.1	Northern Caucasus	Novodonskaya	CKBN*
9.2	Northern Caucasus	Novodonskaya	CKBN*
9.3	Northern Caucasus	Novodonskaya	CKBN*
10.1	Northern Caucasus	Zolotaya volna	TKNT
10.2	Northern Caucasus	Zolotaya volna	TKNT
10.3	Northern Caucasus	Zolotaya volna	TKNT
11.1	Northern Caucasus	Elizavetinskaya	TKPT
11.2	Northern Caucasus	Elizavetinskaya	TKPT
11.3	Northern Caucasus	Elizavetinskaya	TKPT
12.1	Central Russia	Albidum	TKNP*
12.2	Central Russia	Albidum	TKNP
12.3	Central Russia	Albidum	TKNP*

in each region. Pathogen populations from the Northern Caucasus have shown the maximum value of the H-index (2,245). Thus, relatively distinct populations were defined by virulence.

RAPD-PCR, which was successfully used earlier to estimate DNA polymorphism in rust fungi, including *P. graminis* f.sp. *tritici* (Chen et al. 1993, 1995; Kolmer et al. 1995; McCallum et al. 1999; Kolmer et al. 2000; MacDonald et al. 2000; Maleeva et al. 2003), was used to evaluate the degree of molecular variation between isolates collected from different geographic populations. DNA was extracted by the CTAB method (Griffith and Shaw 1998) from 28 isolates, marked in Table 1. The RAPD-PCR reactions primers Core (5'-GAGGGTGGXGGXTCT-3') and PR3 (5'-(GTG)₅-3') were used separately and in combination (Maleeva et al. 2003). In all cases, polymorphism of the amplification products was found (Fig. 1, p. 114). Dendrograms from the UPGMA clustering (Treecon for Windows) grouped the isolates in almost the same manner. The dendrogram constructed on the data using primer Core by an index bootstrap was the most stable (Fig. 2, p. 114).

Puccinia graminis f.sp. *tritici* isolates tested by RAPD tend to be divided in relation with their geographical region. In the dendrogram, North Caucasus isolates generated one cluster (C) with a high degree of association (93 % at a level of 0.2 relative genetic units). Isolates from Central Russian Federation and the Ukraine formed another cluster (A) (96 % probability of association at a level of 0.1 relative genetic unit). A separate group (B) was formed from isolates allocated with barberry (Fig. 2, p. 114).

Table 1 (continued). Races of *Puccinia graminis* f.sp. *tritici* in different regions of the Russian Federation and the Ukraine (2001). An * indicates isolates used for RAPD analysis. Table is continued from p. 111.

Isolate No.	Region	Wheat cultivar	Race
13.1	Central Russia	Underwood of hard wheat	TKNT*
13.2	Central Russia	Underwood of hard wheat	TKNT*
13.3	Central Russia	Underwood of hard wheat	TKNT*
14.1	Central Russia	Hard wheat	PKJG
14.2	Central Russia	Hard wheat	PKJG*
14.3	Central Russia	Hard wheat	PKPN*
15.1	Central Russia	Two-rowed barley	TKNT
15.2	Central Russia	Two-rowed barley	TKNT
15.3	Central Russia	Two-rowed barley	TKNT
16.1	Central Russia	Barberry	MKDT*
16.2	Central Russia	Barberry	MCBQ*
16.3	Central Russia	Barberry	PKDT*
17.1	Central Russia	Barberry	MKBT*
17.2	Central Russia	Barberry	MKBT*
17.3	Central Russia	Barberry	MKBT
18.1	Central Russia	Couch grass	MKBT
18.2	Central Russia	Couch grass	MKBT
18.3	Central Russia	Couch grass	MKBT
19.1	Ukraine	Mironovskaya	TKNT
19.2	Ukraine	Mironovskaya	TKNT
19.3	Ukraine	Mironovskaya	TKNT
20.1	Ukraine	Kharkovskaya 23	TKNT
20.2	Ukraine	Kharkovskaya 23	TKPT
20.3	Ukraine	Kharkovskaya 23	TKPT*
21.1	Ukraine	Poltavskaya	PKST
21.2	Ukraine	Poltavskaya	TKNT*
21.3	Ukraine	Poltavskaya	TKNT
22.1	Ukraine	Mirich	TKNS
22.2	Ukraine	Mirich	TKNT
22.3	Ukraine	Mirich	TKNT
23.1	Ukraine	Rannaya	TKNS
23.2	Ukraine	Rannaya	TKNS
23.3	Ukraine	Rannaya	TKNS*
24.1	Ukraine	Odesskaya 267	TKPS
24.2	Ukraine	Odesskaya 267	TKPS
24.3	Ukraine	Odesskaya 267	TKPS
25.1	Ukraine	Pallidum	TKNT*
25.2	Ukraine	Pallidum	TTNT
25.3	Ukraine	Pallidum	TKNS

The analysis of the population structure in *P. graminis* f.sp. *tritici* shows relative geographic separation of the tested isolates. The existence of some common races (on virulence data) and similar RAPD patterns assumed some genetic exchange between populations of *P. graminis* f.sp. *tritici* by air during infection of wheat. Distinguishing the Northern Caucasus population in 2001 and the maximum number of races was accompanied by the presence of early local sources of *P. graminis* f.sp. *tritici* infection (Lekomtseva 1996). Allocation of separate group of isolates from barberry (RAPD-PCR data) showed that the sexual process contributed to variability in the fungus.

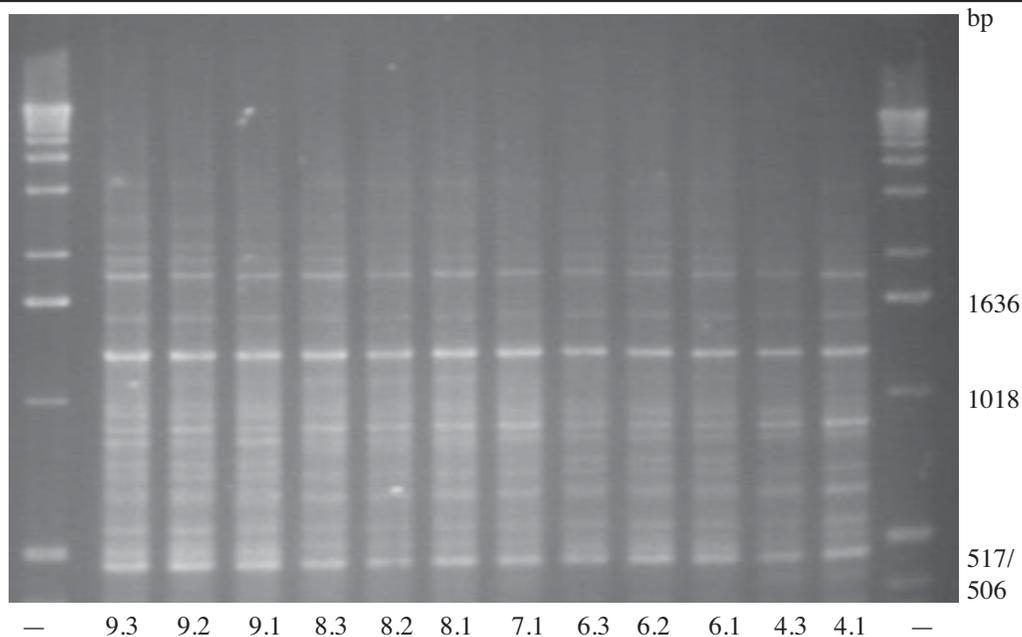


Fig. 1. Randomly amplified polymorphic DNA of *Puccinia graminis* f.sp. *tritici* from the Northern Caucasus of the Russian Federation with primer Core.

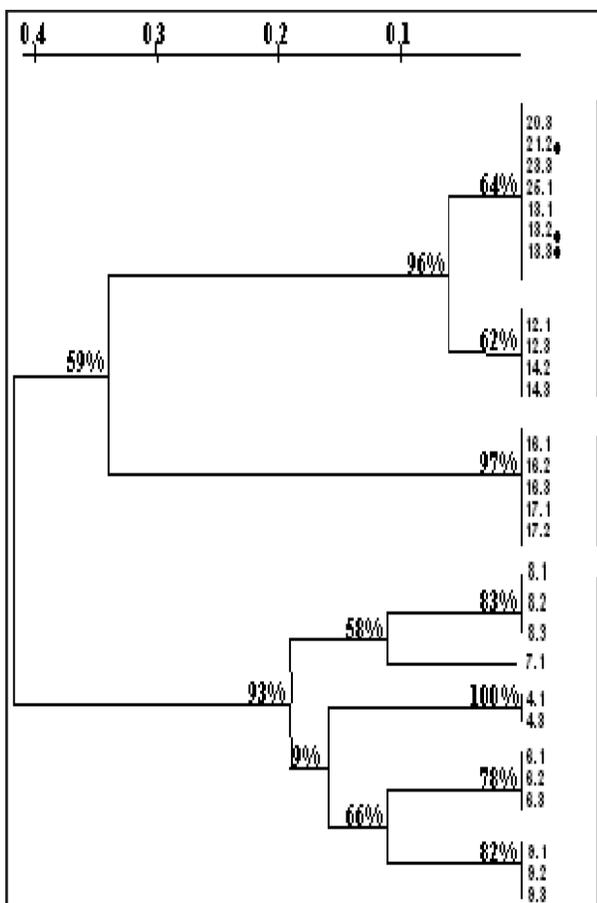


Fig 2. Dendrogram of 28 isolates of *Puccinia graminis* f.sp. *tritici* based on RAPD-analysis with primer Core.

Table 2. Frequency of *Puccinia graminis* f.sp. *tritici* races (%) in the Russian Federation and the Ukraine in 2001. H is the Shannon diversity index

Race	Region		
	Northern Caucasus	Central Russian Fed.	Ukraine
TKNT	27.3	28.6	45.8
TKNS	9.1	—	20.8
MKLS	9.1	—	—
RTDS	9.1	—	—
PKBS	3.0	—	—
CKCS	3.0	—	—
MKCS	3.0	—	—
MTNS	3.0	—	—
RTNS	6.1	—	—
MKNS	9.1	—	—
CKBN	9.1	—	—
TKPT	9.1	—	12.5
TKNP	—	14.3	—
PKJG	—	9.5	—
PKPN	—	4.8	—
PKDT	—	9.5	—
MKDT	—	4.8	—
MKBT	—	28.6	—
PKST	—	—	4.2
TKPS	—	—	12.5
TTNT	—	—	4.2
H	2,245	1,753	1,468

The UPGMA method was used for cluster analysis (Program Treecon for Windows (the version 1.3b)). The cluster analysis and calculation of genetic distance after Link et al. (1995). The analysis of reliability was by the bootstrap method on 100 repetitions. A, B, and C the main clusters noticed on the dendrogram. Dominant race was TKNT.

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Pathogenicity of the stem rust Puccinia graminis f.sp. tritici in the central Russian Federation in 2003.

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In last 20 years, development of a stem rust on wheat in the Russian Federation was serious. A large variability in environmental conditions in the expansive territory provided a background for the occurrence and reproduction of the stem rust infection on wheat (Elansky and Lekomtseva 1996; Maleeva et al. 2003).

Outbreaks of rust on grain crops is known to completely depend on resistance of the plant hosts, favorable climate conditions, infection sources, and susceptibility to the pathogen of plants at different vegetative stages. The interaction between infection period, resistance of wheat cultivars, and environments conditions is difficult to determine (Eversmeyer and Kramer 2000). Resistance is controlled by genes of different genetic origin, many of which have been transferred to wheat plant from wild cereal species (Hulbert et al. 2002).

In the Russian Federation and the countries of the former Soviet Union, *P. graminis* f.sp. *tritici* races were studied for 60–80 years in the 1900s (Lekomtseva 1996). Overwintering regions and pathogen reproduction were determined. The race structure was determined at sexual and asexual reproduction stage. Genes for resistance in some of wheat cultivars and virulence genes in the pathogen were determined. Cultivars resistant to stem rust were introduced. Because of a decreased level of stem rust infection on wheat, interest in the populations structure of the fungus has declined. Recent stem rust outbreaks make studying the population structure of the pathogen on different host plants in different regions of the Russian Federation a necessity.

During the last decade, interest in studying stem rust development on barberry (the intermediate host) and wild species (additional host-plants) increased. The barberry can be source new fungus genotypes as a result of sexual recombination. The wild species, in addition to a decreasing level of perennial cereal grasses, can help infection accumulation of the pathogen. We wanted to study *P. graminis* f.sp. *tritici* races structure in the central Russian Federation on barberry plants and wild cereal species.

In 2003, no stem rust developed on wheat was not observed because of dry, hot conditions. Separate *P. graminis* f.sp. *tritici* aecia were collected from the end of May to June from barberry plants in the botanical garden of Moscow University, the main botanical garden of the Russian Academy of Sciences, and in private plots in the Moscow region. Fungal uredinia were collected on couch grass (*Elytrigia repens*) and barley at the end of July and August at different sites in the Moscow regions. A total of 14 samples (eight from barberry, five from couchgrass, and one from barley) were collected. Aeciospores and urediniospores were used to infect the susceptible wheat cultivar Khakasskaya. Five monouredinial clones were isolated and reproduced for each isolate. Races were screened according the Pgt system using 16 isogenic wheat lines (Roelfs and Martin 1998; Long et al. 2004).

In 2003, stem rust on wheat was not found. The aecia of the fungus was found on barberry, barley, and couch grass. Seven pathogen races were identified using 16 *Sr*

Table 3. Races of *Puccinia graminis* f. sp. *tritici* in the Central Russian Federation on different host plants in 2003.

Race	Susceptibility of <i>Sr</i> wheat genes	Number of monouredinial isolates	Percent
TTNT	5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 30, 9a, 9d, 10, Tmp	40	38.1
MKNS	5, 7b, 6, 8a, 9g, 36, 30, 9a, 9d, 10	25	23.7
TTNS	5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 30, 9a, 9d, 10	15	14.3
PKNT	5, 9e, 7b, 6, 8a, 9g, 36, 30, 9a, 9d, 10, Tmp	10	9.5
TKNT	5, 21, 9e, 7b, 6, 8a, 9g, 36, 30, 9a, 9d, 10, Tmp	5	4.8
PTNT	5, 9e, 7b, 11, 6, 8a, 9g, 36, 30, 9a, 9d, 10, Tmp	5	4.8
MKNT	5, 7b, 6, 8a, 9g, 36, 30, 9a, 9d, 10, Tmp	5	4.8
Total		105	100

wheat lines (Table 3). Two races, TTNT (virulence to *Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr9a*, *Sr9d*, *Sr9e*, *Sr9g*, *Sr10*, *Sr11*, *Sr21*, *Sr30*, *Sr36*, and *SrTmp*) and MKNS (*Sr5*, *Sr6*, *Sr8a*, *Sr7b*, *Sr9a*, *Sr9d*, *Sr9g*, *Sr10*, *Sr30*, and *Sr36*) dominated in the central Russian Federation

and comprised 38.1 % and 23.7 %, respectively, of the total population. Race TTNS (*Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr9a*, *Sr9d*, *Sr9e*, *Sr9g*, *Sr10*,

Sr11, *Sr21*, *Sr30*, and *Sr36*) made up 14.3 % of the population. Other races were present in much lower quantities. Races TTNT, MKNS, and TKNT were found on wheat in the central Russian Federation, the Northern Caucasus and in the Ukraine in 2001, a season favorable for development of wheat stem rust. Six races on barberry, two races on couch grass, and one on barley were identified (Table 4). All races were characterized by high virulence. In the dominate race TTNT, 14 virulence genes were identified.

Evaluation of isogenic wheat lines indicated that the majority of the genes except *Sr9b* and *Sr17* were susceptible to the stem rust in 2003 (Table 5). The resistance of wheat lines with *Sr9b*, *Sr9e*, *Sr11*, *Sr21*, *Sr30*, and *Sr36* were susceptible in the hot growing conditions in 2003 (Lekomtseva 2004).

Based on the race structure in 2003 during a light stem rust infection on wheat, we assumed that under unfavorable conditions the

Table 5. Virulence of *Puccinia graminis* f. sp. *tritici* isolates from different host plants on *Sr* wheat isolines in 2003. Data are percent of monouredinial isolates on host plants virulent to wheat isolines with *Sr* genes.

Line	Barberry	Barley	Couch grass
<i>Sr5</i>	100.0	100.0	100.0
<i>Sr6</i>	100.0	100.0	100.0
<i>Sr7b</i>	100.0	0.0	100.0
<i>Sr8a</i>	100.0	0.0	100.0
<i>Sr9a</i>	100.0	0.0	100.0
<i>Sr9b</i>	0.0	0.0	0.0
<i>Sr9d</i>	100.0	100.0	100.0
<i>Sr9e</i>	88.0	100.0	100.0
<i>Sr9g</i>	100.0	100.0	100.0
<i>Sr10</i>	100.0	100.0	100.0
<i>Sr11</i>	60.0	100.0	100.0
<i>Sr17</i>	0.0	0.0	0.0
<i>Sr21</i>	60.0	100.0	0.0
<i>Sr30</i>	100.0	100.0	100.0
<i>Sr36</i>	100.0	100.0	100.0
<i>SrTmp</i>	73.3	100.0	100.0

Table 4. Races of *Puccinia graminis* f. sp. *tritici* on different host plants in 2003 (%) in the central Russian Federation.

Race	Barberry	Couch grass	Barley
TTNT	56.7	66.7	100.0
MKNS	26.7	—	—
PKNT	6.7	—	—
TTNS	—	33.3	—
TKNT	3.3	—	—
PTNT	3.3	—	—
MKNT	3.3	—	—

fungus maintains virulence phenotypes on the intermediate (barberry) and additional host-plants (wild cereals). Comparing the race structure of *P. graminis* f. sp. *tritici* in the central Russian Federation and the race structure in the U.S. during the same year, Long et al. (2004) showed a significant difference between populations of the fungus in the European and American continents. Long-term observations of the decline in the stem rust pathogen on the host and a simultaneous increase of fungus development on wild cereals species can indicate that during years of low infection, infection potential changes from agrocenosis to biocenosis.

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Cytogenetic and phytopathologic evaluation of bread wheat–alien lines.

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Bread wheat–alien lines were produced in the Agricultural Research Institute for South-East Regions by crossing cultivars of bread wheat with *T. turgidum* subsp. *durum* cultivars L1078 and L2505, *T. turgidum* subsp. *dicoccoides* line L2560, *Ae. speltoides* lines L2608 and L2166, *Ae. umbellulata* lines L1046/2 and L1074/2, *S. cereale* line L2075, *Ag. intermedium* lines L1740, Multy 6R, and L1059, and *Ag. elongatum* lines L1015 and L1016. All investigated lines have 42 chromosomes. C-banding patterns of the abovementioned lines showed that some of the lines have a substitution of bread wheat chromosomes by alien chromosomes: L1059 and Multy 6R, 6Agi (6D); L1015 and L1016, 3Age (3D); and L1046/2 and L1074/2, 2U (2A). Other lines have translocations: L2608, T2BL-2SL (these lines were kindly provided by I.F. Lapochkin); L2166, T2D-2S; and L2075, T1RS-1BL.

Analysis of the first meiotic metaphase in the F₁ hybrids ‘L1078/Saratovskaya 29’ and ‘L2505/Saratovskaya 29’ shows that chromosome pairing is normal, but 2 to 4 univalents were observed in the ‘L2560/Saratovskaya 29’ F₁ hybrid. Line L2560 may have an inversion in the from *T. turgidum* subsp. *dicoccoides* chromosome, which carries a gene for resistance to leaf rust.

Phytopathologic evaluation of perspective spring bread wheat–alien lines were made. The bread wheat cultivars Saratovskaya 55 and L 503 served as controls. For resistance to leaf rust in 2003–04, we selected lines Multy 6R, L487, L484, and L2870, which had the infection type (IT) 0;. The IT in lines L784, L856, and the check cultivars was 3. To evaluate powdery mildew resistance, all lines had an IT = 3, with the exception of L487, which had an IT = 2. The IT of Saratovskaya 55 was 4 and that of L 503 was 1. For stem rust resistance, lines L784 (IT = 1) and L856 (IT = 0;) were considered resistant; all other lines were IT = 3.

Study of embryogenic processes in the wheat somatic callus with the use of genetic models.

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In vitro somatic embryogeny is used widely in investigations of specific gene expression and their translation products, which are representative of the embryogenic potential of plant callus cells. To solve these problems, we need to create experimental genetic models based on isogenic lines with a known functional relationship between the gene expression and the phenotypic behavior of the protein encoded.

Previously, we described the relationship between the embryogenic potential of wheat calli and the content of the proliferative antigen of initial cells (PAI) therein by a genetic model including a tall cultivar (Saratovskaya 29) of soft spring wheat and its NILs differing in the dwarfing gene *Rht-B1c*. This genetic model is extended here by investigating lines with *RhtB1b* and *Rht14* alleles and their tall sibs. Relative, semiquantitative analysis of PAI content was done on a basis of a solid-phase immunoassay using monospecific antibodies to PAI. Dwarf lines have a higher embryogenic potential than their tall sibs and Saratovskaya 29 in PAI content at certain stages of the callus genesis (24 and 30 days). In addition, the embryogenic potential of dwarf line *RhtB1c* exceeds that of the other dwarf lines (*RhtB1b* and *Rht14*), which have less embryogenic potential in the PAI content. This fact can be used in bioengineering work when evaluating the embryogenic potential of newly produced wheat lines in an *in vitro* culture.

SIBERIAN INSTITUTE OF PLANT PHYSIOLOGY AND BIOCHEMISTRY**Siberian Division of the Russian Academy of Sciences, Lermontov str., 132, Irkutsk-33,
P.O Box 1243, Russian Federation, 664033.***The impact of early autumn frost on the efflux of nitrous substances in spring wheat.*

A.K. Glyanko, N.V. Mironova, and G.G. Vasilieva.

Introduction. Frost during the period of ripening and maturing of spring wheat grain is a typical natural phenomenon in many parts of the world (Russian Federation, Scandinavian countries, Canada, and the USA). The short-term impact of negative temperature on plants at this time (particularly during milky ripeness) is a decrease in the technological and sowing qualities of the grain resulting in the formation of so-called 'frost-beaten grain' with low germinating capacity, low test weight, and low flour production (Lamb 1967; Razumovsky and Zabolina 1969; Reimers and Illi 1974). Wheat from such grain have a low initial intensity of growth and morphological distortions (Pavlov 1967; Illi et al. 1979). Such properties of the grain after an early autumn frost are apparently conditioned by the negative influence of cold on the physiological-biochemical processes that are responsible for grain ripening and maturing. Among these processes, those of nitrous substances efflux from vegetative parts of the plants to reproductive organs in the period of kernel formation are very important. The present work was aimed at determining the degree of impact of early autumn frost at milky ripeness on the nitrogen efflux from leaves, stems, and spike (chaff) towards the kernel.

Materials and Methods. The spring wheat Skala was grown in a climatic chamber with the temperature regime of $19 \pm 1/1.5 \pm 1^\circ\text{C}$ (day/night). Illumination was by incandescent lamps DRL-700 with a light intensity of 14 ± 0.5 kl and a 16-hour light period. Infrared radiation of the lamps was precluded by a water screen. Sandy soil with a low content of total nitrogen (0.009 %) was used for plant growth. Macroelements from Gelrigel's mixture and microelements as per Hogland (Grodzinsky and Grodzinsky 1973) were introduced in the enameled vessels with soil (tank capacity was 9 kg of dry soil). Soil moisture was maintained at 70 % of complete soil moisture capacity (SMC). Ten days prior to termination of the experiment, soil moisture was reduced to 45–50 % SMC. Each vessel contained five plants at the end of the experiment.

The plants were subjected to an artificial frost with the intensity of $-6.5 \pm 0.5^\circ\text{C}$ at milky ripeness stage (moisture 55–60 %). The frost was simulated at night, in the dark. All temperature regulating operations in the freezer were performed automatically in compliance with the program, which included a gradual reduction of temperature from 15 to 0°C ($1^\circ\text{C}/12$ min). Further reduction down to a minimum negative temperature of $-6.5 \pm 0.5^\circ\text{C}$ was at $1^\circ\text{C}/22$ min (Kurets 1974). After 1.5-h exposure at $-6.5 \pm 0.5^\circ\text{C}$, the temperature was increased to 0°C and then to 15°C with a speed $1^\circ\text{C}/12$ min. Total duration of negative temperature impact on the plants was 6 hours. The plants did not show any visible damage.

Control plants were placed in the chamber with optimal growth temperature. One and one-half hours after the termination of extreme temperature factor impact on the test plants, both sets of plants were fed via roots with ^{15}N -labeled $\text{Ca}(\text{NO}_3)_2$ at a dose of 100 mg of nitrogen/vessel. Nitrogen enrichment was 95.05 atomic % ^{15}N . Further growth of both control and test plants was conducted at the temperature conditions preceding the frost.

Samples for analysis were selected after 7 days (beginning at waxy ripeness phase, grain moisture 38–40 %) and 19 days (end of waxy ripeness phase, grain moisture 17–19 %). The plants were divided into leaves, stems, shaft, and grain. After drying at 105°C , the samples were reduced to a powder. Plant material samples were burned in concentrated sulfuric acid with the catalyst (selenium). Nitrogen was determined per K'eldal's micromethod (Ermakov et al. 1987). The samples were enriched with heavy-isotope nitrogen on an MI-1305 mass-spectrometer (Optical-mechanical Plant, Sumy, Ukraine). The content of marked nitrogen in the plants samples was calculated according to the formula of isotope dilution (Korenkov 1977). The test was repeated six times. The results are presented as average arithmetic means with the standard error identified. Reliability of differences was assessed by Student criterion.

Results and Discussion. Depending on the state in which plants overcome the impact of negative temperature, supercooling or ice formation in the cells, the degree of damage will vary (Drozov et al. 1977). Our test wheat plants went through the artificial frost apparently in the state of supercooling, because they showed no visible damage. The absolute

Table 1. Change in the nitrogen content (mg/plant) in different organs of spring wheat plants under the impact of early autumn frost.

Treatment	Start of waxy ripeness stage			End of waxy ripeness stage			Complete grain ripeness		
	Total	Excess atom ¹⁵ N %	Marked	Total	Excess atom ¹⁵ N %	Marked	Total	Excess atom ¹⁵ N %	Marked
LEAVES									
Control	7.5 ± 0.30	5.7 ± 0.30	0.5 ± 0.02	5.7 ± 0.30	2.6 ± 0.2	0.2 ± 0.01	4.9 ± 0.10	2.9 ± 0.20	0.2 ± 0.01
Early frost	8.4 ± 0.50	6.4 ± 0.40	0.6 ± 0.02	4.8 ± 0.20	3.8 ± 0.2	0.2 ± 0.01	4.8 ± 0.20	4.7 ± 0.40	0.2 ± 0.01
STEMS									
Control	22.7 ± 1.00	9.1 ± 0.05	2.2 ± 0.10	12.9 ± 0.60	6.8 ± 0.1	0.9 ± 0.01	10.8 ± 0.60	6.9 ± 0.10	0.8 ± 0.04
Early frost	19.3 ± 0.80	10.6 ± 0.50	2.2 ± 0.20	7.8 ± 0.40	4.6 ± 0.2	0.4 ± 0.01	7.1 ± 0.40	6.6 ± 0.05	0.5 ± 0.02
CHAFF									
Control	7.0 ± 0.30	7.2 ± 0.10	0.5 ± 0.03	3.1 ± 0.10	5.8 ± 0.1	0.2 ± 0.01	2.2 ± 0.05	6.8 ± 0.30	0.2 ± 0.01
Early frost	5.2 ± 0.05	6.2 ± 0.05	0.3 ± 0.02	2.7 ± 0.20	4.8 ± 0.3	0.1 ± 0.01	2.2 ± 0.05	6.8 ± 0.30	0.2 ± 0.01
GRAIN									
Control	<u>28.5 ± 0.60*</u> 2.2 ± 0.10	17.2 ± 0.70	5.2 ± 0.20	<u>46.7 ± 1.00</u> 3.3 ± 0.05	18.1 ± 0.9	8.9 ± 0.40	<u>51.5 ± 0.90</u> 3.0 ± 0.10	17.2 ± 0.50	9.3 ± 0.30
Early frost	<u>22.4 ± 0.90</u> 2.2 ± 0.05	13.7 ± 0.30	3.3 ± 0.10	<u>51.0 ± 1.00</u> 3.5 ± 0.10	14.0 ± 0.8	7.6 ± 0.40	<u>47.1 ± 1.30</u> 2.9 ± 0.05	16.6 ± 0.50	8.3 ± 0.30

* numerator = mg/plant; denominator = % from dry weight of grain

nitrogen content in wheat kernel of the control constantly increased until the end of plant growth (Table 1). In the variant with frost, we observed a tendency towards reduction of nitrogen content in the kernel, from the end of the waxy ripeness phase through complete kernel ripeness. Relative nitrogen content in the kernel (% of dry mass) increased in both test variants by the end of waxy ripeness phase and was reduced at complete kernel ripeness. However, this reduction was statistically reliable only in the variant with frost (P > 0.95).

With ripening and maturing of kernels, the total nitrogen content in leaves, stems, and shaft was reduced (Table 1). Nevertheless, in the period from the end of waxy ripeness until complete kernel ripeness, the frost variant demonstrate an increase in excessive ¹⁵N % and marked nitrogen content in stems and shaft. In the leaves, analogous changes in these parameters were statistically unreliable. The rise of marked nitrogen content in the vegetative organs apparently was related to the absorption of nitrogen introduced in the soil during fertilization.

The highest nitrogen content in the kernel from the stems by complete kernel ripeness was observed in both control (62 %) and treated (65 %) plants (Table 2). The leaves had 13 % (control) and 19 % (treated) and the shaft 25 % (control) and 16 % (treated) total stored nitrogen. Similar results characterized the period from the beginning to end of

Table 2. Change in total and marked nitrogen efflux from vegetative organs of spring wheat under the impact early autumn frost. Numerator = mg/plant; denominator = % of total nitrogen.

Treatment	Efflux of total nitrogen				Efflux of marked nitrogen			
	Total	from leaves	from stems	from chaff	Total	from leaves	from stems	from chaff
START TO END OF WAXY RIPENESS PHASE.								
Control	<u>15.5 ± 1.3</u> 100	<u>1.8 ± 0.4</u> 12	<u>9.8 ± 1.2</u> 63	<u>3.9 ± 0.3</u> 25	<u>1.9 ± 0.1</u> 100	<u>0.3 ± 0.02</u> 16	<u>1.3 ± 0.10</u> 68	<u>0.3 ± 0.03</u> 16
Early frost	<u>17.6 ± 1.7</u> 100	<u>3.6 ± 0.5</u> 21	<u>11.5 ± 0.9</u> 65	<u>2.5 ± 0.2</u> 14	<u>2.4 ± 0.2</u> 100	<u>0.4 ± 0.02</u> 17	<u>1.8 ± 0.20</u> 75	<u>0.2 ± 0.01</u> 8
START OF WAXY RIPENESS TO COMPLETE RIPENESS.								
Control	<u>19.3 ± 1.2</u> 100	<u>2.6 ± 0.3</u> 13	<u>11.9 ± 1.2</u> 62	<u>4.8 ± 0.3</u> 25	<u>2.0 ± 0.1</u> 100	<u>0.3 ± 0.05</u> 15	<u>1.4 ± 0.10</u> 70	<u>0.3 ± 0.02</u> 15
Early frost	<u>18.8 ± 1.0</u> 100	<u>3.6 ± 0.5</u> 19	<u>12.2 ± 0.9</u> 65	<u>3.0 ± 0.1</u> 16	<u>2.2 ± 0.2</u> 100	<u>0.4 ± 0.02</u> 18	<u>1.7 ± 0.20</u> 77	<u>0.1 ± 0.02</u> 5

waxy ripeness. We concluded that during both interphase periods, frost resulted in a reliable reduction of nitrogen efflux from shaft to kernel ($P \geq 0.99$). We also observed a reliable ($P > 0.95$) increase in nitrogen efflux from the leaves during the period from beginning to end of waxy ripeness. Nevertheless, these changes were unreliable for the beginning of the waxy ripeness phase.

Further data analysis showed that an increase in nitrogen content in the kernel of the plants subjected to frost by complete ripeness amounted to 24.7 ± 1.6 mg/plant (range 22.4 ± 0.9 – 47.1 ± 1.3 mg/plant), including marked nitrogen 5.0 ± 0.3 mg/plant (range 3.3 ± 0.1 – 8.3 ± 0.3 mg/plant) (Table 1). Consequently, an increase in total and marked nitrogen content in the kernel exceeded the amount of nitrogen arriving from the vegetative organs by 24 % ($P > 0.95$) and 57 % ($P > 0.99$ for total and marked nitrogen, respectively) (the decrease in total and marked nitrogen from vegetative organs amounted to 18.8 ± 1.0 and 2.2 ± 0.2 mg/plant, respectively). In other words, plants subjected to frost used both nitrogen accumulated in the vegetative organs and nitrogen from soil solution for kernel formation, a considerable part of which was marked nitrogen.

The control plants showed similar regularities but used soil nitrogen to lesser extent. Thus, the total nitrogen increase in the kernel exceeded its efflux from vegetative organs by 16 % (the difference is unreliable) and marked nitrogen increased to 51 % ($P > 0.99$).

If we calculate the value of parameters considered in relation to the period beginning to the end of waxy ripeness, we find that total nitrogen inflow into the kernel will exceed its efflux from the vegetative organs by 38 % (11.0 ± 2.1 mg/plant; $P > 0.99$) in the frost variant and 15 % (2.7 ± 18 mg/plant; the difference is unreliable) in control plants. Consequently, between the beginning and end of waxy ripeness, nitrogen from the soil also was used for kernel formation.

In the plants subjected to frost, use of total nitrogen from soil for kernel formation from the end of waxy ripeness to complete ripeness declined from 38 % (11.0 ± 2.1 mg/plant) to 24 % (5.9 ± 1.9 mg/plant) (the difference is unreliable) and that of marked nitrogen increased from 44 (1.9 ± 0.1 mg/plant) to 57 % (2.8 ± 0.2 mg/plant) ($P > 0.95$). We conclude that frost stimulated the use of marked nitrogen for kernel formation. However, frost did not produce a reliable impact on the total nitrogen content in the kernel at maturity (Table 1). We note a reduction in the relative content of total nitrogen in the kernel from the end of waxy ripeness to complete ripeness (Table 1). Nitrogen loss from the kernel at maturity has been described (Kumakov 1987; Korovin 1984). Korovin (1984) indicated that this phenomenon may be accounted for by increased moisture and low environmental temperature. The concrete physiological-biochemical mechanism of this fact is unknown.

Our tests on early autumn frosts produced a significant impact on the efflux of total and marked nitrogen from the vegetative organs to the kernel. Total nitrogen efflux from the leaves increased and from the shaft was reduced. We observed an increased of marked nitrogen efflux from the stems between the beginning and end of the waxy ripeness stage. Stimulating or hampering physiological functions after stress may be connected with the ability of the plant and depend on the character and intensity of the stress (Drozdov et al. 1977; Al'tergot 1981). For the spike rachis and the closeness of the nitrogen source used by maturing kernels, then a reduction in nitrogen after a frost could impact protein synthesis in the kernel. Earlier investigations showed that an early autumn frost causes changes in the proportion of vital reserve proteins of spring wheat kernels (glutenin and gliadin) (Glyanko and Trufanov 1999). Similar changes may affect baking qualities of wheat grain.

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Influence of low-intensity laser radiation on lipid peroxidation in wheat callus cultures.

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During the last two decades, low-intensity laser radiation in the visible and near-infrared ranges is finding an increasing application in biological studies including biotechnological research (Grishko et al. 1999). However, up to now, the data are scarce on the mechanism of the effect of laser light on biological subjects because of difficulties encountered in analyzing light energy transformations in cells and the complexity of the response of a complex living system, multilevel in its organization, to laser effects. At present, we are actively pursued the effect of low-intensity laser radiation on animal and human tissues (Skobelkin 1997). At the same time, we emphasize that plants are evolutionary more adapted to the perception and assimilation of light energy, because they include a multitude of light-sensitive compounds, such as phytochromes and photosynthetic pigments. In our opinion, plants are particularly interesting for investigating the mechanisms of laser radiation.

Previously, we showed that low-intensity laser radiation stimulates morphogenetic processes in tissue cultures of wheat and wild grasses, such as rhizogenesis and the formation of morphogenic calli and regenerated plants (Salyaev 2001a and b). However, laser light can be regarded not only as a stimulant, but also as a stress agent damaging cells and tissues, most notably at the initial stages of radiation (Rogatkin and Chernyi 1999).

Generation of reactive oxygen species and an enhancement in lipid peroxidation processes are known to be the primary responses to many stress agents (Baraboi 1991; Smirnov 1993; Kurganova et al. 1997; Kuznetsov 2000). In this connection, establishing whether or not the low-intensity laser radiation can induce lipid peroxidation in plant tissues was important. An analysis of accumulation of the primary and secondary products of lipid peroxidation can be regarded as one of possible lines of approaching such problem. Therefore, the objective of this work was to investigate the effect of low-intensity laser radiation on the process of accumulation of secondary lipid peroxidation products in wheat tissue culture.

The wheat cultivar Skala (bred in Siberia) tissue culture was used in this work. A mature embryo with half of endosperm was used as an explant. Callus formation was induced on a modified Murashige-Skoog medium complemented with 2 % sucrose and 2 mg/l 2,4-D. The calli were irradiated on the 6th day of the first subculture.

Calli irradiation was performed during 5 min using a LG-79 helium-neon laser with a radiation wavelength of 632.8 nm and an intensity of 10 mW at the sample level. Irradiation intensity was measured using a LM-2 device (Carl Zeiss, Germany). The calli were irradiated directly in glass test tubes (10 mm in diameter) used for their culturing, and light-intensity losses in passing through the glass did not exceed 12 %. Before and after irradiation, the calli were grown in the dark. In each independent experiment, 100 calli were used; half of them served as a control, and the other half was treated by the helium-neon laser.

The content of the compounds under investigation in the calli was determined immediately following radiation and also after 48 h. The lipid peroxidation level was assessed spectrophotometrically (Kurganova et al. 1997) as an amount of lipid peroxidation products that react with thiobarbituric acid (TBA-reactive products). To this end, 0.2 g of callus tissue was ground in a homogenizer with 9 ml of heptane:isopropanol (1:1, v/v) mixture. The homogenate thus

obtained was supplemented with 0.1 ml of saturated TBA solution and centrifuged at 4000 rev/min for 10 min. The supernatant diluted with distilled water was shaken; after phase separation, the upper heptane fraction was collected. This fraction was supplemented with ethanol (1:5, v/v), and the optical density of the mixture at 532 nm was determined using an SF-46 spectrophotometer (LOMO, Russia). In all cases, the amount of secondary lipid peroxidation products in the samples was calculated from the spectrophotometric readings using molar extinction coefficient. Means from three experiments (each including nine biological replications) and their standard errors are shown in the figure. Each biological replication included from four to seven calli.

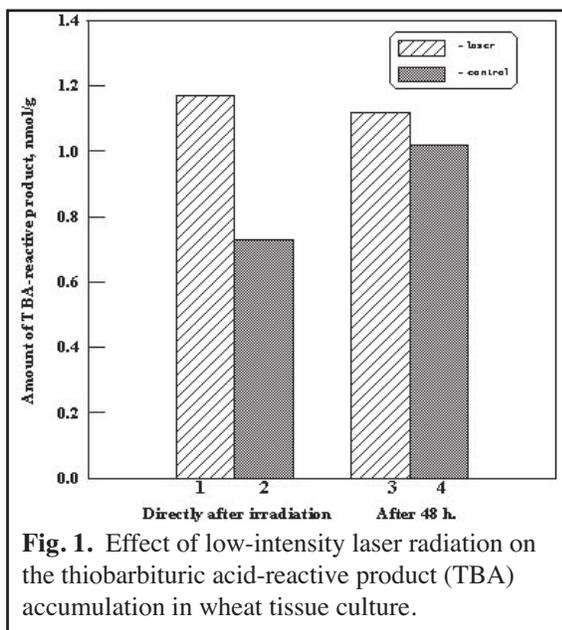


Fig. 1. Effect of low-intensity laser radiation on the thiobarbituric acid-reactive product (TBA) accumulation in wheat tissue culture.

The changes in the accumulation of secondary lipid peroxidation products in wheat tissue culture induced by low-intensity laser radiation are sufficiently clear-cut (Fig. 1). TBA-reactive product content in the tissue increased from 0.73 nmol/g fr wt in the control to 1.17 nmol/g fr. wt immediately after irradiation. Within 2 days after irradiation, this difference somewhat decreased, but nevertheless remained quite significant: TBA-reactive product contents in the control and irradiated samples were 1.02 and 1.12 nmol/g fr. wt, respectively. Thus, after 48 h, the content of TBA-reactive products in control samples somewhat increased as compared to that observed immediately after irradiation. Judging from the published evidence (Smirnov 1993), this can be related to a cyclic stress-response pattern in the wheat tissue culture caused by subculturing to a new culture medium. In this case, the phases of these cycles in the control and laser-irradiated samples most likely do not coincide with each other.

The data suggest that low-intensity laser radiation can induce lipid peroxidation in wheat tissue culture. We showed that the initial response of a plant tissue to irradiation involved an increase in the content of secondary lipid peroxidation

products (see Fig. 1). Moreover, we showed that, as an aftereffect, laser light stimulated the morphogenetic processes in plant tissues (Salyaev et al. 2001a and b). In our opinion, this stimulation could result from the metabolic changes induced by the alterations in the contents of compounds, such as lipid peroxidation products, formed in the primary photoreactions. The changes in cells produced by the accumulation of these compounds could serve as a signal triggering not only respective defense mechanisms, but also some secondary responses including those on the transcription level (Karu 2001). This suggestion is indirectly supported by stimulation of the morphogenetic processes in the tissue cultures of wheat and wild grasses under the effect of low-intensity coherent radiation (Salyaev et al. 2001a and b).

Thus, we suggest that a general cell response induced by laser-light irradiation can be divided into two specific responses, which do not coincide in time. The first one consists of a rapid stress effect resulting in an increase in the amount of lipid peroxidation products, and the second and longer one, are the secondary reactions related to the adaptive metabolic changes and apparently accompanied by the stimulation of morphogenetic processes.

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Nitrate reductase activity at different levels of nitrate nitrogen availability for spring wheat.

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Nitrate reductase (NR) is an enzyme well-known to plant biologists, which acts as a limiting link in the chain of nitrate reduction to ammonium and catalyzes the first stage of nitrate reduction to nitrite. Most of the studies of NR physiological and biochemical properties were published in the 1960s–80s. In particular, plant physiologists revealed the connection between NR activity and cultural plants productivity, protein synthesis in crops grain, as well as intensity of nitrogen assimilation by the plants (Deckard et al. 1973; Rautou et al. 1977; Peshkova and Khavkin 1980). Pekker and Tokarev (1984) suggest use of NR activity for diagnosing plants nitrogen nutrition. In the last few years, interest in NR has been fostered by the discovery of NR ability to reduce nitrite to nitric oxide (NO), a molecule that plays an important role in the vital processes of animals and plants (Yamasaki and Sakihama 2000; Desikan et al. 2002; Garcia-Mota and Lamathina 2003). This article sums up the results of tests on determination of NR activity during the vegetation period of spring wheat (Skala) depending on the dose of nitrogen fertilizer. The work was targeted at finding the dependence between NR activity and the degree of nitrate nitrogen availability for the plants.

Materials and Methods. The tests were performed in vegetation vessels with the capacity of 4 kg of dry soil with low content of total nitrogen (0.009 %). The mixture with nitrate source of nitrogen was used as a nutritious mineral mixture (Thomas et al. 1979). The content of mineral nitrogen introduced in the vessels varied from 0 to 800 mg / vessel. Soil moisture in the vessels was maintained at the level of 65–70 % of total moisture capacity. 10 plants grown in the vegetation house from June to August were left in each vessel.

NR activity was determined in anaerobic medium according to the method described in Mulder et al. (1959) using disks (0.05–0.1 mm) cut out from the leaves. The samples (0.3 g of fresh tissue) were placed in retorts containing the following: 0.1 M phosphate buffer with pH 7.8 (5 ml); neutralized 0.1 M solution of malic acid (1 ml); 5 % solution KNO₃ (1 ml) and 3 ml of distilled water. The retorts were placed in the vacuum-desiccator, with air pumped out, and kept for 1 h at 27°C. Enzyme reaction was terminated by adding 1 ml of 10 % acetic acid and 2 ml of saturated ammonium sulfate solution. In the control sample the enzyme reaction was terminated prior to incubation of the reaction mixture. NR activity was determined on the basis of the quantity of nitrites formed as a result of enzyme reaction; their content was determined in the filtrate with 0.6 % sulfanilamide in HCl and 0.6 % solution of N-(1-naphthyl) ethylenediamine hydrochloride. The color intensity was measured on the photocolormeter (FEK-56M) at wave length of 546 nm. The samples for determination of enzyme activity were taken during the phases of two, three, four leaves, to come into ear and blooming. The upper growing and lower green leaves were subjected to analysis. The test was repeated five times; the analytical repetition is three times. Data are presented as mean ± standard deviation.

Results and Discussion. Table 3 (p. 125) shows that NR activity on the medium without nitrogen introduction is minimal during all the phases of plants development as compared to the variants with nitrogen introduced. The highest enzyme activity in this option was observed in the phase of two leaves, then with the plants growth and development it gradually came down, particularly in the lower leaf. Thus, at anthesis, NR activity in the upper leaf reduced by 2.6 times, in the lower leaf, by eight times compared to two-leaf phase.

In the options with nitrogen introduction regardless of the dose, enzyme activity in the upper and lower leaves either did not differ, or was slightly higher in the lower leaf than in the upper leaf. Starting from the three-leaf phase, NR activity in the variants with nitrogen dose 40 and 100 mg/vessel gradually decreased, and more so in the lower leaf. In the variant with nitrogen dose 200 mg/vessel the enzyme activity in the upper leaf remained approximately at the same level up to the blooming phase, whereas in the lower leaf it decreased. In the variants with high nitrogen content (400

and 800 mg/vessel), NR activity by the blooming phase in the upper leaf increased and in the lower leaf did not change significantly. Thus, the lowest NR activity is characteristic of the variant without nitrogen fertilizer introduction, and in the variants with low nitrogen content, NR activity by the blooming phase decreased significantly both in upper and lower leaves.

A nitrogen dose of 200 mg/vessel is a transient value between the variants with insufficient level of nitrogen in the medium and its high

level. In the plants of this variant, NR activity in the upper leaf did not decrease significantly by the blooming phase, though it fell remarkably in the lower leaf. Apparently, in this case regulation of nitrogen nutrition by the plant is directed primarily at providing growing organs with mineral nitrogen, which causes the flow of newly absorbed nitrates to go to the growing leaf ensuring fairly high enzyme activity.

In the upper and lower leaves at low nitrogen doses (0, 40, and 100 mg) there were observed identical regularities of NR activity change: by the blooming phase NR activity in the upper leaf decreased in accordance to the doses by 2.6; 3.8, and 3.2 times, and in the lower leaf by 8.0; 7.1, and 5.4 times. In the variant with nitrogen dose of 200 mg activity, only insignificantly decreased in the upper leaf by the blooming phase (approximately by 1.2 times), in the lower leaf the enzyme activity decreased by 2.3 times. At high nitrogen availability (400 and 800 mg/vessel), NR activity was, as a rule, high in both upper and lower leaves.

The lack of nitrogen results in a decrease of NR activity first in the lower leaf, whereas in the upper leaf, enzyme activity remains higher. This effect is well seen particularly in the variant with nitrogen dose of 200 mg/vessel. Attention should be paid to the intensification of NR activity during spike formation in the variants with high nitrogen availability for the plants, which, apparently, may be accounted for by intense nitrates absorption by the plants during this vegetation period, as formation of reproduction organ creates a powerful attracting center and new growth points, which require inflow of nitrous substances for synthetic processes.

Sufficient nitrogen availability allows the plant to maintain NR activity at a high level both in the upper and lower leaves. This, apparently, leads to prolonging of the lower leaves life via activation of nitrogen assimilation processes, and, consequently, to a slowdown of the plant ageing process and a prolonging of the wheat plant's vegetation period.

Thus, the acquired data speak in favor of the connection between NR activity in the leaves and the level of mineral nitrogen availability for the plants. These results are of interest for elaboration and specification of methods of diagnostics of grain-crops nitrogen nutrition using NR activity. In particular, according to our data in the course of diagnostics of nitrogen availability for spring wheat by NR activity, it is efficient to determine enzyme activity not only in the upper growing leaf, but also in the lower green leaf, which has completed growth, for the sake of earlier identification of soil nitrogen identification.

Table 3. Nitrate reductase activity in spring wheat leaves in different phases of plant development depending on the level of nitrogen nutrition 9 nmol NO₂⁻/g (fresh weight)/1 h). Note top values are for the upper leaf; bottom values are for the lower leaf.

N dose mg/vessel	Phase of plant development				
	2-leaf	3-leaf	4-leaf	heading	anthesis
0	<u>154 ± 10</u>	<u>132 ± 10</u>	<u>90 ± 7</u>	<u>73 ± 3</u>	<u>59 ± 32</u>
	100 ± 12	126 ± 12	51 ± 4	36 ± 2	0 ± 1
40	<u>610 ± 45</u>	<u>485 ± 35</u>	<u>400 ± 31</u>	<u>213 ± 18</u>	<u>162 ± 9</u>
	700 ± 71	315 ± 30	306 ± 19	126 ± 10	98 ± 8
100	<u>675 ± 7</u>	<u>555 ± 45</u>	<u>606 ± 66</u>	<u>335 ± 22</u>	<u>210 ± 20</u>
	745 ± 26	325 ± 29	340 ± 22	224 ± 14	139 ± 7
200	<u>695 ± 29</u>	<u>600 ± 20</u>	<u>663 ± 40</u>	<u>962 ± 51</u>	<u>600 ± 35</u>
	800 ± 39	400 ± 28	383 ± 15	681 ± 31	350 ± 24
400	<u>655 ± 40</u>	<u>586 ± 59</u>	<u>684 ± 9</u>	<u>1,560 ± 179</u>	<u>1,148 ± 56</u>
	655 ± 30	510 ± 41	600 ± 19	900 ± 45	542 ± 34
800	<u>645 ± 30</u>	<u>510 ± 43</u>	<u>726 ± 21</u>	<u>1,015 ± 79</u>	<u>1,060 ± 54</u>
	610 ± 25	462 ± 40	569 ± 25	840 ± 29	685 ± 31

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Impact of disulfidreductase from the wheat caryopsis on certain technological characteristics of wheat flour and dough.

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The technological quality of wheat flour is known to be largely dependent on highly-molecular subunits of glutenin containing the biggest amount of SH-groups, capable of forming S-S links (Payne 1987). Dependence of gluten quality on the content of disulfide links was experimentally proven by Kretovich et al. (1978), Vakar et al. (1972), Bloksma (1975), and Kaczkowski et al. (1980). In our laboratory, we extracted the enzyme with thiol:protein disulfide oxidoreductase (EC 1.8.4.2) activity from wheat corn (Trufanov et al. 1999). The enzyme catalyzes dissociation of protein disulfides using restored glutathione as a cofactor. Our model experiment has shown that wheat disulfidreductase reduces aggregation ability of gluten proteins, which is apparently caused by dissociation of proteins SS-links and consequent weakening of gluten matrix rigidity (Osipova et al. 2004). The investigation was focused on the impact of disulfidreductase preparation introduction in the course of dough kneading on certain technological characteristics of flour and dough.

Materials and Methods. Disulfidreductase was extracted and purified from flour of Siberian selection wheat, Tulunskaya 12 variety as per the earlier described methods (Trufanov et al. 1999). In the tests there were used partially purified disulfidreductase preparation containing admixture of glutathionreductase (N1) and preparation of disulfidreductase with enzyme purity, that is not containing admixtures of other thiol-disulfide metabolism (N2) enzymes. Fifty mg of enzyme preparation containing approximately 3-5 units of disulfidreductase activity and NaCl were added to 50 g of flour of cultivar Mironovskaya 808. A similar portion (50 mg) of NaCl was added to control. Technical parameters were analyzed in compliance with the methods of the State Variety Testing of Agricultures (Methods 1988). The physical properties of dough were studied using Chopin alveograph, which was used to determine specific work for dough deformation (strength of flour, W; units of alveograph, u.a.), tenacity (P, mm) and extensibility (L, mm), and P/L ratio. Table 4 presents average data of three independent tests \pm standard error.

Results and Discussion. The addition of enzymes only insignificantly increases dough tenacity (P) (Table 4). Dough extensibility (L) values increase remarkably, N2 preparation addition increases extensibility by 7 %, whereas the addition of partially purified preparation (N1) by approximately 17 %. High efficiency of N1 preparation may be accounted for by the fact that presence of glutathionreductase ensures fairly high amount of restored glutation required for catalytic activity of disulfidreductase. Flour strength reliably increases with the addition of enzyme preparations. P/L proportion, however, practically does not change, so on the whole gluten quality of the Mironovskaya 808 wheat does not improve. Nevertheless, significant, up to 17 %, increase of dough extensibility is of interest. These data allow us to infer that partially cleaned disulfidreductase preparation containing glutathionreductase activity may be used to improve qualities of excessively strong, short-tearing gluten.

Table 4. Alveograph parameters with enzyme additives introduction (Significant at * P > 0.05; ** P > 0.01).

Sample	Alveograph parameters			P/L ratio
	Flour strength W, u.a.	Tenacity P, mm	Extensibility L, mm	
Control	188 ± 8	60 ± 0.5	112 ± 9	0.5 ± 0.04
TREATMENT:				
Preparation N1	223 ± 6**	64 ± 4.0	131 ± 10*	0.5 ± 0.10
Preparation N2	231 ± 2**	67 ± 4.0	120 ± 15	0.6 ± 0.10

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*Isoforms of glutathione reductase in wheat grains *Triticum aestivum*.*

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Glutathione reductase (GR) catalyzes the NADPH-dependent reduction of oxidized glutathione (Foyer et al. 1997). The primary function of glutathione reductase is to maintain a high GSH/GSSG ratio in cells, which is crucial for a variety of cellular functions, including the biosynthesis of DNA (Noctor et al. 1998). This flavoprotein oxidoreductase has a central role in maintaining GSH within the cellular environment, particularly during stress. Most, if not all, stresses include an oxidative stress component (Prasad et al. 1994; Wise 1995) that leads to tissue damage if antioxidative defenses are insufficient.

Wheat leaves contain two charge/mass-separable isoforms of glutathione reductase, one chloroplastic and the other probably cytosolic (De Lamotte et al. 2000). The endosperm of durum mature kernels contained a single form of glutathione reductase; it appeared about the 18th day after anthesis, whereas another isoform, present at the early stages of grain development, disappeared between the 20th and 30th days after flowering (Lascano et al. 2001).

Materials and Methods. Grains of the wheat cultivar Tulunskaya-12 were used in this research. The soluble, enzymatically active protein fraction of the wheat grains was extracted with a 0.1 Tris buffer, pH 7.5, containing 2 mM EDTA, from standard ground flour in the proportion 1:2 (weight:volume). The extract was subjected to chromatography on DEAE-Sephadex A-50. The protein fraction was eluted in a 0-0.6 M NaCl gradient. Fractions obtained were separated according in 7 % PAAG to the method of Davis (1964) at the basic pH. Molecular forms of glutathione reductase were

identified immediately on gel slabs by specific coloring (Ye et al. 1997). Bromophenol-blue was used as the marker for estimating Rf values, indicating the mobility of enzyme bands relative to the mobility of the bromophenol-blue front.

Results and Discussion. Selective enzyme coloring after native electrophoresis in the PAAG slabs manifested its presence in all the protein fractions acquired through ion-exchange chromatography of DEAE-sephadex A-50 (Fig. 2), revealing that the enzyme is represented by two molecular forms with different relative electrophoretic mobility (Rf) 0.36 and 0.32 (GR 1 and GR 2, respectively), which split during proteins desorption from anions by a linear gradient of NaCl concentration. The initial enzyme extract contained both enzyme isoforms (Fig. 2, lane 1).

During ion-exchange chromatography, the first to eluate from the sorbent was glutathione reductase molecular form (GR 2) with Rf 0.32 (Fig. 2, lanes 25–32), then GR 1, with Rf 0.36 (Fig. 2, lanes 33 and 34). Thus, unlike the grain of complete maturity *T. turgidum* subsp. *durum* (Lascano et al. 2001), *T. aestivum* subsp. *aestivum* grains contain two isoforms of glutathione reductase that may be split with the help of ion-exchange chromatography.

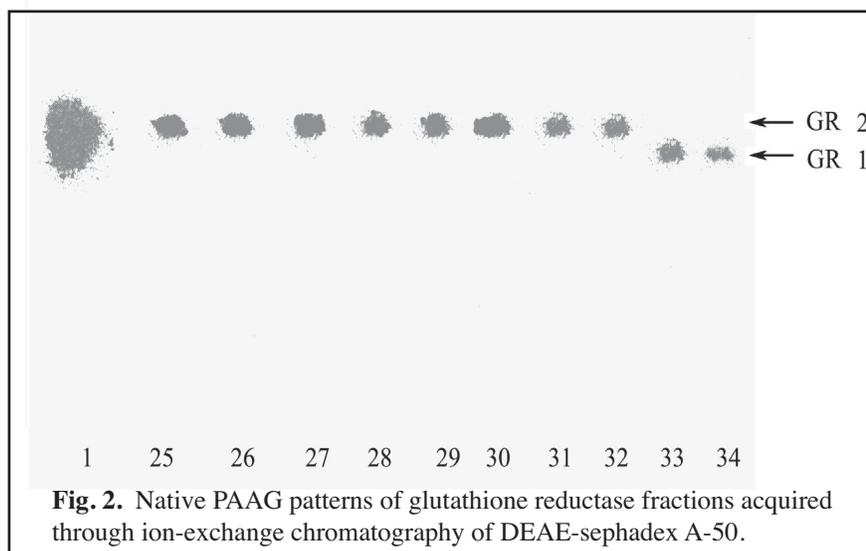


Fig. 2. Native PAAG patterns of glutathione reductase fractions acquired through ion-exchange chromatography of DEAE-sephadex A-50.

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The oxidative phosphorylation uncoupling of winter wheat mitochondria by saturated fatty acid and participation of ADP/ATP-antiporter.

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Oxidative phosphorylation is the main ATP source in aerobic organisms. In certain conditions, the uncoupling of oxidative phosphorylation (substrate oxidation without phosphorylation) occurs. Fatty acids are natural uncouplers of oxidative phosphorylation. Different mechanisms through which fatty acids cause uncoupling are known (Jezek 1999; Skulachev 1999; Wieckowski et al. 2000; Kadenbach 2003). Recent studies indicates that fatty acids are also involved in cell death pathways (Penzo et al. 2002). Fatty acid uncoupling is inhibited by addition of bovine serum albumin (BSA), purine nucleotides, and by ATP/ADP antiporter inhibitor, carboxyatractyloside (CAT) (Skulachev 1991).

As shown previously, unsaturated free fatty acids in winter wheat mitochondria plays not only a role of uncouplers (oleic, petrozelinic, linoleic and erucic acids), but also could be the only oxidation substrate for them (linoleic acid) (Grabelnych et al. 2004). Although unsaturated fatty acids have more effect on the mitochondrial membrane potential, the role of saturated fatty acid in energetic cell metabolism is significant too (Pastore et al. 2000; Penzo et al. 2002). Lauric and palmitic acids have caused more significant Δy decrease rate in durum wheat mitochondria among the saturated acids (Pastore et al. 2000). Fatty acid-dependent uncoupling of oxidative phosphorylation plays an adaptive role during hypothermia and oxidative stress in the plant mitochondria (Casolo et al. 2000; Pastore et al. 2000).

The aim of the present investigation is to study the influence of saturated fatty acid on the winter wheat mitochondria function and determine the ADP/ATP participation in fatty acid-dependent uncoupling.

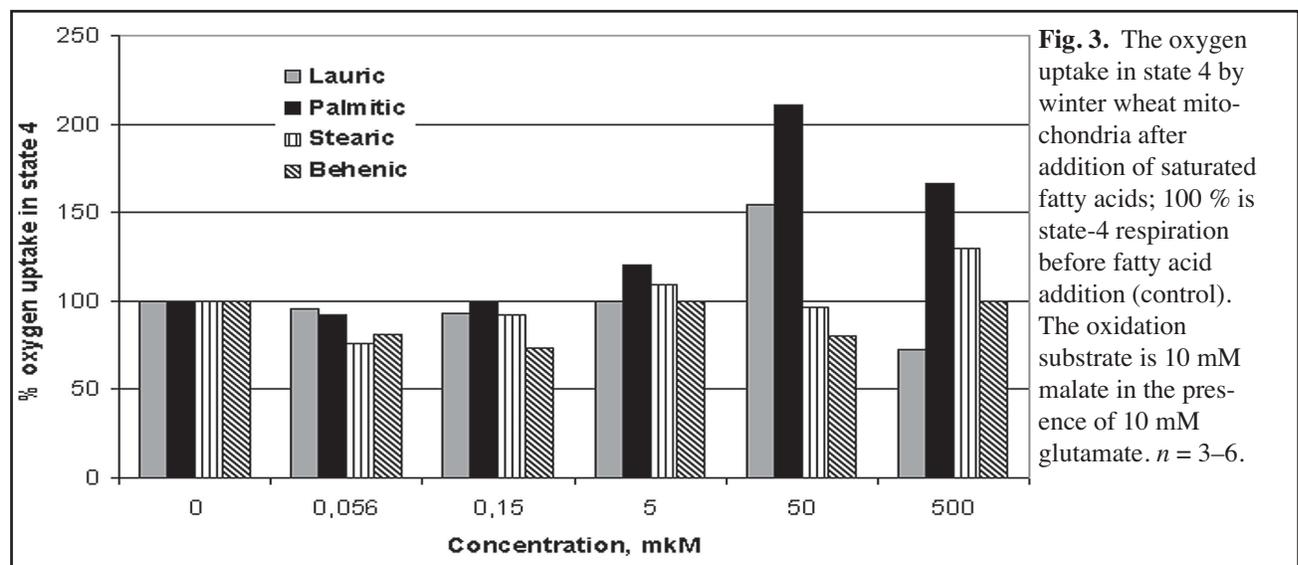
Materials and Methods. Three-day-old etiolated shoots of winter wheat cultivar Zalarinka germinated on moist paper at 26°C, were used in this work. Mitochondria were extracted from winter wheat shoots by differential centrifugation as describes previously (Pobezhimova et al. 2001). Isolated mitochondria were resuspended in the following medium: 40 mM MOPS-KOH buffer (pH 7.4), 300 mM sucrose, 10 mM KCl, 5 mM EDTA, and 1 mM $MgCl_2$.

Mitochondrial activity was recorded polarographically at 27°C using a platinum electrode of a closed type in a 1.4 ml volume cell (Estabrook 1967). The reaction mixture contained 125 mM KCl, 18 mM KH_2PO_4 , 1 mM $MgCl_2$, and 5 mM EDTA, pH 7.4. 10 mM Malate in the presence of 10 mM glutamate was used as an oxidation substrate. Lauric (C 12:0), palmitic (C 16:0), stearic (C 18:0), and behenic (C 22:0) acids were used in concentrations from 0,056 mkM to 10 mM. 1 mkM carboxyatractyloside (Catr) was used as ADP/ATP inhibitor.

Polarograms were used to calculate the rates of phosphorylative respiration (state 3), nonphosphorylative respiration (state 4), the rate of respiration after fatty acid addition, the rate of respiration after Catr addition, respiration control by Chance-Williams, and the ADP:O ratio (Estabrook 1967). The concentrations of mitochondrial protein and CSP 310 were analyzed by Lowry method (Lowry et al. 1951). All the experiments were made in three-six preparations. The data obtained were analyzed statistically, i.e., arithmetic means and standard errors were determined.

Results and Discussion. To determine saturated fatty acid uncoupling effect we added different amounts of fatty acid to state 4 mitochondria, because fatty acid-dependent increase of state 4 oxygen consumption is one of the main indicators of fatty acid-dependent uncoupling together with coefficient RC and ADP:O ratio decrease.

We found that an addition to state-4 mitochondria physiological concentrations (0.056 and 0.15 mkM) of lauric and palmitic acids didn't cause any changes in the rate of oxygen consumption but addition physiological concentrations of stearic and behenic acids caused even a decrease of oxygen consumption (Fig. 3). Behenic acid did not cause any changes in the rate of oxygen consumption at higher concentrations added. The most influence of lauric acid on the rate of oxygen consumption the was found at concentration 50 mkM, which caused about 54 % stimulation of non-phospho-



rylative respiration (Fig. 3, p. 129). Palmitic acid also has the most uncoupling activity at concentration 50 mkM, which cause twofold increase of non-phosphorylative respiration. The uncoupling effect of stearic acid become apparent only at high concentrations and didn't exceed 30 % of stimulation of state 4 respiration at concentration 500 mkM (Fig. 3, p. 129). Recoupled effect of Catr (about 39 % decrease of non-phosphorylation respiration rate) in winter wheat mitochondria shows participation of ADP/ATP-antiporter in this process. The uncoupling effect of palmitic and stearic acid was fully associated with participation of ADP/ATP-antiporter. The uncoupling effect of lauric acid also was partially associated with participation of ADP/ATP-antiporter.

The other picture of respiration stimulation in fatty acid-treated mitochondria after ADP addition was observed. In second cycle of phosphorylation the uncoupling action of all studied saturated fatty acids, including behenic acid, was found. Figure 4 shows the data about fatty acid influence on the oxygen consumption at concentrations which cause maximal uncoupling in the second cycle of phosphorylation.

Fatty acids can be divided into two groups: 1) lauric and palmitic acid (Fig. 4A and 4B) that cause immediate stimulation of state-4 respiration and the same or smaller stimulation of state-4 respiration in second cycle of phosphorylation; 2) stearic and behenic acids (Fig. 4C and 4D), that did not cause stimulation of state-4 respiration immediately but cause stimulation of state-4 respiration in second cycle of phosphorylation.

An addition of 50 mkM lauric acid caused the increase in state-4 respiration (Fig. 4A, 3), the subsequent addition of ADP caused the transfer of mitochondria to phosphorylative state (Fig. 4A, 4) and then to nonphosphorylative state (Fig. 4A, 5), whose value was equal to respiration rate in first cycle of phosphorylation after addition lauric acid. Palmitic acid also caused the increase in state-4 respiration (Fig. 4B, 3) and subsequent addition of ADP accompanied transfer to state 3 (Fig. 4B, 4), but its value was smaller than in first cycle of phosphorylation. State-4 respiration was higher (Fig. 4B, 5) as compared with state-4 respiration before palmitic acid addition, but smaller then state-4 respiration after palmitic acid addition in first cycle of phosphorylation (Fig. 4B, 2).

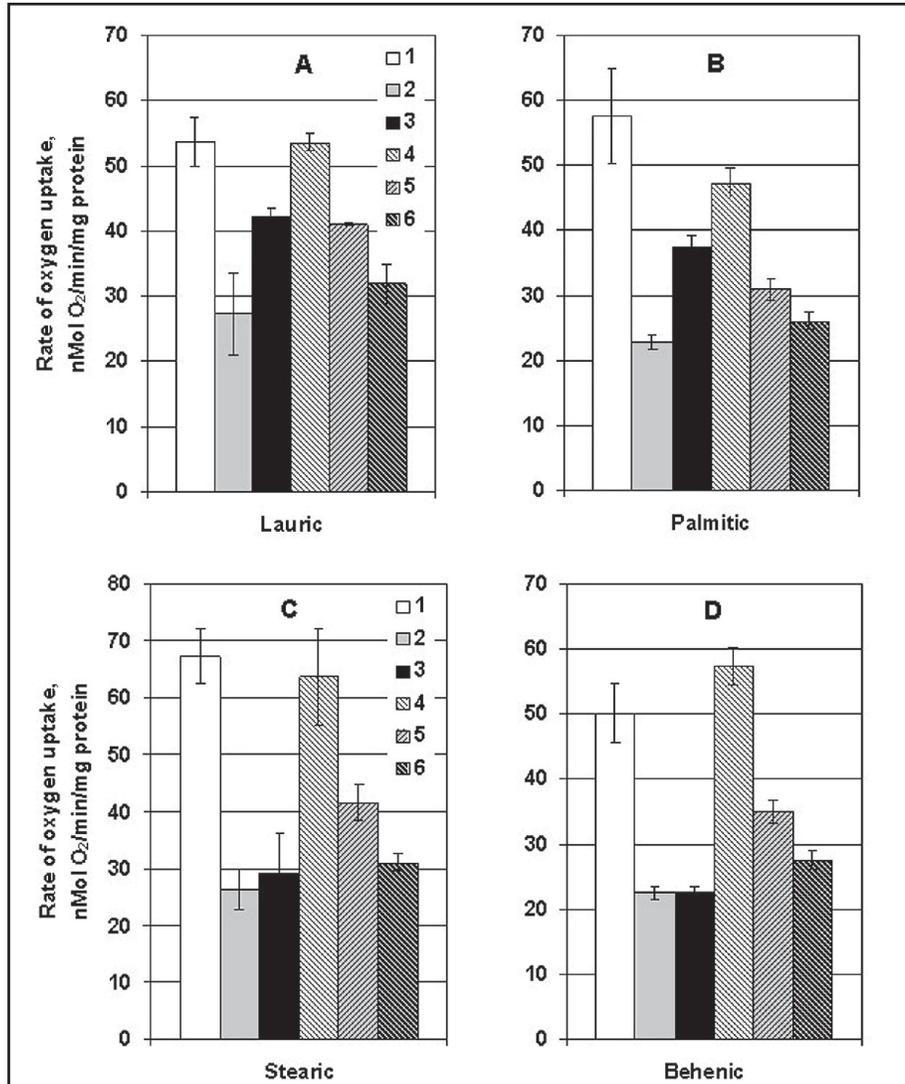


Fig. 4. The influence of saturated fatty acids and carboxyatractyloside (Catr) on the oxygen uptake by winter wheat mitochondria at first (1, 2, and 3) and second (4, 5, and 6) cycles of phosphorylation. Oxidation substrate is 10 mM malate in the presence of 10 mM glutamate. $M \pm SE, n=3-6$. Graph A, 50 mkM lauric acid; B, 10 mkM palmitic acid; C, 5 mkM stearic acid; and D, 5 mkM behenic acid. Lane 1, state-3 respiration; 2, state-4 respiration before addition of fatty acid; 3, state-4 respiration after addition of fatty acid; 4, subsequent state-3 respiration after 200 mkM ADP addition; 5, subsequent state-4 respiration; and 6, subsequent state-4 respiration after addition of 1 mkM Catr.

Although 5 mkM stearic acid addition in state 4 did not cause statistically significant stimulation of respiration (Fig. 4C, 3; p. 130), we observed 58 % increase of non-phosphorylative rate of respiration in second cycle of phosphorylation (Fig. 4C, 4; p. 130). Likewise the influence of stearic acid, behenic acid addition in state 4 didn't cause stimulation of respiration (Fig. 4D, 2; p. 130), but we observed 56 % increase of non-phosphorylative rate in the second cycle of phosphorylation (Fig. 4D, 4; p. 130). In all cases the increase of non-phosphorylative respiration rate in second cycle of phosphorylation was accompanied by decrease of RC coefficient and ADP:O ratio (Table 5). RC coefficient decrease was 26–49 % and ADP:O ration decrease was 27–33 %. So, all saturated fatty acids studied cause the uncoupling of oxidative phosphorylation in second cycle phosphorylation; the increase of non-phosphorylation respiration and decrease of RC coefficient and ADP:O ratio.

The stimulation of respiration by lauric acid was fully associated with ADP/ATP-antiporter participation (Fig. 4A, 6; p. 130). At the same time, the stimulation of respiration by palmitic, stearic and behenic acids decreased after addition of Catr only about 50 % (Fig. 4B, C, D, 6; p. 130). Therefore, we conclude that different carrier proteins participate in these fatty acid-dependent uncoupling.

All saturated fatty acids studied were able to cause the increase of winter wheat mitochondria respiration. Lauric, palmitic, and stearic acids caused oxidative phosphorylation uncoupling immediately, at the same time the uncoupling effect of behenic acid started after subsequent ADP stimulation. In our experiments with *T. aestivum* subsp. *aestivum*, it is shown that palmitic acid have the most uncoupling effect, whereas Pastore with coauthors (2000) found using *T. turgidum* subsp. *durum* mitochondria that lauric acid have the most uncoupling effect. ADP/ATP-antiporter participated in fatty acid-dependent uncoupling the all studied saturated acids.

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Table 5. The decrease of RC coefficient and ADP:O ratio in winter wheat mitochondria in presence of fatty acids during second cycle of phosphorylation. 100 % is RC coefficient or ADP:O ratio of mitochondria at first cycle of phosphorylation before fatty acid addition.

Saturated acid concentration	RC coefficient %	ADP:O ratio %
Lauric, 50 mkM	66.16	72.47
Palmitic, 10 mkM	51.00	71.27
Stearic, 5 mkM	58.01	67.21
Behenic, 5 mkM	73.87	66.86

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Seasonal changes in the content of dehydrin-like proteins in mitochondria from crowns of field-grown winter wheat plants

I.V. Stupnikova, G.B. Borovskii, A.I. Antipina, and V.K. Voinikov.

Cold stress adaptation has been established to induce dehydrin synthesis and accumulation (Close 1996). These proteins belonging to the family of the late embryogenesis proteins are characterized by the presence of highly conserved Y, S, and K segments. A peculiarity of dehydrin amino-acid composition provides for their functions. Amphiphilic-helices found in dehydrins are considered to bind to intracellular molecules, especially membranes and proteins, mainly by hydrophobic interactions (Close 1997). These associations may protect the functions of membranes and proteins by preventing coagulation during environmental stress and maintain water in dehydrated cells (Hoekstra et al. 2001). A correlation was observed between the endogenous concentration of dehydrins and frost tolerance of plant species, organs, or tissues (Sarhan et al. 1997). The patterns of dehydrin accumulation in trees coincided with seasonal fluctuations in their frost resistance (Wisniewski et al. 1996). In tissues of leaves (deciduous species), flower buds, blueberry bark, peach, black current, poplar, willow, and acacia, the accumulation of dehydrin-like proteins was reported in autumn and winter. However, the seasonal changes in the composition and content of these proteins in wintering grasses have not been studied. The presence of dehydrins in the nucleus, cytoplasm, cytoskeletal elements, and plasma membrane of various cells in seedlings and adult plants was demonstrated by immunolocalization and subcellular fractionation (Houde et al. 1995). Earlier, we detected two dehydrin-like proteins, which accumulated in mitochondria of the freezing-resistant wheat seedlings during cold hardening under laboratory conditions (Borovskii et al. 2000). Taking into account all facts above the objective of the study was to follow possible seasonal fluctuations in amount and spectra of dehydrins in winter wheat crowns being the vitally important winter wheat part, which determines winter survival.

Materials and Methods. Experiments were performed on field-grown winter wheat plants cultivar Irkutskaya ozimaya hardened under natural conditions. Mitochondria were isolated by differential centrifugation, as was described in (Borovskii et al. 2000). The crowns were ground with a mortar and pestle in the medium containing 0.3 M sucrose, 40 mM Mops (Sigma, United States), 2 mM EDTA, 10 mM KCl, 1 mM MgCl₂, 0.05 % polyvinylpyrrolidone, 0.5 % cysteine, and 0.5 % BSA (ICN, United States), pH 7.5. All other procedures were performed similarly as during mitochondria isolation from seedlings. Mitochondria from the crowns were purified using a discontinuous Percoll gradient comprising 21 % and 35 % Percoll. Purified mitochondria were used for protein isolation. SDS-PAGE was run in 11 % acrylamide gel in the modified Laemmli system (Laemmli 1970). Immunodetection was performed by the method of Timmons and Dunbar (1990). Polyclonal antibody against K fragment of dehydrins was kindly presented by T.J. Close (University of California, Riverside).

Results and Discussion. All our previous experiments concerning the detection of mitochondrial dehydrins were carried out with seedlings. Therefore, elucidating whether or not similar accumulation of dehydrins occurred in mitochondria of overwintering adult plants was important. Mitochondria were isolated from winter wheat crowns during autumnal hardening, in winter, and during spring deadadaptation. The mitochondria from crowns contained proteins with molecular weights being similar to proteins from seedlings, 63 and 52 kD (Fig. 5). Any other proteins related to dehydrins were not detected. The content of these proteins increased substantially during autumnal hardening. Peshkova et al. (1998) showed that the crowns of this winter wheat

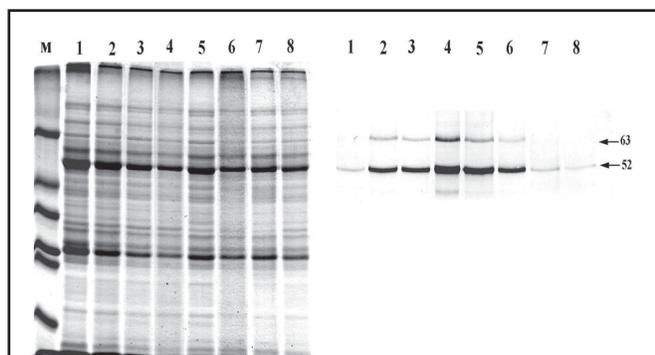


Fig. 5. Mitochondrial dehydrins from the crowns of winter wheat grown in the field. Proteins were fractionated in 11 % SDS-PAAG (left, gel) and transferred to nitrocellulose membrane (right, blot), which was incubated in the solution of antibody against dehydrins (1:1,000). Immunoreactive groups were detected with secondary antibody conjugated with alkaline phosphatase. Time of sampling: (1) Sept.; (2) Oct.; (3) Nov.; (4) Dec.; (5) Feb.; (6) March; (7) Apr.; and (8) May. Molecular weights (kD) of dehydrins are indicated on the right.

cultivar lost up to 67–69 % of water during hardening. The highest content of mitochondrial dehydrins was in December, and thereafter it began to decline gradually. In spring, the content of dehydrins in mitochondria from wheat crowns decreased to their level in autumn. In April, dehydrin content was similar to that in September; in May, it was even lower (Fig. 5, p. 132). We know that winter wheat cryotolerance increases during cold acclimation starting from early autumn and achieves its highest value by the beginning of winter, indicating the interrelation between dehydrin content and winter wheat freezing tolerance, what is in agreement with the results of other researchers (Sarhan et al. 1997). Earlier we showed that in contrast to mitochondrial dehydrins, the spectra of those in total cell water-soluble fraction contained much more protein groups. In the total water-soluble protein fraction, both the amount of dehydrins in each group and the number of dehydrin groups of a particular molecular weight increased with autumnal hardening and attained the highest values by the end of January. At this time, dehydrins with molecular weights of 209, 196, 66, 50, 41, 24, 22, 17, 15, and 12 kD were detected among soluble proteins (Stupnikova et al. 2004). Two protein groups (66 and 50 kD) had molecular weights close to those of mitochondrial dehydrins (63 and 52 kD). The methods used did not permit us to decide whether these proteins with close molecular weights were similar or different proteins.

Adaptation process is known to include two stages: unspecific stress-response and specific adaptation (Kuznetsov et al. 1987). During the first stage, mobilization and development of plant defensive systems occur, which provide for organism short-term surviving. During the second stage, specific adaptation mechanisms develop, which are responsible for plant life under the conditions of long-term stress (Kuznetsov et al. 1987). The accumulation of these dehydrins during cold adaptation of field-grown plants and the absence of their further changes in autumn and winter indicate evidently that these proteins operate like cryoprotectors during both stages of adaptation.

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An effect of varied stresses on alternative oxidase and plant uncoupling mitochondrial protein expression in winter wheat seedlings.

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The different stress effectors are able to create similar damages in cell: the increased viscosity of membranes, formation of gel phases, degradation, and destruction of proteins, phospholipids and other macromolecules. An identical phenomenon was shown during treatment of cells by superoxide (Vanlerberghe et al. 1992). Accumulation of active oxygen and its derivatives also have been shown in plants in response to various environmental stresses. Oxidizing stress can be

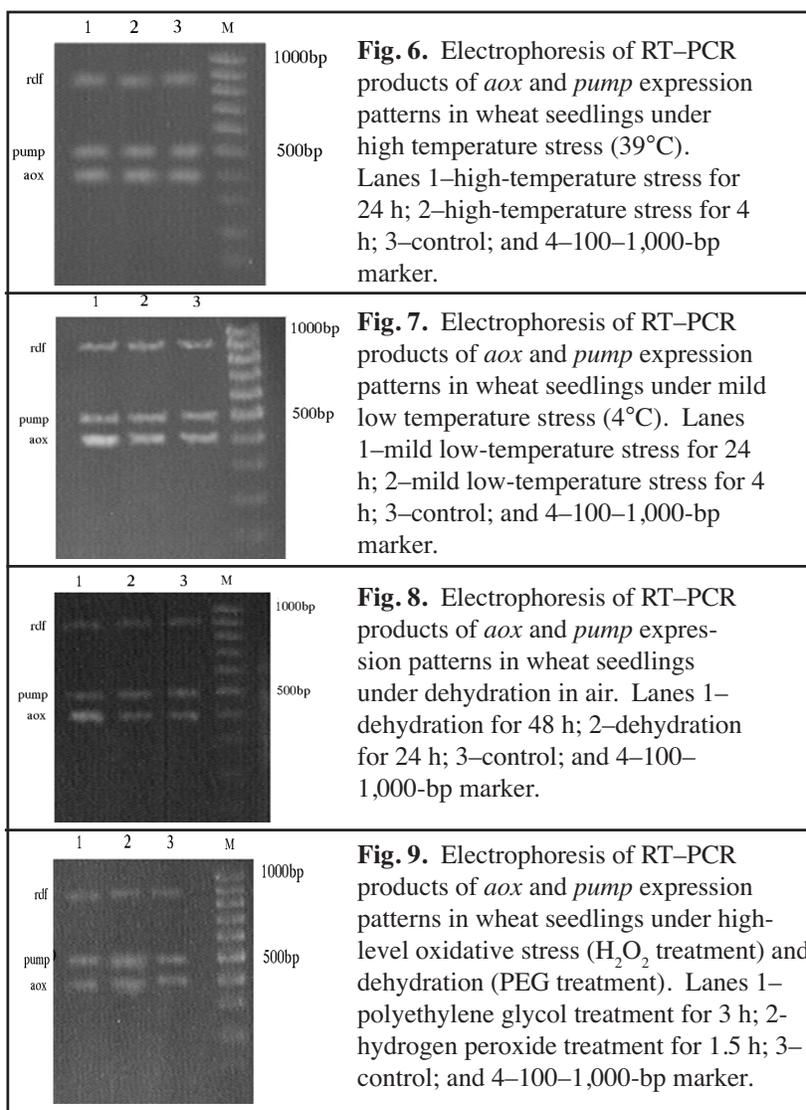
considered as secondary answer to damage of cell organelles and activation of redox-enzyme system in the cell (Vanlerberghe et al. 1997). Recent reports show the existence of various pathways of this process leading to similar results – decreasing of transmembrane potential on inner membrane of mitochondria and reduction of mitochondrial reactive oxygen species (ROS) generation. Protein participation in this type of regulation depends on plant species, stage of the development, type of stress, and some other factors. Alternative oxidase (AOX) and plant uncoupling mitochondrial proteins (PUMP) seems to play a significant role in ROS production regulation processes. These proteins decrease transmembrane potential in different ways. Activation of the AOX pathway leads to uncoupling of oxidation and phosphorylation in three of four respiratory complexes. On the other hand, PUMP (like other members of UCP (uncoupling protein) family) does not block phosphorylation, but stimulates the spreading of potential via transport of fatty acids ions (Umbach 1993). The correlation of these two ways in decreasing of transmembrane potential is of great interest. We note that AOX and PUMP can work as antagonists because fatty acids that necessary for PUMP functioning depresses AOX activity (Jezek et al. 2000). The aim of the present study was to demonstrate the amounts of AOX and PUMP expression patterns in winter wheat under different stressing conditions using multiplex RT-PCR method.

Materials and methods. After growing for 3 days, wheat seedlings were subjected to environmental stress treatments. Low temperature heat and dehydration treatments were conducted by transferring of etiolated seedlings to separate conditions: 4 or 39°C, 30 % H₂O₂ solution for 1.5 h, polyethylene glycol (PEG MW8000) solution treatment for 3 h, and dehydration in air for 24 and 48 h. Samples were immediately frozen in liquid nitrogen and stored at -70°C until processed for RNA extraction. Transcript levels of *aox* and *pump* was also controlled under unstressed conditions.

Primers were designed from published sequence data of genes encoding AOX, PUMP (EMBL database) and RDF (Monstein et al. 2002) to amplify 415-bp, 505-bp, and 890-bp fragments of corresponding transcripts from *T. aestivum* cv. Irkutskaya ozimaya. Using a SV Total Isolation System (Promega, Madison, WI), 200 ng of total RNA was isolated and used for the first-strand cDNA synthesis with REVERTA kit (Amplisense, Moscow) and 3'-primer. The amount of total RNA in reactions was identical in experiments which results we compared. The transcript levels of the constitutively expressed *rdf* gene were also monitored on corresponding gels to confirm that amount of RNA in compared experiments was equal.

Results and Discussion. The early events of plant adaptation to environmental stresses are perception and subsequent stress-signal transduction that lead to the activation of various physiological and metabolic responses, including the development of oxidation stress. Therefore, they can cause induction of *aox* and *pump* genes expression since AOX and PUMP have been shown efficiently reduce the generation of mitochondrial reactive oxygen species (Kowaltowski et al. 1998; Umbach 1993).

The expression patterns of *aox* and *pump* were analyzed under various



stresses. Expression of *aox* and *pump* were not included under high temperature treatment (Fig. 6, p. 134) or short-time cold stress (Fig. 7, p. 134), but *aox* expression was induced after 24 hour of low temperature treatment whereas *pump* gene expression was not affected (Fig. 7, p. 134). Expression of *aox* was induced after 48 h of dehydration (Fig. 8, p. 134). The strongest and fastest accumulation of *aox* and *pump* mRNAs within 1,5 h was observed when wheat seedlings were subjected to high oxidative stress with hydrogen peroxide (Fig. 9, p. 134).

These results demonstrate that expression of *pump* was induced in winter wheat seedlings mainly by strong oxidative stress. Previously, Maia et al. (1998) reported that cold-inducible AtPUMP protein may play a role in heat-requiring physiological events in *Arabidopsis*. Although, two cDNAs encoding UCP-like proteins have been isolated from potato (StUCP) (Laloi et al. 1997). Because the expression of StUCP was detected mainly in flowers and fruits, it has been hypothesized that StUCP can be associated with burst of respiration in flowering and fruit ripening in combination with AOX (Laloi et al. 1997). The cold treatment at 4°C for 12 h of skunk cabbage plants (*Symplocarpus foetidus*) was enough for expression of two UCP-like genes SfUCP and SfUCP encoding uncoupling proteins (Ito 1999).

Only powerful oxidative stress with hydrogen peroxide could induce strong and simultaneous expression of *aox* and *pump* genes in winter wheat seedlings (Fig. 9, p. 134). We propose from our results that *aox* induction most likely and mainly occurs as part of the acclimation process and is necessary for adaptation to stress conditions. *aox*, therefore, is a gene of adaptation. This possibility is supported by other investigators (Djajanegara et al. 2002). On the other hand, it may be that two energy-dissipating systems, both PUMP and AOX are involved in the regulation of the oxidative stress development in extreme situation of oxidative burst as it was in our experiment with hydrogen peroxide treatment.

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Dehydrin localization in mitochondria of winter wheat seedlings.

I.V. Stupnikova, G.B. Borovskii, A.I. Antipina, and V.K. Voinikov.

Among proteins related to cold acclimation and cold stress tolerance, the dehydrin family presents peculiar interest. This family, known as a group-2 late embryogenesis abundant (LEA) proteins, is one of the ubiquitous water-stress-responsive proteins in plants (Close 1997). These proteins are hydrophilic, thermostable and glycine-rich and possess unique repeated sequences that are believed to form putative amphiphilic α -helices. The putative multiple functions of dehydrins have been described from the viewpoint of deduced secondary structures. They were hypothesized to function by stabilizing large-scale hydrophobic interactions such as membrane structures or hydrophobic patches of proteins. Highly-conserved polar regions of dehydrins have been suggested to hydrogen-bond with polar regions of macromolecules, acting essentially as a surfactant, to prevent coagulation under conditions of cellular dehydration or low tempera-

tures (Ismail et al. 1999). Immunolocalization and subcellular fractionation results have showed that members of the dehydrin family are present in the nucleus, cytoplasm, and plasma membrane (Danyluk et al. 1998). We have found that two dehydrin-like proteins (dlps) accumulate in mitochondria of cereals in response to cold (Borovskii et al. 2000). We have also demonstrated a positive correlation between accumulation of the two mitochondrial dlps and cold-tolerance of the species studied. The aim of the study was to elucidate whether dehydrins penetrate mitochondria or they associate with the surface of the organelles.

Materials and Methods. Experiments were performed on etiolated winter wheat seedlings of the cultivar Irkutskaya ozimaya. Seedlings were grown on moistened filter paper at 22°C for 4 days. Mitochondria were isolated by differential centrifugation, as was described in (Welin et al. 1994). Mitochondria from wheat shoots were purified using a discontinuous Percoll gradient (De Virville et al. 1994). This gradient comprised 18, 23, and 35 % Percoll in the medium containing 0.3 mM sucrose, 40 mM Mops-KOH, pH 7.4, and 0.1 % BSA. Mitochondria were collected from the interface between 23 and 35 % Percoll. Purified mitochondria were used for protein isolation or they were treated with pronase E (1 mg/ml) at 37°C for 1 h in order to determine proteins localized at the outer membrane. Purified mitochondria were placed in the sample buffer, and proteins were extracted at 60°C for 15 min. Similar procedure was applied to the proteins from membrane fraction. SDS-PAGE was run in 10–12 % acrylamide gel in the modified Laemmli system (Laemmli 1970). Immunodetection was performed by the method of Timmons and Dunbar (1990). Polyclonal antibody against K fragment of dehydrins was kindly presented by T.J. Close (University of California, Riverside).

Results and Discussion. Earlier we found that dehydrins are able to associate with mitochondria very rapidly, as it was detected during seedling freezing (Borovskii et al. 2002). We supposed that these proteins did not penetrate inside the mitochondria but associated with their outer membrane. To verify this assumption, we used seedlings acclimated to 4°C for a week. Such a term for acclimation was chosen to permit dehydrins to be transported into mitochondria if such a transport could take place in the cell. In order to elucidate dehydrin

localization, we treated isolated and purified mitochondria with pronase E. The results obtained permitted us to conclude that, during seedling cold acclimation, two dehydrins accumulated in mitochondria being localized at their outer membrane because they were accessible for protease (Fig. 10). Earlier, outer localization of other protective proteins, maize low-molecular-weight heat-shock proteins, was detected in mitochondria in the test with protease (Borovskii and Voiniko 1993).

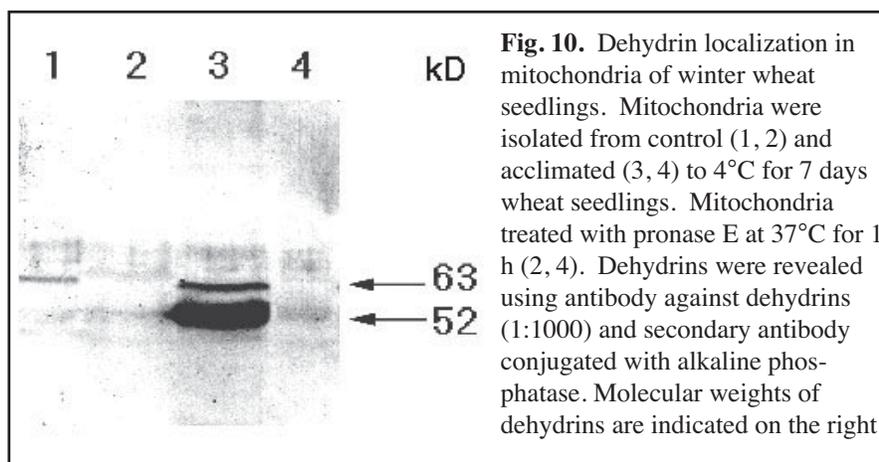


Fig. 10. Dehydrin localization in mitochondria of winter wheat seedlings. Mitochondria were isolated from control (1, 2) and acclimated (3, 4) to 4°C for 7 days wheat seedlings. Mitochondria treated with pronase E at 37°C for 1 h (2, 4). Dehydrins were revealed using antibody against dehydrins (1:1000) and secondary antibody conjugated with alkaline phosphatase. Molecular weights of dehydrins are indicated on the right.

Dehydrins evidently fulfill a defensive function in the mitochondria. Understanding the mechanism of this defense is difficult, because it is unknown whether membrane lipids or proteins are the targets for dehydrin action. A dehydrin WCOR410 is known to associate with the plasma membrane. This dehydrin is supposed to protect this very frost-sensitive structure (Danyluk et al. 1998). Thomashow (1999) believes that dehydrin can suppress lipid phase transition from lamellar to hexagonal state, which implies its interaction with membrane lipids. Hara et al. (2003), relying on their data, suggested that dehydrin facilitates plant cold acclimation by acting as a radical-scavenging protein to protect membrane systems under cold stress. Such type of protection is especially important for mitochondria because reactive oxygen species arise therein inevitably during respiration. Mitochondria convert about 2 % of consumed oxygen into hydrogen peroxide, which could damage membranes (Scandalios et al. 1997). On the other hand, dehydrin protective effects on proteins are not less significant. Dehydrin protection of some enzymes against denaturing was demonstrated in experiments *in vitro* (Wisniewski et al. 1999).

Thus, our results showed that the mitochondrial dehydrins are peripheral proteins and associated with the surface of the outer mitochondrial membrane. We suppose that dehydrins interact with membrane lipids and their stabilizing effect during cell dehydration is exerted via the retardation of lipid layer phase transition or due to reduction

of lipid peroxidation. It seems likely that dehydrin association with mitochondria is an important mechanism of cold stress resistance.

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Association of dehydrins with membranes of winter wheat cells during cold treatment

I.V. Stupnikova, G.B. Borovskii, A.I. Antipina, and V.K. Voinikov.

Primary sites of cold injury in plants are cell membrane systems (Thomashow 1999). Therefore, protecting membrane structures is one of the important parts of efficient cold adaptation and tolerance mechanisms, in which cold-regulated (COR) proteins play significant role, functioning as cryoprotectors or regulators. Among COR-proteins, presumably protecting cell membranes the dehydrin family presents particular interest. Danyluk et al. (1998) found acidic dehydrins WCOR 410, a subtype of the dehydrin family, which are largely hydrophilic and have high content of charged residues and hydroxylated residues. WCOR 410 accumulates in the vicinity of the plasma membrane of cells in the sensitive vascular transition area where freezing-induced dehydration is likely to be more severe (Danyluk et al. 1998). Hara et al. (2003) showed that a dehydrin protein, purified from *E. coli* expressing citrus dehydrin cDNA prevented peroxidation of soybean liposomes in vitro. They suggested that the dehydrin facilitates plant cold acclimation by acting as a radical-scavenging protein to protect membrane systems under cold stress (Hara et al. 2003). We also found total cell dehydrin-like proteins with molecular weights of 209, 196, 169, 66, 50, and 41 kD, which are characteristic for hardening state of winter wheat plants (Stupnikova et al. 2000; Borovskii et al. 2002a), and mitochondrial proteins ranging from 52 to 63 kD depending on the species of cereals, which accumulated during cold adaptation (Borovskii et al. 2002b). Our next task was to elucidate whether and which dehydrins associate with other (nonmitochondrial) cell membranes at low temperature.

Materials and Methods. Experiments were performed on etiolated winter wheat seedlings of the cultivar Irkutskaya ozimaya. Seedlings were grown on moistened filter paper at 22°C for 4 days. For hardening, 3-day-old seedlings grown

under optimal conditions were exposed to low temperature for 7 days at 4°C. Mitochondria were isolated by differential centrifugation, as was described in (Welin et al. 1994). Mitochondria from wheat shoots were purified using a discontinuous Percoll gradient (De Virville et al. 1994). Purified mitochondria were used for membrane protein isolation. Membranes were sedimented by ultracentrifugation at 105,000 g for 90 min. Membrane proteins were extracted with sample buffer at 50–60°C for 20 min and separated in 12 % SDS–PAAG. Proteins were transferred to the nitrocellulose membrane and detected with antibody against dehydrins. Immunodetection was performed by the method of Timmons and Dunbar (1990). A polyclonal antibody against the K fragment of dehydrin was kindly provided by T.J. Close (University of California, Riverside).

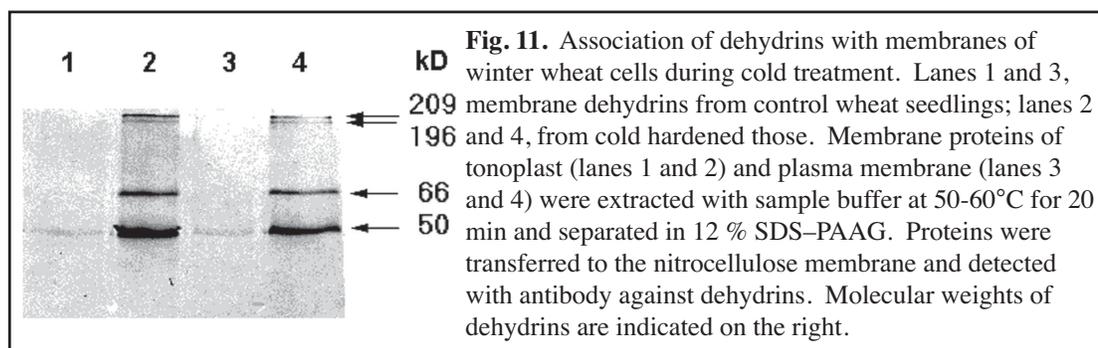
Results and Discussion. To study dehydrins associated with other (nonmitochondrial) cell membranes at low temperature, we isolated the fractions of such cell membranes as tonoplast and plasma membrane from wheat seedlings exposed to low temperature for 7 days and from seedlings grown at 22°C (control). Among wheat membrane proteins, we detected four dehydrins with molecular weights of 209, 196, 66, and 50 kD (Fig. 11). Dehydrin-like proteins with similar molecular weights were found earlier among total cell water-soluble dehydrins, isolated from winter wheat seedlings and crowns (Borovskii et al. 2002a; b). The main part of these proteins has membrane localization. Both total cell and

membrane dehydrins accumulated during cold adaptation (Fig. 11), what taking together with their hydrophilic and heat-stable nature allows suggesting that they protect

and stabilize membranes structures of wheat cells during cold stress. We also believe that a limited number of dehydrin types could exert stabilization of diverse membrane structures, which could be injured by cold. Other dehydrin-like proteins (Borovskii et al. 2002a) revealed in total cell fraction possibly are water-soluble proteins protecting possibly cold-sensitive cell proteins. Two membrane dehydrin groups (66 and 50 kD) had molecular weights close to those of mitochondrial dehydrins (63 and 52 kD). The methods used did not permit us to decide whether these proteins with close molecular weights were similar or different proteins. Therefore, so far we can not answer the question whether dehydrins associated with mitochondria can be detected in other cell compartments. On the basis of the data we may conclude that dehydrins with molecular weights 209, 196, 66, and 50 kD could interact with lipids of cell membrane structures during cold treatment and stabilize them preventing lipid phase transition.

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Identification of HMW and LMW subunits of glutenin and their impact on various technological parameters.

E.V. Berezovskaya, V.A. Trufanov, T.N. Mitrofanova, and L.S. Kazmiruk.

Wheat reserve proteins gliadin and glutenin are the key components of gluten, which are responsible for rheological and baking qualities of wheat flour. Glutenins belong to the class of polymeric proteins, whose individual polypeptides are known as high- and low-molecular weight subunits (HMW- and LMW- glutenin subunits). Subunits of these two groups differ in amino-acid composition, molecular mass (from 23 to 68 kDa for LMW-glutenin subunits and from 77 to 160 kDa for HMW-glutenin subunits), and structure (Kasarda 1999). HMW-glutenin subunit genes are located on the long arms of chromosomes 1A, 1B, and 1D, loci *Glu-A1*, *Glu-B1*, and *Glu-D1*, respectively (Payne 1987). LMW-glutenin subunit genes are on the short arms of chromosomes 1AS, 1BS, and 1DS (Singh et al. 1988). Many researchers have shown that allele variations of HMW- and LMW-glutenin subunits are associated with differences in technological parameters of flour and dough (Payne 1987; Autran et al. 1987; Gupta et al. 1989). Both LMW- and HMW-glutenin subunits, whose number is normally three-four times that of LMW-glutenin subunits, make a significant impact on viscose-elastic properties of dough and other technological parameters of flour (Pogna et al. 1990; Metakovsky et al. 1997). Variations in the composition of these subunits among different genotypes are in fact the major factor, which determines differences in baking qualities of dough and bread.

This work summarizes the results of complex investigation of grain reserve protein, glutenin, in connection with productivity and quality of gluten of two unrelated cultivars of soft spring wheat and four hybrid F₉ and F₁₀ from their interbreeding, establishment of the impact of various alleles coding glutenins, and the results of quantitative determination of HMW- and LMW-glutenin subunits and their correlation and role in determination of rheological properties.

Materials and Methods. The objects of investigation were the varieties of soft spring wheat Rollo (R) and Drott (D) and four constant hybrid forms from their interbreeding: RxD-I, RxD-II, RxD-III, and RxD-IV in F₉ and F₁₀ generations. The spring wheat cultivar Drott (Fylgia II/Sviöf 0990) has high resistance to fungal diseases, it is a highly productive variety with fairly strong straw. The Early Tyumenskaya cultivar was selected by mass selection method from the cultivar Rollo (K-45657, Norway).

For glutenin extraction, we used a Tris-HCl buffer, pH 6.8 containing sodium dodecylsulphate and 10 % β-mercaptoethanol following complete gliadin extraction by 70 % ethanol. Restoration of S-S-bonds was conducted for 15 hours at 40°C. SDS-PAAG electrophoresis was performed according to Laemmli (1970), overnight, at constant current strength and stable voltage. Concentrating and separating gels (3 and 10 %, respectively), containing acrylamide, methylenbisacrilamide, Tris-HCl (pH 6,8), TEMED, ammonium persulphate, and 10 % SDS. The gels were scanned and density-measured. A standard protein mixture was used to determine molecular mass (Mr) of glutenin subunits. HMW-glutenin subunits were identified according to Payne et al. (1983). Aggregation ability of gluten proteins, as one of the major characteristics of gluten rheological properties, was determined by the modified method of Arakawa and Yonezawa (1975), which is widely used for classifying cultivars by gluten quality. The tests were conducted directly with flour and gluten washed in 1 % NaCl solution and in distilled water. Washed gluten was extracted by 0.01 M acetic acid for 16–20 h at 4°C with constant agitation. The extracts then were centrifuged at 7,000 g for 15 min. Protein concentration was determined by spectrophotometric Calcar's method and calculated as per the following formula:

$$1,45D_{280} - 0,74D_{260} = C \text{ mg/ml,}$$

where 0.74 and 1.45 = recalculation ratios,
C = protein concentration in the solution,
D_{280 and 260} = optical solution density.

Aggregating ability was determined by the change of absorption at turbidity increase, for 10 min with the interval of 30 sec, protein solution + 0.2 M phosphate buffer, pH 5.65, (Arakawa and Yonezawa 1975) or pH 9.5

(Trufanov 1994) containing 2 M NaCl. The constant of aggregation speed K was calculated at the first stages of aggregation by the turbidity change using the following equations:

$$K = r/C^4, \quad r = t^3/3t, \text{ where}$$

K = constant of the initial aggregation stage;

t = solution turbidity index;

t = time in sec;

C = protein concentration, %; and

t_{10}/C = aggregation constant after 10 min of processing, calculated per unit of protein concentration in the solution.

Result and Discussion.

Electrophoresis of proteins from standard cultivars (Hope, Bezostaya (unawned) spring, Novosibirskaya 67, and Chinese Spring) allowed the identification

of high-molecular-weight subunits according to Payne (1983). The genes encoding glutenin HMW-glutenin subunits are distributed on the long chromosome arms 1A, 1B, and 1D. Three loci, *Glu-A1*, *Glu-B1*, and *Glu-D1*, jointly make up a locus i and encode HMW subunits. Two subunits of loci *Glu-B1* and *Glu-D1* have two subgroups x and y, subunits differing in mobility and amino-acid composition, including cystein content. Standard cultivars with identified subunits (Table 6) allowed us to establish the position of each subunit via comparison of densitograms (Fig. 12) and Rf in the course of electrophoresis in SDS-PAAG. We also used alternative identification system using molecular masses (Ng and Bushuk 1989) (Table 7).

The data on the composition and molecular weight of functional glutenin subunits and density-measuring scanning of electrophoretic spectra of the standard wheat cultivars show that there were identified hypothetical genotypic formulas for HMW-glutenin subunits of the cultivars Rollo and Drott and their four hybrids. We demonstrated redistribution of inherited information between hybrids (Table 8).

Table 6. Genotype of high-molecular-weight glutenin subunits of some standard wheat cultivars.

Chromosome arm	Locus	HMW-glutenin subunit composition			
		Novosibirskaya 67	Bezostaya	Hope	Chinese Spring
1AL	<i>Glu-A1</i>	a (1)	b (2*)	a (1)	c (null)
1BL	<i>Glu-B1</i>	b (7+8)	c (7+9)	c (6+8)	b (7+8)
1DL	<i>Glu-D1</i>	a (2+12)	—	d (5+10)	a (2+12)

Table 7. Molecular weight of the glutenin subunits of some standard wheat cultivars.

Subunit	Molecular weight
1	114.7
2*	106.6
2	103.7
5	98.5
6	86.6
7	84.9
8	75.8
9	72.8
10	71.8
12	69.3

Table 8. Allelic variation in glutenin subunits in the cultivars Rollo and Drott and their hybrids.

Chr Arm	Locus	Rollo	Drott	Rollo/Drott hybrid			
				I	II	III	IV
1AL	<i>Glu-A1</i>	c (null)	a (1)	a (1)	c (null)	a (1)	a (1)
1BL	<i>Glu-B1</i>	b (7+8)	c (6+8)	c (6+8)	c (6+8)	b (7+8)	b (7+8)
1DL	<i>Glu-D1</i>	a (2+12)	d (5+10)	d (5+10)	a (2+12)	d (5+10)	a (2+12)

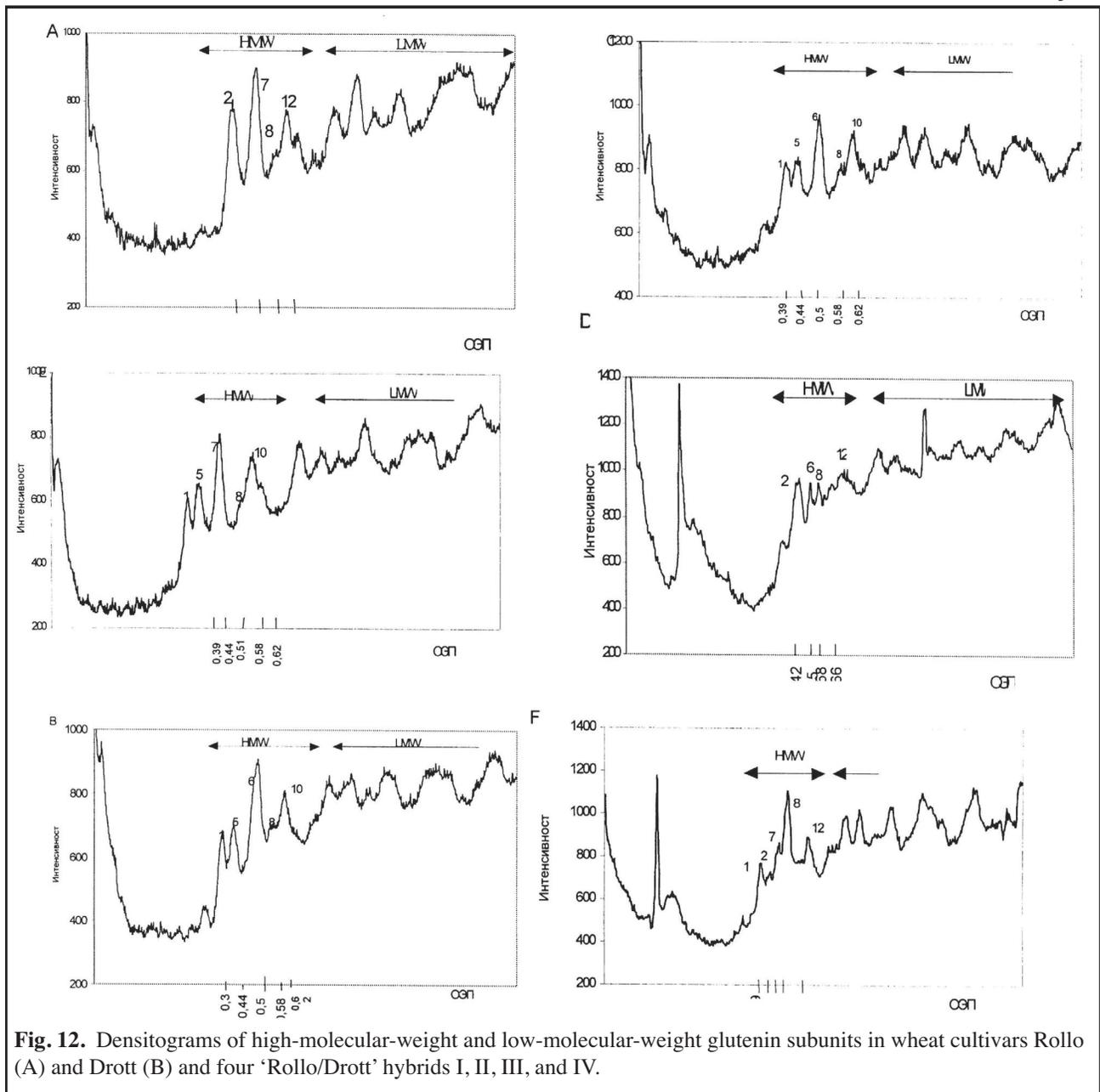


Fig. 12. Densitograms of high-molecular-weight and low-molecular-weight glutenin subunits in wheat cultivars Rollo (A) and Drott (B) and four 'Rollo/Drott' hybrids I, II, III, and IV.

All the four hybrids produced by crossing of two parental cultivars Rollo and Drott have significant differences in their HMW-glutenin subunits. Thus, the I hybrid completely correspond to the Drott HMW-subunits, but manifests differences in the realm of LMW-glutenin subunits (Fig. 12). In the other three hybrids, parental HMW-glutenin subunits were redistributed: the 'Rolo/Drott' hybrid II inherited from Rollo subunits 2+12 of locus *Glu-D1* and nonexpressing subunit of null-locus *Glu-A1*, and from Drott subunits 6+8 of locus *Glu-B1*; 'Rolo/Drott' hybrid III is closer by component composition to Drott, but is different from it by the single subunit 7, typical of Rollo and belonging to locus *Glu-B1*; 'Rolo/Drott' hybrid IV is closer to Rollo, differing by one subunit (1) encoded by locus *Glu-A1* (Table 8).

Subunit 1 is associated with high baking quality in contrast to the null allele. This subunit might possess a unique primary structure, which participates in the formation of stable gluten aggregates. Subunits 5+10 also correlate with good baking quality, in contrast to subunits 2+12. Differences in amino-acid sequence and secondary structure between allele subunits 5+10 and 2+12 also were revealed (Anderson 1996). We identified genotypic formulas that contain both 'good', 5+10 and 1 (in Drott), and 'bad' subunits, 2+12 (in Rollo).

Apparently, Drott is likely to produce higher dough quality than Rollo, which is confirmed by aggregation and technological qualities parameters. ‘Rolo/Drott’ hybrids I and III might also yield good parameters, as their spectrum contains HMW-glutenin subunits 5+10 obtained from Drott. We assumed that ‘Rolo/Drott’ hybrid IV will manifest poor baking qualities, because it inherited subunits 2+12 from both parents, which do not have good parameters of technological properties.

High-molecular-weight subunits represent only 10 % of all the gluten proteins, but their role in determining baking quality in accordance with different subunit types is immense. Another important factor in determination and more precise definition of various technological parameters is the second group of polymeric proteins LMW-glutenin subunits. HMW- and LMW-glutenin subunits differ in the values of amino-acid composition, molecular weight (from 23 to 68 kDa) and structure (Kasarda 1999).

The quantity of LMW-glutenin subunits exceeds that of the HMW-glutenin subunits by 3–4 times (Kasarda 1999). Their characterization is complicated, because they are controlled by a large number of genes and their subunits are often insoluble after restoration of intermolecular disulfide bonds.

To investigate their participation in the characterization of the cultivars, we determined their relative quantity and calculated the proportion between the quantities of LMW- and HMW-glutenin subunits. According to Hou et al. (1996), this proportion in flour proteins may act as an important parameter in the evaluation of rheological and baking qualities of wheat.

Table 9. Percentage of high-molecular-weight (HMW) and low-molecular-weight (LMW) glutenin subunits in the cultivars Rollo and Drott and their hybrids.

Line	HMW-GS	LMW-GS	LMW-GS/HMW-GS
Rollo	25.5	75.4	2.95
Drott	46.4	53.6	1.16
Rollo/Drott I	30.5	69.5	2.27
Rollo/Drott II	23.6	76.4	3.23
Rollo/Drott III	25.0	75.0	3.00
Rollo/Drott IV	24.7	75.3	3.04

Quantitative determination of the LMW- and HMW glutenin subunits was conducted with the help of density-measuring analysis DDS-PAAG of electrophoretogram (Fig. 13). Quantitative content of HMW- and LMW-glutenin subunits was calculated with the help of software; the results are presented in relative units (Table 9). The data demonstrate the fact that LMW-glutenin/HMW-glutenin subunit proportion is significantly higher in Rollo (2.95) compared to Drott and lower in their hybrids (3.0–3.23, Table 9). This value has a positive correlation with the data on the flour

Table 10. Technological properties and aggregation parameters of Rollo and Drott and four ‘Rollo/Drott’ hybrids. Data is from 2001; t_{10}/C - aggregation constant at the 10th minute of the process.

	Rollo	Drott	Hybrid I	Hybrid II	Hybrid III	Hybrid IV
Technological parameter	c (null)	a (1)	a (1)	c (null)	a (1)	a (1)
	b (7+8)	c (6+8)	c (6+8)	c (6+8)	b (7+8)	b (7+8)
	a (2+12)	d (5+10)	d (5+10)	a (2+12)	d (5+10)	a (2+12)
LMW-GS/HMW-GS	2.95	1.16	2.27	3.23	3.0	3.04
aggregation constant	4.84	12.63	13.60	8.91	13.51	5.9
t_{10}/C	30.26	26.11	28.68	34.82	39.57	33.09
sedimentation (ml)	42	29	35	36	29	31

sedimentation analysis: The higher the LMW-glutenin/HMW-glutenin subunit index, the higher the sedimentation parameters. Rollo has a much higher index (2.95) than

that of Drott, with a high sedimentation index (42 ml). So, we see a tight correlation of LMW-glutenin/HMW-glutenin subunit proportion and parameters of sedimentation flour analysis. Hybrids do not manifest significant differences in these parameters (Table 10).

Comparative investigation of aggregating ability of the vinegar-soluble fraction of flour reserve proteins of hybrids in the F_9 and F_{10} with various types of electrophoretic spectra showed that among the biotypes under study, the

ratio of initial aggregation stage (K) ranges between 4.84–13.6. Genotypes, whose electrophoretic spectrum contains glutenin subunits 6+8 (Drott and hybrids I and II), regardless of the presence of other subunits, had aggregation ratio 12.63, 8.91, and 13.6, respectively; with the final aggregation stage index (t_{10}/C) equaling 26.11, 28.68, and 34.82, respectively.

Thus, we performed a complex investigation of the grain-reserve protein glutenin. We identified correlations between the composition of HMW-glutenin subunits, the proportion of HMW- and LMW-glutenin subunits, and a number of technological properties. HMW-glutenin subunit 1 was shown to be associated with high baking quality, in contrast to the null allele. Subunits 5+10 also correlate with high baking qualities in contrast to subunits 2+12.

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Subunits of functional glutenin as structural elements of complex gluten proteins.

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Native wheat glutenin is a complex set of biochemically diverse polypeptides connected by different forces of protein-protein interactions. The interest in identifying peculiarities in the genetically determined composition of functional glutenin subfractions is because of their immediate participation in the formation of a gluten-protein complex that is responsible for baking properties in flour. High-molecular-weight glutenin subunits of the majority of industrial wheat cultivars of many countries have been well-studied. The participation of these structural elements in the formation of compositionally complicated, multicomponent protein-gluten complex responsible for dough and bread quality are known.

Glutenin is known to consist of at least 15–17 subunits with different molecular mass, amino-acid composition, and primary and secondary structure are associated into a single permolecular protein complex via intermolecular disulfide bonds stabilizing a three-dimensional structural gluten matrix. The number of these bonds is a genotypically determined trait of a genetically specific character that determines gluten physical properties and dough rheological characteristics (Trufanov 1994).

To find favorable alleles, we were interested in the impact of chromosomes of homoeologous groups 1 and 6, which control storage protein synthesis, in wheat lines with intervarietal substitutions. These lines have contrasting baking qualities based on the quantitative content of HMW-glutenin subunits present.

Materials and Methods. Soft wheat lines with intervarietal substitutions of chromosomes 1A, 1B, 1D, 6A, 6B, and 6D of the cultivar Novosibirskaya 67 (N67), a strong wheat, and the high-protein recipient cultivar Diamant 1 (Dm) with low technological quality were used (Obukhova et al. 1997; Maystrenko et al. 1993).

Glutenin subfractions (GN) were obtained from freshly ground flour after removal of albumins, globulins, and gliadins by successive extraction with 0.05 M acetic acid (subfraction GN-1), 4 M urea (subfraction GN-2), and 4 M urea in the presence of 2-mercaptoethanol (subfraction GN-3) (Trufanov 1994). Protein fractions were dialyzed against 0.01 M acetic acid and lyophilized. Subunits composition in the subfractions GN-1, GN-2, and GN-3 was studied after SDS electrophoresis in 9 % polyacrylamide gels according to Laemmli (1970). Evaluation of quantitative content of PAGE zones in glutenin subfractions of each substituted line fraction was by densitometric analysis of electrophoretograms (Fig. 13). Quantitative content of five HMW-glutenin subunits was calculated with the help of a computer program. The results were expressed in units and in % relative to the recipient cultivar Dm (Fig. 14, p. 145).

Result and Discussion. The donor cultivar N67 exceeds the recipient Dm in relative content of subunits 2 and 3 in the easily soluble GN-1 fraction (by 10–12 %); in sparingly soluble GN-2 fraction, subunits 4 and 5 by 13 and 45 %, respectively; and in the insoluble (without restoration of SS bonds) GN-3 fraction, subunits 1 and 2 by 25 and 10 %, respectively) (Fig. 14, p. 145). Simultaneously, the donor N67 is inferior to the recipient Dm in terms of GN-1 content of subunits 1 and 4 by 11 and 20 %, respectively; in GN-2, subunits 1 and 3 by 17 and 7 %, respectively; and in GN-3, subunits 3, 4, and 5 by 5, 20, and 36 %, respectively.

Obukhova et al. (1997) and Maystrenko et al. (1993) showed that in Dm and N67, subunits 1 and 2 are controlled by genes on chromosomes 1A, 1B, and 1D, whereas subunit 3, which contains three components, is controlled by genes on chromosomes 1B and 1D. One component of subunits 3 belongs to N67 and is controlled by genes on chromosome 1B; the second belongs to Dm and also is controlled by genes on 1B; and the third component, common for both cultivars, is controlled by genes on chromosome 1D. Several authors (Obukhova et al. 1997; Maystrenko et al. 1993; Payne 1979, 1997) assume that chromosomes of different homoeologous groups participate in genetic control of compositionally complicated subunits 4 and 5 (Fig. 15, p. 146).

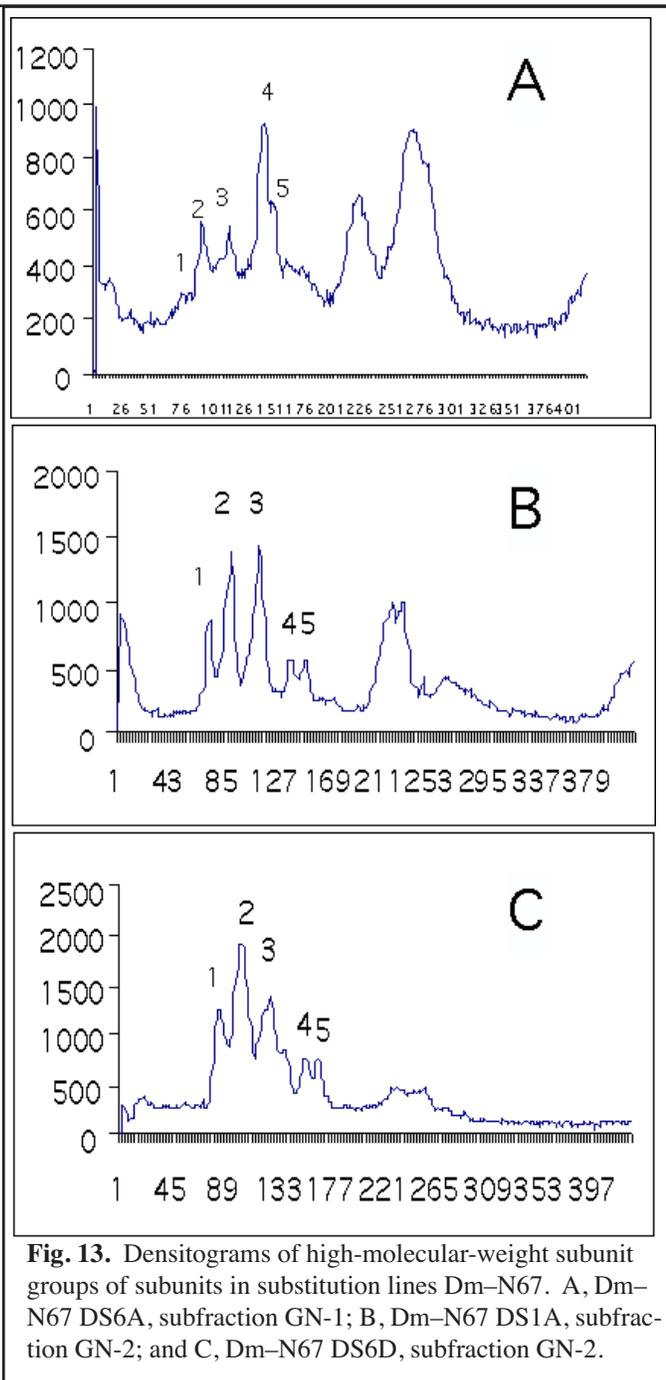


Fig. 13. Densitograms of high-molecular-weight subunit groups of subunits in substitution lines Dm-N67. A, Dm-N67 DS6A, subfraction GN-1; B, Dm-N67 DS1A, subfraction GN-2; and C, Dm-N67 DS6D, subfraction GN-3.

Compared to Dm, subunit 1 prevails in the subfraction GN-1 in N67–Dm DS1A DS6A, and DS6B; in subfraction GN-2 in Dm–N67 DS1B; and in subfraction GN-3 in Dm–N67 DS1B, DS1D, DS6A, and DS6B. Subunit 2 prevails in Dm–N67 DS6A in the GN-1 subfraction, in all substituted lines of the GN-2 subfraction, and in Dm–N67 DS1A, DS1B, and DS6A in subfraction GN-3. Subunit 3 content was higher only in Dm–N67 DS6A in subfraction GN-1 and in Dm–N67 DS6B and Dm–N67 DS6D. Subunit 4 content was higher in all the lines of subfractions GN-1 and GN-3. Subunit 5 prevailed only in the subfraction GN-1 in substituted lines with chromosomes 1D and 6A and was slightly higher in Dm–N67 DS1A in subfraction GN-3.

The positive influence of the subunit content on glutenin in the recipient cultivar Dm was to varying degrees caused by chromosomes from the donor cultivar N67. A considerable substitution effect was evident in the case of chromosome 6A. Substitution of this chromosome resulted in intensifying synthesis of all the subunits in soluble subfraction GN-1, two in the sparingly soluble subfraction GN-2, and two in the insoluble fraction GN-3. Extraction of GN-3 subunits is possible only after complete restoration of inter- and intramolecular SS links that stabilize the gluten structural matrix. Simultaneously, the content of individual subunits in these substitution lines was lower than in the recipient, particularly that of subunits 3, 4, and 5.

Differences in solubility of subfractions GN-1, GN-2, and GN-3 are known to be associated with the density of their spacial structure, which determines the ability of these proteins to form permolecular protein associates, characteristic of gluten (Trufanov 1994). Formation of protein glutenin macroassociates in the grains *in vivo* happens with the participation of various intermolecular forces: ion-electrostatic interactions conditioned by acidic and basic amino acids (AA), hydrophobic contacts (hydrophobic AA), and SS links (cystine). Consequently, the combined impact of nonvalent and covalent forces are determined by the quantity and biochemical properties of individual polypeptides (subunits), primarily by the content and location in polypeptide chains of reactive SH groups that are able to form intermolecular SS bonds. The folding of proteins of various origin and the chaperon-dependent assembling of protein macroassociates in the cell *in vivo* are known to happen in cotranslation and/or posttranslation periods and to be catalyzed by the system of SH/SS-metabolism enzymes, in particular, by protein disulfide isomerase (Chi-Chen Yong 1998; Marusich 1998; Fisher 1998) responsible for formation, splitting, and isomerization of SS bonds in the proteins. The significant changes observed in the substitution lines in the quantitative content and proportion of HMW-glutenin subunits in functional glutenin fractions of contrasting in technological properties wheat varieties may play an important role in the formation (assembling) and stabilization of functionally important glutenin complexes as a structural basis of gluten.

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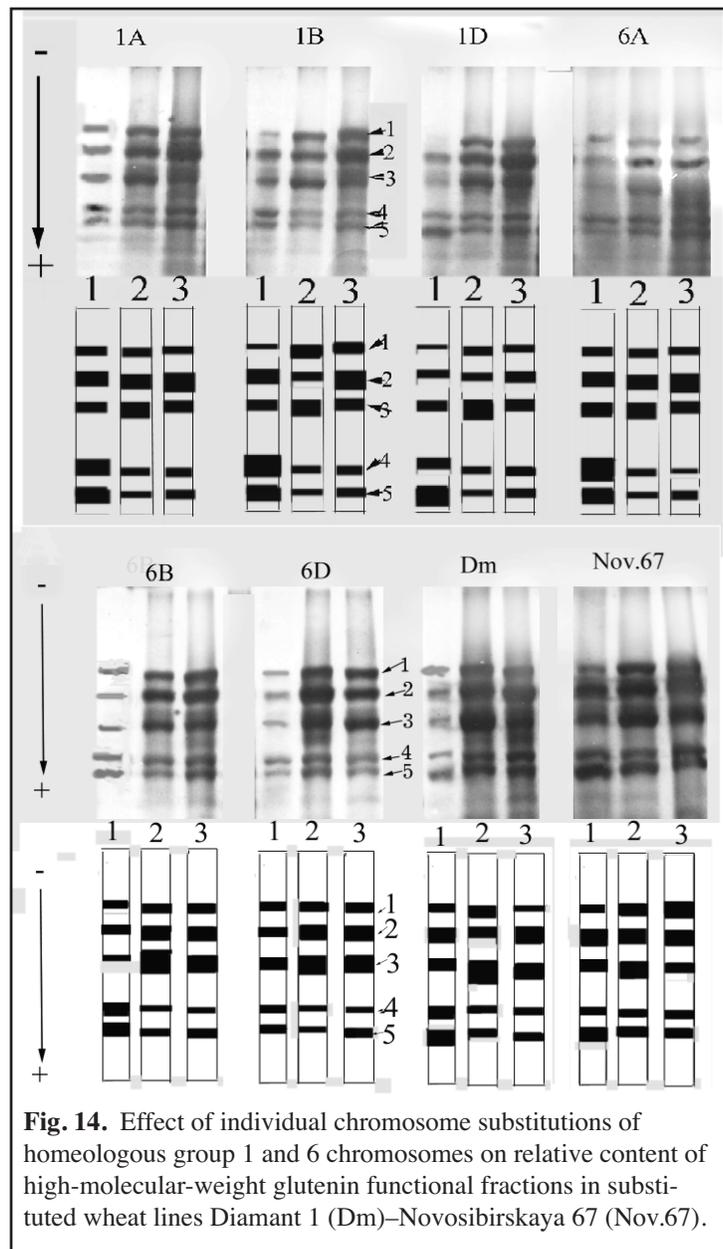


Fig. 14. Effect of individual chromosome substitutions of homeologous group 1 and 6 chromosomes on relative content of high-molecular-weight glutenin functional fractions in substituted wheat lines Diamant 1 (Dm)–Novosibirskaya 67 (Nov.67).

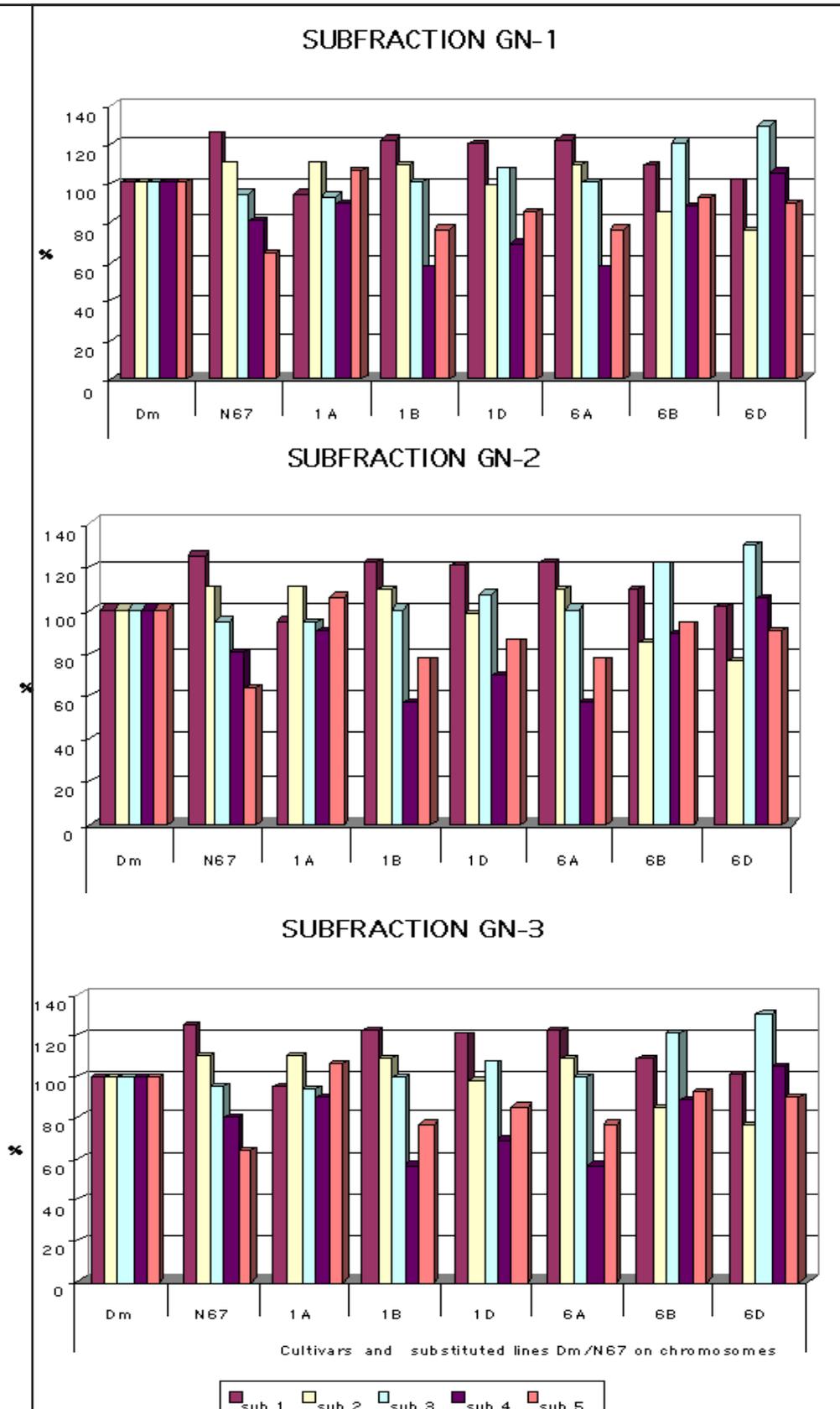


Fig. 15. SDS-PAGE of high-molecular-weight glutenin subunits. Subfraction GN-1 glutenin extracted from flour by 0.05 M acetic acid; subfraction GN-2 glutenin extracted from flour by 4 M urea; and subfraction GN-3 glutenin extracted from flour by 4 M urea in the presence of 2-mercaptoethanol.

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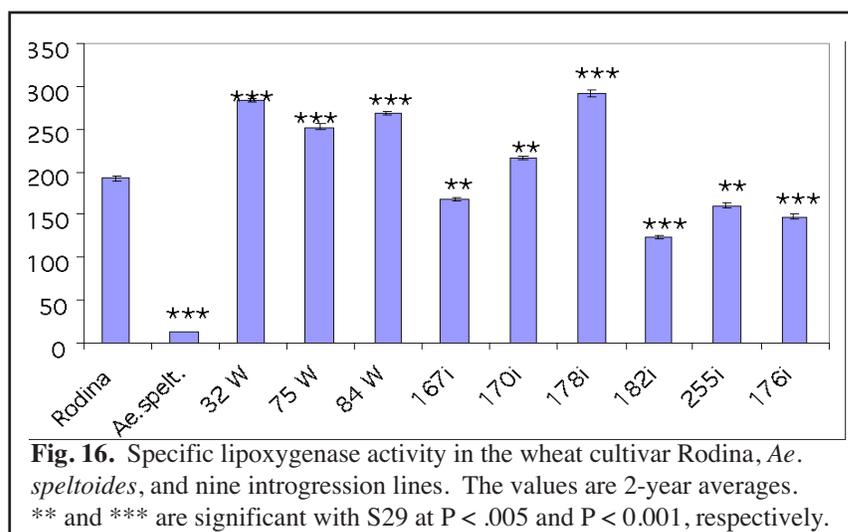
Specific lipoxygenase activity in Triticum aestivum subsp. aestivum / Aegilops speltoides introgression lines.

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Lipoxygenase (linoleat:oxygen oxidoreductase, EC 1.13.12, Lpx) is known to catalyze the oxidation of unsaturated fatty acids resulting in formation of peroxide and hydroperoxide compounds. This enzyme is widely distributed in plant cells. Lipoxygenase reactions may initiate the synthesis of signaling molecule or be involved in inducing structural or metabolic changes in the cell (Siedow 1991; Grechkin 1999).

Our research involved *T. aestivum* subsp. *aestivum* / *Ae. speltoides* introgression lines. Species belonging to the genus *Aegilops*, a wild relative of wheat, are widely used for introducing agriculturally important traits, such as resistance to fungal diseases and pests and tolerance to high salinity and temperature, into the genome of common wheat. The common spring wheat Rodina was used as the female parent in producing the introgression lines. This line is part of the ‘Arsenal’ collection produced by I.F. Lapochkina (Institute of Agriculture of Central Regions in Non-Chernozem Zone of Russia, Nemchinovka and Lapochkina 2001).

Lipoxygenase activity was analyzed in Tris-soluble flour extracts according to Doderer et al. (1992). The average values of specific Lpx activity in the parental cultivars and eight introgression lines for 2 years are in Fig. 16. Enzyme activity is a level lower in wild cereals than that in wheat lines. This difference possibly is because Lpx genes are in triplicate in hexaploid wheat, although this point requires further study. Introgression causes a significant increase in Lpx values in lines 32W, 75W, 84W, 178i, and 170i in a genetic background of Rodina. Lipoxygenase activity in lines 167i, 182i, 255i, and 176i significantly decreased in comparison with Rodina.



Previously, the same introgression lines was characterized by means subtelomeric repeats Spelt 1 and Spelt 52 being specific markers *Ae. speltoides* (Salina et al. 2001). We have found that values of specific Lpx activity are directly connected to Spelt 1 amount (Fig. 17). Any connection Lpx with Spelt 52 was not revealed.

Alien introgression also can lead to change of Lpx activity, which is connected with physiological processes in plants. Lines with alien chromosomes may be a source of certain disease-resistance genes and other traits that change a plants adaptive potential. These transfers into common wheat cultivars may be useful for breeding programs.

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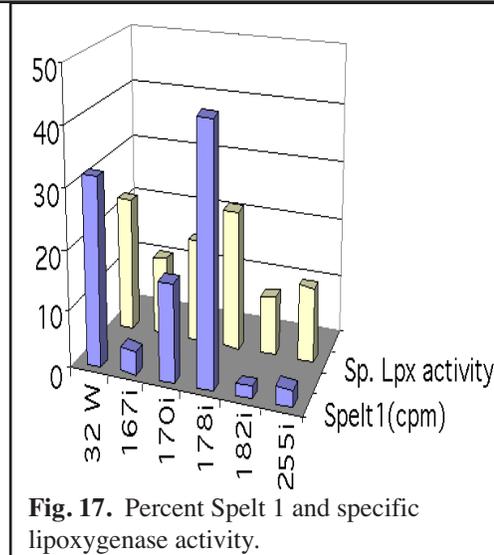


Fig. 17. Percent Spelt 1 and specific lipoxygenase activity.

Specific lipoxygenase activity in intervarietal substitution lines of hexaploid wheat.

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Lipoxygenases (Lpx, EC 1.13.11.12) are nonheme, iron-containing dioxygenases widely distributed in plants, fungi, and animals. Lipoxygenase catalyzes the addition of molecular oxygen to polyunsaturated fatty acids to produce an unsaturated fatty acid hydroperoxide. Products of the Lpx pathway are involved in inducing structural or metabolic changes in the cell. In plants, Lpx has been associated with some processes in number of developmental stages (Siedow 1991) and with mobilization of storage lipids during germination (Feussner et al. 2001). Lipoxygenase also is used as a storage protein during vegetative growth (Tranbarger et al. 1991). Lipoxygenase also influences quality parameters of wheat gluten (Mc Donald 1979; Shiba et al. 1991).

Lipoxygenase expression is regulated by different effectors such as the source/sink status (Feussner et al. 2001), jasmonic acid (Park et al. 1994) and abscisic acid (Melan et al. 1993), and also by different forms of stress, such as wounding, water deficiency, or pathogen attack (Porta et al. 1999; Melan et al. 1993).

Lines with substitutions of individual chromosome pairs of the homoeologous group in wheat are convenient genetic material for the study of donor and recipient gene effects on their expression and enzyme specific activity. The objective of our research is the substitution lines of Saratovskaya 29–Janetskis Probat (S29–JP) for all chromosomes excluding 1. These lines were developed at the Institute of Cytology and Genetics, Novosibirsk, Russian Federation.

In order to measure Lpx activity, proteins were extracted from the flour by Tris buffer 1:1 (w:v) at 4°C. Lipoxygenase activity was assayed spectrophotometrically under the wavelength 234 nm (Doderer et al. 1992). One unit of activity was defined as the change in optical density on 0.001/min. Specific activity was expressed by ratio of activity

units to 1 mg of protein in 1 ml of incubation media. Protein concentration was determined by according to Lowry et al. (1951).

Results of Lpx activity measurement in all investigated lines are shown in Fig. 18. The diagrams present average data of the three biological and three analytical replicates. The donor JP exceeded the recipient S29 by 60 % for the specific activity of Lpx. In all lines excluding 3D and homeologous group 5, this trait is significantly higher than that of the donor parent JP.

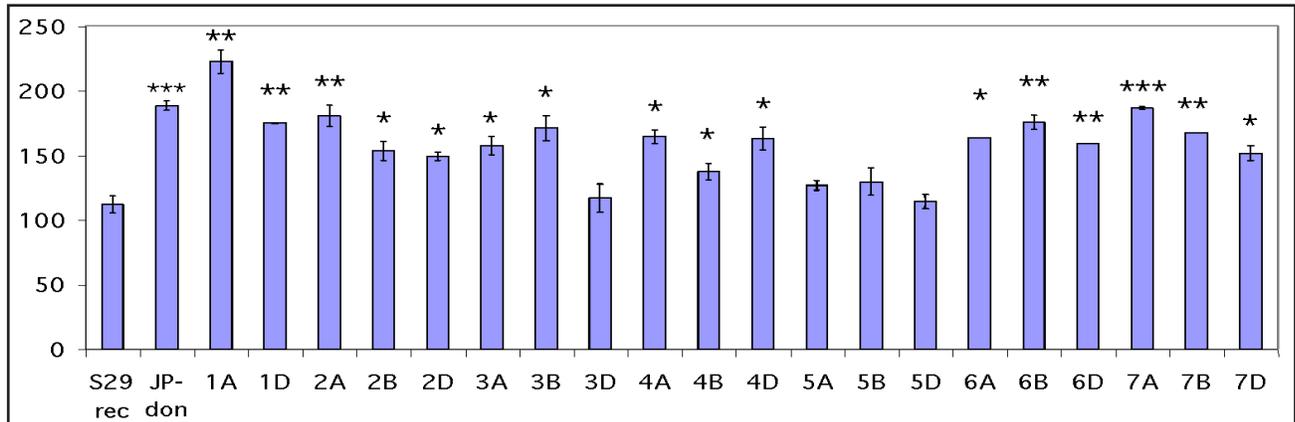


Fig. 18. Specific lipoxigenase activity of individual chromosome substitutions from Janetskis Probat (JP) in the cultivar Saratovskaya 29 (S29). Values are significantly different at $P < 0.005$ (*), $P < 0.001$ (**), and $P < 0.001$ (***)

The genes responsible Lpx synthesis are located on the chromosomes of the homeologous groups 4 and 5 (Hart and Langstone 1977). Judging from our results, chromosomes of different homeologous groups participate in control of this character. We have shown that intervarietal substitution of the homeologous groups 1 and 6 chromosomes affect the functional activity of this enzyme (Trufanov et al. 2001). From this data, we concluded that there are genes or regulators of Lpx activity along with structural genes.

The data from 3 years of Lpx activity measurements are in Fig. 19. Levels of Lpx change over different years, but Lpx activity inheritance in the parental cultivars and the substitution lines is similar during the experimental period. Average data for the 3-year experiment with this set are given in Fig. 20 (p. 150). The most significant influence on enzyme specific activity is from the substitution of chromosome 4A. Substitution of chromosomes from homeologous group 5 does not change Lpx activity. Probably, chromosomes from JP have such Lpx alleles that cannot compensate for the activity of corresponding alleles of S29. The chromosomal location of Lpx structural genes is known, but the regulation of its activity may be under separate genetic control. Taking into account the important physiological role of Lpx, further genetic studies will be extremely important.

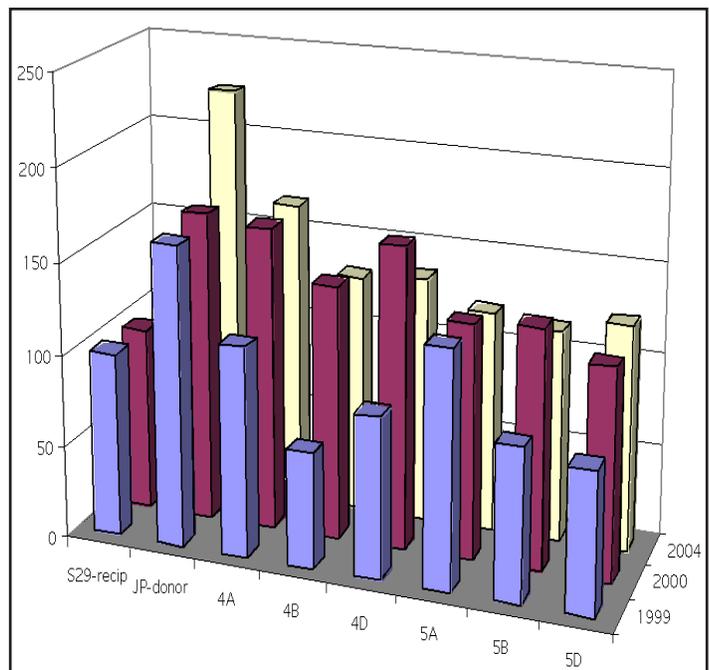


Fig. 19. Specific lipoxigenase activity in percent related to the recipient cultivar Saratovskaya 29 with individual chromosomes from the donor cultivar Janetskis Probat. Data is from 3 years of measurements.

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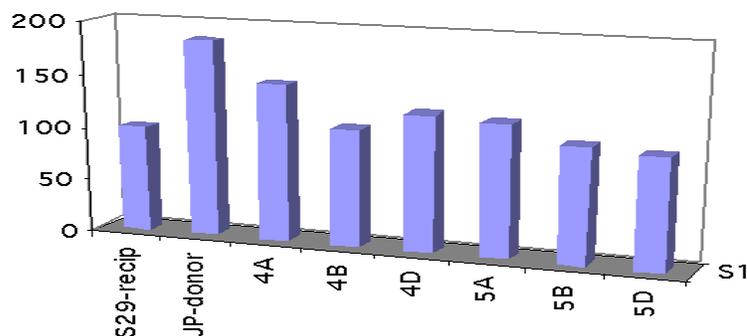


Fig. 20. Specific lipoxygenase activity in the recipient cultivar Saratovskaya 29 (%) relative to the donor cultivar Janetskis Probat. Data are for substituted chromosomes are average values over 3 years of measurements.

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Glycine-rich antimicrobial peptides from seeds of *Triticum kiharae* Dorof. et Migusch.

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Peptides with antimicrobial activity are present in most plant species. Most plant antimicrobial peptides isolated so far contain even numbers of cysteine residues (4, 6, or 8) forming disulfide bridges, thus providing high stability to the peptides. Based on homologies at the primary structure level and cysteine motifs, plant antimicrobial peptides can be

classified into distinct families including thionins, plant defensins, lipid transfer proteins, and hevein- and knottin-type antimicrobial peptides (Garcia-Olmedo et al. 1998). All antimicrobial peptides studied so far exert their antimicrobial activity at the level of the plasma membrane of the target microorganism, but different peptide types are likely to operate via different mechanisms. In a number of plant species, a considerable induction of genes expressing antimicrobial peptides has been observed upon infection with pathogens suggesting their role in inducible defense response of plants. Constitutive expression of heterologous antimicrobial peptide genes, which has resulted in enhanced resistance to particular microbial pathogens, has been achieved for several plant species. New potent antimicrobial peptides are of considerable interest for production of transgenic resistant crops, they also have a considerable potential as novel therapeutic agents, disinfectants and food preservatives. Considerable progress has been made recently in the identification of new antimicrobial peptides in different plant species. However, wild relatives of wheat and related species highly resistant to pathogenic microorganisms are poorly studied. In this work, we analyzed the peptide composition of seeds of *Triticum kiharae*, a synthetic allopolyploid produced by crossing *T. timopheevii* subsp. *timopheevii* with *Ae. tauschii*, which is highly resistant to most fungal pathogens.

Materials and Methods. The peptide fraction was extracted from *T. kiharae* flour with acids (1 % trifluoroacetic acid, 1 M HCl, and 5 % HCOOH) for 1 h at room temperature and precipitated overnight with cold acetone, redissolved, and subjected to chromatography on Heparin Sepharose. Proteins and peptides were eluted with a stepwise NaCl gradient. The unbound, 100- and 500-mM NaCl fractions were desalted on a C8 cartridge, dried on a Speedvac concentrator, and separated by size-exclusion chromatography on a Superdex Peptide HR 10/30 column (Amersham-Pharmacia, Biotech, Uppsala, Sweden). Proteins and peptides were eluted with 0.05 % TFA, containing 5 % acetonitrile at a flow rate of 250 l/min, and monitored by absorbance at 214 nm. The peptide fraction was further separated by RP-HPLC on a Vydac C18 column (4.6 x 250 mm, particle size 5 μ m) with a linear acetonitrile gradient (10–50 %) for 1 h at a flow rate of 1 ml/min and 40°C. Peptides were detected at 214 nm. The chromatographic fractions were tested for the antifungal activity against *Helminthosporium sativum* and characterized by mass spectrometry (MS) and N-terminal sequencing. Mass spectra were acquired on a model Reflex III mass spectrometer (Bruker Daltonics, Bremen, Germany). Dehydrobenzoic acid was used as matrix. N-terminal amino acid sequences were determined by automated Edman degradation on a model 492 Procise sequencer (Applied Biosystems) according to the manufacturer's protocol.

Results and Discussion. Previous analysis showed that the seeds of *T. kiharae* contained several families of antimicrobial peptides with molecular masses from 3 to 6 kD. In this work, we focused on the glycine-rich peptides. We discovered least eight glycine-rich peptides. Their molecular masses varied from 4,295 to 4,750 Da. N-terminal sequencing (from 10 to 50 amino acid residues) showed that they were homologous, extremely glycine-rich, and cysteine-free. In sequenced regions, glycine constituted 70–80 % of amino acids, and only few other amino-acid residues were found (Tyr, His, Pro, and Ala). A remarkable feature of these peptides was the presence of different repeat motifs, such as G_n YPGH, G_n YP or G_n YPGR, where n is a variable number. Similar repeat motifs were discovered in glycine-rich proteins involved in plant defense against biotic and abiotic stress. The glycine-rich peptides are low-abundant constituents of *T. kiharae* seeds, their yields were as follows: 0.4 g/g dry weight for Tk-AMP-G1, 0.6 g/g for Tk-AMP-G2, 0.7 g/g for peptides Tk-AMP-G3 and Tk-AMP-G4. Preliminary antifungal tests with *H. sativum* showed that the Tk-AMP-G peptides caused morphological changes in the fungus at concentrations of 100-150 mg/ml in a dose-dependent manner. These data indicate that *T. kiharae* is a valuable source of glycine-rich peptides, whose biological activities will be investigated in more detail.

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The alterations in several yield-contributing traits in transgenic wheat.

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During the last years, knockout technology based on molecular mechanisms of silencing (RNA interference) is actively used in experiments to study of gene functions. Now this approach has become a tool for wheat research. The method includes transformation of plant with the genetic construct providing the synthesis or endogenous formation in the cells of double-stranded RNA homologous to any transcribed region of the target gene. The appearance in cells of such dsRNA triggers specific defensive mechanisms of restriction of dsRNA and mRNA with homology to it, which results in

silencing of target gene. This process was believed to be very specific. However, recently it was reported that dsRNA might exert unspecific effects on expression of the genome and the genes of the transgenic construct.

Earlier in laboratory the transgenic plants of spring, common wheat cultivar Khakasskaya were obtained using *Agrobacterium* transformation *in planta*. T-DNA of these plants contains inverted repeat of 350-bp fragment of tetracycline resistance gene from pBR322 under the control of 35S CaMV promoter and *nptII* gene as marker. This construct provides the synthesis of untranslatable dsRNA in wheat cells. It is supposed that its nucleotide sequence has no homology to the wheat genome.

In the present study we have investigated morphological stability of 18 independent transgenic lines on T1 selfed progeny. The presence of T-DNA in individual plant has been confirmed by PCR-analyses with primer to inverted repeat sequence from pBR322. Segregation of T-DNA in progeny was observed only in 2 from 18 transgenic lines (with non-Mendelian ratio), in other lines all offspring plants had transgenic insertion. In contrast to T1 plants with normal phenotype, T2 progeny of some lines displayed alteration in several traits in field conditions. Using analysis of variance by statistical program AGROS 2.10 we have estimated a significance of differences between transgenic plants from individual lines and control group of non-transgenic wheat plants of Khakasskaya. The established changes manifested in the decrease of spike length (a significant decrease in 13 from 18 lines, i.e., 16.6 % of transformants), a spikelet number per spike (16.6 %), kernel weight (a significant decrease in 13 from 18 lines, i.e., 72 %), and in the increasing of productive tillering in wheat (11 %).

The reason of these changes might be in the shifts in the hormonal balance in transformants; however, this assumption demands additional investigations. We are studying inheritance of these alterations. With regard to the nature of observed changes, we can conclude that a high frequency of similar-type changes in independent lines cannot result of either insertion mutagenesis or somaclonal variability. We believe that the insertion expressing dsRNA induces unspecific pleiotropic effect on the recipient wheat genome.

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Genealogical analysis of winter bread wheat cultivars resistant and susceptible to Fusarium head blight from Southern Russia and Ukraine.

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We studied winter bread wheat cultivars from southern Russian Federation (Krasnodar, Stavropol, and Rostov regions) and the Ukraine. For the analysis, cultivars with known pedigrees and were used not conflicting with estimates of reaction to *Fusarium spp.* Among the lines were 40 resistant and moderately resistant cultivars (see Table 5, p. 155) and 61 susceptible cultivars from southern Russia including Bezostaya 1 (Borojevic and Dencic 1988, Buerstmayr et al. 1996), Avrora (Borojevic and Dencic 1988), Lgovskaya 167 (Anonymous 1990), Bystritsa, Delta, Podarok Donu, Donskoj Mayak, Tarasovskaya Ostistaya, Zernogradka 10, Zarnitsa, Stanichnaya, Bat'ko, Donskoj Syurpriz, Zernogradka 11, Rodnik Tarasovskij (Anonymous 1994-2003), Sfera (Puchkov et al. 1996), Spartanka (Terekhina 1993), Dialog 1061-10, KN-1221-k-7-2-14, KN-201-90-k-2, KN-2503-h-112-6-11, KN-2503-h-112-6-3, KN-3071-h-16-16, KN-3303-h-117, KN-3385-h-756, KN-4594-h-370-41, KN-5835-h-427, Massiv 809-1016, Rannyaya 47, Zamena, Krasnodarskaya 70, Olimpiya 2, Skifyanka, Soratnitsa, Ejka, Novokubanka, Otrada, Yugtina, Krasnodarskaya 90, Nika Kubani, Ofeliya, Polovchanka, Azau, Aliza, Zimorodok, Nak, Umanka, Kupava, Uskoryanka, Krasota, Selyanka, Krasnodarskaya 99, Yashkulyanka, Fisht (Puchkov et al. 2001); and from Ukraine: Albatros Odesskij, Donetskaya 46 (Anonymous 1989), Kiyanka (Anonymous 1981), Lutescens 7 (Anonymous 1990), Mironovskaya Yubilejnaya (Mesterhazy 2001), Odesskaya 132 (Anonymous 1976-1995), and Odesskaya Polukarlikovaya (Babayants et al. 2001).

For each of cultivars a genetic profile was constructed and transfer of scab resistance from ancestors to the descendants was traced on genealogical trees with the help of the Genetic Resources Information and Analysis System GRIS3.5 (Martynov and Dobrotvorskaya 2000). All cultivars of the analyzed set are the descendants of the most popular cultivars Bezostaya 1 (frequency of presence in pedigrees 94.5 %), Mironovskaya 808 (62.6 %), and/or Odesskaya 16

(65.9 %). To reveal differences in the contributions of these parents in groups of resistant and susceptible cultivars the two-way ANOVA of the parentage coefficients for the randomized design was used (Table 1). The investigated factors were cultivar groups (factor A) with two gradations (resistant and susceptible) and dominant ancestors (factor B) with number of gradations b = 3.

The ANOVA (Table 1) has shown the significance of differences of groups of resistance/susceptibility (factor A) and ancestor contributions (factor B). The greatest interest represents interaction of the investigated factors. The significant interaction (A*B) specifies differences in distribution of the contributions of the same ancestors in groups of resistant and susceptible cultivars. The comparison of average coefficients of parentage in cultivar groups (Table 2) shows, that the contributions of cultivars Bezostaya 1 and Mironovskaya 808 are identical in both groups, and the contribution of Odesskaya 16 is significant higher in group of resistant cultivars. It is known, that Bezostaya 1 is susceptible to FHB (Borojevic and Dencic 1988, Buerstmayr et al. 1996), and the estimates of reaction to *Fusarium spp.* for Mironovskaya 808 are conflicting, from moderately resistant (Javor et al. 1997) up to susceptible (Buerstmayr et al. 1996). The results of our analysis show that most likely Mironovskaya 808, as well as Bezostaya 1, does not carry genes of resistance to FHB. Moderately resistant cultivar Odesskaya 16 (Kazmin and Shindin 1997) most frequently was the donor of resistance of scab-resistance genes in cultivars from southern Russian Federation and the Ukraine. Odesskaya 16 was selected from cultivar Odesskaya 12 (Hostianum 237/Zemka, where Hostianum 237 is selection from Kharkovskaya, a landrace of Kharkov region, and Zemka is local variety from Odessa region). Odesskaya 16 is absent in pedigrees of some resistant cultivars (Dakha, Russa, Echo, and Zimdar 4), but there are other derived cultivars of Hostianum 237. Therefore, we assume that a source of resistance to FHB in cultivars from southern Russian Federation and the Ukraine was the landrace Kharkovskaya via Hostianum 237.

To understand the role of other sources of resistance, we used ANOVA of the contributions of some known donors of resistance, which are in pedigrees of the investigated cultivar set (Table 3). The investigated factors were groups of resistant/susceptible cultivars (factor A) and donors of resistance genes (factor B) with the number of gradation b = 9. The ANOVA (Table 3) shows significant differences in the average parentage coefficients of groups of resistant/susceptible cultivars (factor A) and donors (factor B). The significant 'A x B' interaction specifies differences in distribution of the contributions of the donors in groups of resistant and susceptible cultivars.

Comparing average coefficients of parentage of the donors (Table 4, p. 154) shows that in addition to Odesskaya 16 in the group of resistant cultivars are the significant cultivars Redcoat, Frontana, and Cheyenne. These donor cultivars are in pedigrees of the southern Russian and Ukrainian cultivars via Biserka (Redcoat), Red River 68 (Frontana), and Colt (Cheyenne).

Table 1. Two-way ANOVA of the parentage coefficients of the three most important ancestors of winter bread wheat cultivars from southern Russian Federation and the Ukraine (original data are transformed through $\arcsin\sqrt{x}$). Items with an * are significant at $P < 0.001$.

Source	SS	DF	MS	F
General	40,936.9	202		
Cultivar groups (factor A)	545.6	1	545.6	4.08*
Ancestors (factor B)	12,132.7	2	6,066.3	45.41*
Interaction (A x B)	1,940.3	2	970.1	7.26*
Error	26,318.3	197	133.6	

Table 2. Average coefficients of parentage of important ancestors in groups of resistant to scab and susceptible winter bread wheat cultivars from southern Russian Federation and the Ukraine. Items with an * are significantly different at $P < 0.01$.

Ancestor name	Resistant	Susceptible
Bezostaya 1	0.37	0.38
Mironovskaya 808	0.15	0.16
Odesskaya 16	0.25*	0.10

Table 3. The two-way ANOVA of the parentage coefficients of donors of resistance to scab in pedigrees of bread wheat cultivars from Southern Russia and Ukraine (original data are transformed through $\arcsin\sqrt{x}$). Values with an * are significant at $P < 0.001$.

Source	SS	DF	MS	F
General	23578.6	246		
Groups (factor A)	1669.8	1	1669.8	39.73*
Donors of resistance (factor B)	10716.3	8	1339.5	31.88*
Interaction (AxB)	1568.8	8	196.1	4.67*
Error	9623.7	229	42.0	

Established with the help of the pedigree analysis, the probable donors of resistance genes are given in Table 5 (p. 155). Of the 43 resistant and moderately resistant lines, three of the pedigrees are unknown and two cultivars (Maslovchanka 90 and Kharkovskaya 20) were not established. Most frequently, Odesskaya 16 and its derivatives (Odesskaya 51, Obrij) (55 %) and Frontana (28 %) were donors of resistance genes. Resistance to FHB in the older cultivars Kooperatorka, Novokrymka 102 (Ukraine), and Cheyenne (USA) were selected from the landrace Crimean, which probably was heterogenous for reaction to *Fusarium spp.* The Canadian cultivar Canus (Marquis/Kanred) also most likely received resistance genes from Crimean via Kanred, because Marquis is susceptible to scab.

This analysis has shown that despite of the rather large number of known sources of resistance to FHB in the Russian and Ukrainian wheat breeding programs, they are practically unused. Our analysis was made on the basis of the information about resistance or susceptibility of bread winter wheat cultivars received by the different authors in different time. Therefore, we consider the data about source of resistance and statistical estimations made by comparison of sets of resistant and susceptible cultivars, as preliminary. Nevertheless, the approach, based on the genealogical information, can be useful for the analysis of the comparable data.

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Table 4. Average coefficients of parentage of the donors of scab-resistance genes in sets of resistant and susceptible winter wheat cultivars from southern Russian Federation and the Ukraine. Values with an * are significantly different at $P < 0.05$.

Ancestor name	Resistant	Susceptible
Odesskaya 16	0.25*	0.10
Redcoat	0.09*	0.03
Hope	0.01	0.00
Frontana	0.07*	0.03
Tohoku 34	0.01	0.01
Kooperatorka	0.03	0.02
Cheyenne	0.08*	0.01
Canus	0.01	0.01
Gentil Rosso	0.02	0.02

Table 5. Bread wheat cultivars resistant and moderately resistant to Fusarium head blight from southern Russian Federation and the Ukraine and hypothetical donors of resistance. Incomplete pedigree information is available on lines marked with an asterisk (*).

Cultivar name	Reference	Country	Year	Donor of resistance
RESISTANT				
Basianka	Puchkov et al. 2001	RUS	1985	Redcoat
Charivnitsa Odesskaya	Terekhina 1993	UKR	1990	Odesskaya 16
Dakha	Puchkov et al. 2001	RUS	1993	Canus, Kooperatorka, Kharkovskaya (?)
ErythrospERMum 2582-89	Babayants et al. 2001	UKR	1989	Obrij, Odesskaya 16
ErythrospERMum 2593-90	Babayants et al. 2001	UKR	1989	Odesskaya 16, Odesskaya 51, Frontana
ErythrospERMum 898-91	Babayants et al. 2001	UKR	1991	Obrij, Odesskaya 51, Frontana
Fedorovka	Anonymous 2001 <i>b</i>	UKR	1993	Odesskaya 16, Odesskaya 51
KN-1-1	Puchkov et al. 2001	RUS		Odesskaya 16, Kooperatorka
KNIISKH-22*	Puchkov et al. 2001	RUS		Partizanka
Kolos	Puchkov et al. 2001	RUS	1984	Redcoat
Kooperatorka	Stuchlokova/Kovacicova 1993	UKR	1929	Crimean (?)
Leda	Puchkov et al. 2001	RUS	1995	Obrij
Novokrymka 102	Stuchlokova/Kovacicova 1993	UKR	1929	Crimean (?)
Russa	Anonymous 2001 <i>c</i>	RUS	2000	Kharkovskaya (?)
Zirka	Stuchlokova/Kovacicova 1993	UKR	1984	Odesskaya 16, Frontana
MODERATELY RESISTANT				
Andizhan 1	Puchkov et al. 2001	RUS	2000	Canus
Demetra	Anonymous 1994-2003	RUS	1997	Odesskaya 16, Redcoat
Ekho	Puchkov et al. 2001	RUS	1997	Kharkovskaya (?)
ErythrospERMum 3059-92	Babayants et al. 2001	UKR	1992	Odesskaya 51
Goryanka	Anonymous 1994-2003	RUS	2001	Odesskaya 16 (?)
Hakhodka Odesskaya	Anonymous 2001 <i>b</i>	UKR	2001	Obrij, Odesskaya 51, Frontana
Kharkovskaya 20	Knysh et al. 1989	UKR	1988	?
Khazarka	Puchkov et al. 2001	RUS	1994	Kharkovskaya (?)
KN-2392-h-G-8	Puchkov et al. 2001	RUS		Canus
KN-5755-G-13340	Puchkov et al. 2001	RUS		Odesskaya 16, Kooperatorka
Leleka	Anonymous 2001 <i>b</i>	UKR	1990	Odesskaya 16, Odesskaya 51
Masovchanka 90	Terekhina 1993	UKR	1990	?
Mironovskaya 28	Terekhina 1993	UKR	1994	Odesskaya 16, Kooperatorka
Mironovskaya 64*	Anonymous 1994	UKR	1994	—
Mironovskaya rannespelaya	Anonymous 2001 <i>a</i>	UKR	2002	Frontana, Tohoku 34
Obrij	Babayants et al. 2001	UKR	1983	Odesskaya 51, Frontana
Odesskaya 16	Kazmin and Shindin 1997	UKR	1953	Kharkovskaya (?)
Odesskaya 51	Kazmin and Shindin 1997	UKR	1969	Odesskaya 16
Prikumskaya 115	Anonymous 1994-2003	RUS	1999	Odesskaya 16, Kooperatorka
Prima Odesskaya	Anonymous 2001 <i>b</i>	UKR	2002	Fedorovka, Odesskaya 16
Sirena Odesskaya	Anonymous 2001 <i>b</i>	UKR	2002	Odesskaya 16, Odesskaya 51, Frontana
Starshina	Puchkov et al. 2001	RUS	2003	Cheyenne
Verna	Puchkov et al. 2001	RUS	1998	Cheyenne, Hope
Viktoria Odesskaya*	Anonymous 2001 <i>b</i>	UKR	1998	—
Zastava Odesskaya*	Anonymous 2001 <i>b</i>	UKR	2002	—
Zimdar 4	Puchkov et al. 2001	RUS	1987	Canus, Kharkovskaya (?)
Zlagoda	Anonymous 1976-1995	UKR	1992	Odesskaya 16, Odesskaya 51, Frontana
Zolotava	Anonymous 1976-1995	UKR	1992	Odesskaya 16, Odesskaya 51, Frontana