drograms (mean taxonomic distance, UPGMA), irrespective of their changes caused by experimental stress. In the dendrogram (Fig. 15), three groups of grasses, namely the amphiploids, *H. vulgare*, and *B. secalinus*, are distinctly separated. Appriopriate pairs (s vs. z) also are distinctly separated. Only the AGA amphiploid is differentiated more by the environmental stress. Development of the abaxial epidermis of palea and lemma was most often disturbed in amphiploids. Under drought conditions, high temperature, and starvation, the grass plant developed only one tiller with short spike or poor panicle. Tissues were highly sclerified. Under sufficient watering, the plants were often infested by fungi and setting of caryopses was defective. In conclusion, both environments can create a stress of various nature. Thus, the microstructure of cereals is shifted under heavy stress, but its general pattern is preserved. Then, this pattern can be a good basis for any taxonomic comparisons.

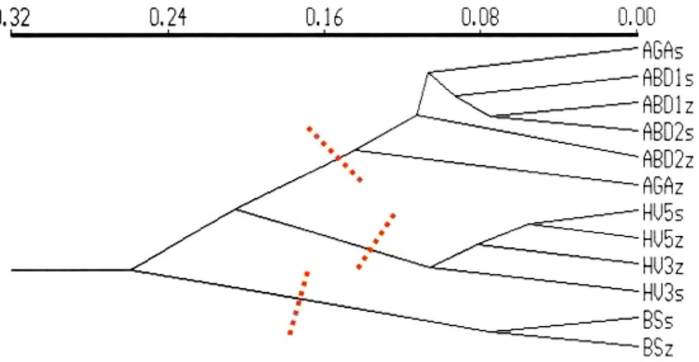

Fig. 15. A dendrogram (Canberra distance, UPGMA) of cereal OTUs described by glumellae and lodicule microstructure (*Triticum* amphiploids having genomes AAGGAA (AGA) and AABBDD (ABD1 and ABD2), and two cultivars of *Hordeum vulgare* (HV3 and HV5) and *Bromus secalinus* (BS)). Taxa are distinctly clustered (red dashed lines).

ITEMS FROM THE RUSSIAN FEDERATION

AGRICULTURAL RESEARCH INSTITUTE FOR THE SOUTH-EAST REGIONS
Department of Genetics, Laboratory of Genetics and Cytology, 7 Toulaikov St., Saratov, 410010, Russian Federation.

The evaluation of spring bread wheat cultivars, NILs, and introgression lines in the hard, drought conditions of 2009–10.

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

For the recommendation of introgression lines with identified combinations of genes for resistance to pathogens in practical breeding some prebreeding research is necessary. These research includes determining resistance to abiotic stresses and bread-making qualities. The conditions of the growing periods of 2009 and 2010 allowed estimating the set of introgression lines for drought resistance. The two-year-old data for grain productivity in NILs in the extremely hard drought conditions have shown the following results. The combination of *Lr9+Lr19*-translocations in the genotypes of cultivars L503, Dobrynya, and line L2032, do not depress yielding ability, but *Lr19+Lr26* significantly improves grain productivity, and *Lr19+Lr24* and *Lr19+Lr25* significantly depresses yield ability. A neutral reaction for grain productivity in the introgression lines with substitution 6Agi (6D) is detected. The incorporation of genetic variability from *T. turgidum* subsps. *dicoccoides* and *dicoccum* to the spring bread wheat cultivars Saratovskaya 58 and Saratovskaya 55 (lines L196 and L2870) does not depress drought resistance, but incorporation of genetic variability from durum wheat (cultivars Saratovskaya zolotistaya, Lyudmila, and Saratovskaya 57) to bread wheat (lines L200/09 and L211/09), and their combination improves this trait. The NILs with combinations of translocations *Lr9+Lr19*, *Lr19+Lr24*, *Lr19+Lr25*, substitution 6Agi (6D), and also lines L196, L2870, L200/09, and L211/09 have good bread making quality at the level of cultivars. The NIL of L503 with combination of *Lr19+Lr26* translocations was exception in which the flour strength was reduced.

The evaluation of spring bread wheat introgression lines of the Genetics and Cytology Laboratory at ARISER in breeding for resistance to leaf and stem rust.

S.N. Sibikeev, A.E. Druzhin, L.I. Laikova (Institute of Cytology and Genetics, Novosibirsk, Russian Federation), D. Singh (KARI, Njoro, Kenya), and A.A. Morgounov (CIMMYT, Turkey).

In the 2010 growing season, a set of introgression lines was estimated for resistance to leaf and stem rusts on the experimental fields of the Institute of Cytology and Genetics (Academgorodok, Novosibirsk, Russia) and for resistance to race Ug99 + *Sr24* (TTKST) of stem rust in the KARI, Njoro, Kenya under natural epidemic conditions. We detected that the NILs with translocation combinations *Lr19+Lr24* and *Lr19+Lr26*, and also lines L196, L2870, L200/09, and L211/09, are resistant to leaf and stem rusts, including to Ug99. Thus, the efficiency of combinations *Lr19/Sr25+Lr24/Sr24* and *Lr19/Sr25+Lr26/Sr31* and also the unidentified leaf and stem rust genes in lines L196 and L2970 has been shown. In lines L196 and L2870, the probability is very high that the leaf and stem rust genes are linked, because during breeding of these lines, selections were conducted only for resistance to leaf rust.

The dynamics of population change Puccinia triticina at ARISER, Russian Federation, during 2008–10.

S.N. Sibikeev and A.E. Druzhin.

The climatic change in the Volga Region was all the more noticeable by its influence on the composition of the population *P. triticina*, which is considered one of the most virulent. Analysis of *P. triticina* population dynamics during 3 years (2008–10), showed that it is quite responsive to increases in air temperature. The study of leaf rust population composition was carried out in a greenhouse on NILs of Thatcher with differing *Lr* genes. Inoculations in the grenhouse were performed at the optimum temperature (20–22°C) using uridinospores that were collected in the field on susceptible cultivars of winter wheat. During 2008, the air temperature did not exceed the critical value for the pathogen (31°C) for virtually the entire season (Fig. 1); and a majority of virulent pathotypes were present in the population (Table 1).

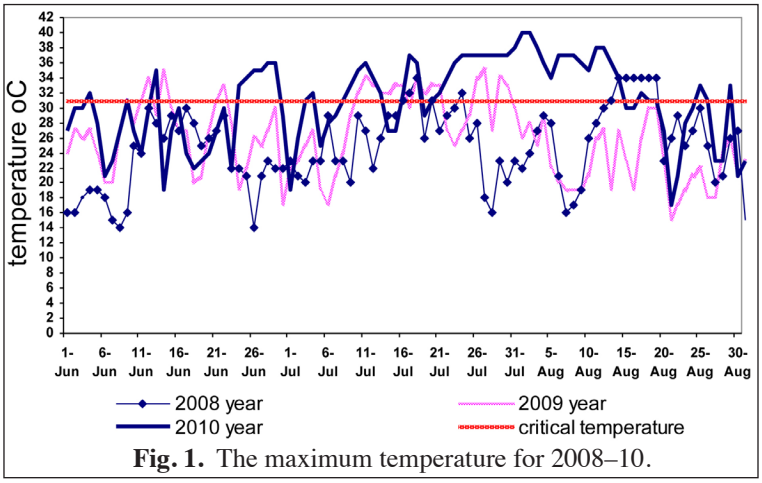

Fig. 1. The maximum temperature for 2008–10.

Since 2009, the has situation changed. The air temperature during the period of infection in field conditions is often higher than the critical indicator for the pathogen or is above the optimal value. This has led to the fact that in the population of the fungus eliminated or reduced their virulence the following pathotypes: pp11, pp14b, pp19, pp24, pp32, and pp40. In 2010, when temperatures during the vegetative period were very high (35-40°C) and in a hard drought (Fig. 1), significant changes were noticed

Table 1. The dynamics of change of the formula avirulence/virulence in populations of *Puccinia triticina* at the Agricultural Research Institute for the South-East Regions, Russian Federation, in 2008–10.

Year	Avirulence/virulence formula
2008	**9, 17a, 29, 28, 41, 42**/2a, 2b, 2c, 3a, 3bg, 3ka, 10, 11, 12, 13, 14a, 14b, 14ab, 15, 16, 18, 19, 20, 21, 22a, 23, 24, 25, 26, 27+31, 30, 32, 33, 34, 37, 38, 40, b, H
2009	**9, 11, 14b, 19, 24, 28, 29, 32, 40, 41, 42**/2a, 2b, 2c, 3a, 3bg, 3ka, 10, 12, 13, 14a, 14ab, 15, 16, 17a, 18, 20, 21, 22a, 23, 25, 26, 27+31, 30, 33, 34, 37, 38, b, H Infection type: 0; 1^-: 14b, 19, 32 22^+: 40 2^+3: 11
2010	**2b, 2c, 3a, 3bg, 3ka, 9, 10, 11, 12, 13, 15, 16, 17a, 19, 21, 24, 28, 29, 30, 32, 41, 42, H**/2a, 14a, 18, 20, 22a, 23, 27+31, 37, 38, 14b, 14ab, 33, 34, b Infection type: 0;1: 9, 21, 32 1^-: 2c, 12, 13, 29 11^+: 2b, 16 1^+2: 3a, 11 2^+: 3bg, 3ka, 10, 15, H 2^+3^-: 30

in the *P. triticina* population. In the pathogen population, there was eliminated or decreased virulence for the following pathotypes: pp2b, pp2c, pp3a, pp3bg, pp3ka, pp10, pp11, pp12, pp13, pp15, pp16, pp17a, pp19, pp21, pp24, pp30, pp32, and ppH. It is interesting that the following virulent pathotypes remained in the population: pp2a, pp14a, pp18, pp20, pp22a, pp23, pp27+31, pp37, pp38, pp14b, pp14ab, pp33, pp34, and ppb, which showed high adaptability to high temperature and were drought resistant.

Effects of interaction 6Agi (6D) chromosomes from Thinopyrum intermedium and Lr19 translocation from Th. elongatum on flour protein content spring bread wheat.

O.V. Krupnova, S.A. Voronina, V.A. Krupnov, and A.E. Druzhin.

On leached, chernozem soil with a crop rotation (a bare fallow–spring bread wheat), flour protein content varied from 13.9% up to 20.3% and gluten content from 30% up to 48%. In these conditions, near isogenic lines for chromosome 6Agi (6D) from *Th. intermedium* and an *Lr19* chromosome 7D translocation from *Th. elongatum* had a positive influence on flour protein content in spring bread wheat, both within a leaf rust epidemic and without.

In a population from crosses between parents Л1400R and 6Agi(6D) and Л1089 and *Lr19*-T7D, we selected recombinant inbred lines Л1204 and Л1205, which have the combination *Lr19*-T7D and 6Agi(6D). In a population from crosses between parents Л2032 (*Lr19*-T7D) and Л1400R, we are selected RILs Л108 and Л396, which have only 6Agi (6D). All four lines (Л1204 and Л1205, Л108, and Л396) are resistant to the Saratov population of a leaf rust and, on a grain yield and a flour protein yield per unit area, exceed that of the parents. For flour protein content, they are less than that of the parents. The mechanism of interaction, 6Agi(6D)/*Lr19* and *Lr19*/6Agi(6D), in a *T. aestivum* background, and the control of the decrease in flour protein content in the RILs, compared with the parents, are unknown.

Laboratory of Spring Durum Wheat Breeding, 7 Tulaikov Street, Saratov, 410010, Russian Federation.

A new spring durum wheat cultivar 'Nikolasha' has been released in the Russian Federation.

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The State Commission on the Test of Breeding Achievements approved a new cultivar of spring durum wheat named 'Nikolasha' (137/00-5) for use in agricultural production in 2009–10. Nikolasha appears well adapted to southern and southeastern areas of European part of the Russian Federation, such as the Krasnodar, Rostov, and Saratov regions. The cultivar was developed thanks to the joint breeding program between P.P. Lukyanenko Krasnodar Research Institute for Agriculture (KRIA) and Agricultural Research Institute for the South-East Regions (ARISER).

Cultivar Nikolasha was developed as a result of individual plant selection in the F_2 generation from the hybrid population obtained by crossing the line D-2033 with the cultivar Nick (D-2029) at ARISER. The line D-2033 was derived from a cross between two highly drought-resistant local lines Leucurum 1863 and Leucurum 1945. The cultivar Nick was derived from a cross between Saratovskaya zolotistaya and Altayskaya Niva. The local cultivar Saratovskaya zolotistaya has very high quality grain and pasta products. The cultivar Altayskaya Niva originated from the Altay region and is highly resistant to common bunt and loose smut. The elite plant was selected in the F_8 generation at KRIA in 2001. The field test of the line 137/00-5 was conducted in Krasnodar in 2004–05.

The spike of Nikolasha is white with white awns, pyramidal in shape, and of medium length (6–8 cm) and density (26–27 spikelets/10-cm rhachilla). Kernels are amber and vitreous. The 1,000-kernel weight was 38–46 g and test weight was 770–822 grams/L. Plants have good resistant to lodging. Plant height is 100–115 cm, which is 5 cm lower than that of the standard cultivar Novodonskaya. Plant heading is earlier than that of Novodonskaya by 1–2 days.

The cultivar is very drought resistant. Nikolasha has a high level of disease resistance, particularly to common bunt and loose smut; good field resistance to leaf, stripe. and stem rust; septoria leaf spot; and tolerant to root rot if sown after such fore crops as winter wheat and barley.

Nikolasha durum wheat is a widely adapted cultivar. The cultivar combines high potential productivity and drought resistance. In 2008, the yield in main trials at KRIA (Krasnodar) reached up to 6.28 t/ha against 4.74 t/ha for the check Kharkovskaya 17. In 2004–06, the average productivity of Nikolasha in the main trial was 5.14 t/ha, which was higher than that of the check cultivar Novodonskaya by 0.37 t/ha. In the Saratov field test in the 2010 spring wheat growing season when the hydrothermal coefficient for May–July in the Volga River Region was very low (0.1–0.2), which corresponds to an extremely strong drought, Nikolasha gave a grain yield of 0.75 t/ha, compared to 0.33 t/ha for the Saratovskaya zolotistaya check. This new cultivar has good physical grain parameters and strong gluten quality. For 2008–10, the average SDS-sedimentation index was estimated up to 50 mL, similar to that of Saratovskaya zolotistaya. Durum wheat Nikolasha is good achievement of ARISER and KRIA shuttle breeding program and according to the technological suitability for the pasta industry after the official testing it was also approved as an original cultivar for the dry, southeast areas of the Russian Federation (Saratov) in 2010 from the State Variety Testing Commission.

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Anther culture method of creating initial breeding stocks for triticale selection at ARISER.

T.I. Djatchouk, O.V. Khomyakova, V.N. Akinina, Yu.V. Italianskaya, N.F. Safronova, and L.P. Medvedeva.

The generation of doubled haploid (DH) plants via anther culture is an important biotechnological method, which permits significant shortening of the breeding process. This technique speeds up the time of cultivar development by several years. Different intervarietal and wheat-triticale hybrids (F_2–F_3 generation) based on the local triticale and wheat cultivars were used for haploid production in this study. The undoubled haploid plants were served by microclonal propagation using a somatic embryogenesis method.

The created DH lines were studied in a traditional breeding process. The winter hexaploid cultivar Student from the Volga region serves as standard cultivar. The triticale breeding program at ARISER works to solve the problems of reducing abiotic and biotic stress influence on the plant growth and increasing yield capacity and grain quality.

In a short time, using traditional and biotechnological approaches, some advanced DH lines of hexaploid triticale were developed. They differ from each other by several botanical and agronomical characteristics, yield capacity, quality of the grain, plant height, and vegetative period. In 2010, Sviatosar, a new winter triticale created by combining conventional and haploid breeding was submitted to the state variety tests. This cultivar was derived from cross of local line with the Krasnodar cultivar Strelets. The higher yield capacity of Svaiatosar is mainly due to a higher 1,000-kernel weight (Table 2).

Table 2. Grain yield, 1,000-kernel weight, and plant height of the new triticale cultivar Sviatosar.

	Grain yield (t/ha)					1,000-kernel weight (g)	Plant height (cm)
Cultivar	200	2008	2009	2010	Average	Average 2007–10	
Sviatosar	3.21	3.69	3.23	1.62	2.94	44.4	130
Student-St	2.81	3.17	2.89	1.08	2.48	38.2	130
LSD_{05}	0.36	0.38	0.30	0.30	0.30	2.4	—

Laboratory of Spring Bread Wheat Breeding, 7 Tulaikov Street, Saratov, 410010, Russian Federation.

Breeding of spring wheat in Saratov.

R.G. Sayfullin and F.V. Sirenko, Yu.V. Lobachev and L.G. Kurasova (Saratov State Agrarian University named after N.I. Vavilov, Department of Plant Growing, Plant Breeding and Genetics, Teatralnaya Square, 1, Saratov, 410012, Russian Federation).

In 2009–10, spring bread wheat cultivars from different wheat breeding centers of the Russian Federation, Germany, Belarus, and Kazakhstan were studied in the field trials at the Agricultural Research Institute for the South-East Region (ARISER, Saratov). The modern cultivars developed in the ARISER were used as a check. Grain yield of old Saratov cultivars (introduced into agricultural production in 1924–57) was 43.8% that of the modern cultivars. The closest yields to those the modern ARISER cultivars were those from Samara (Russia), the grain yield of which reached 66.9%. Yield capacity of cultivars from the relatively dry regions of Russia (Ufa, Orenburg, Kurgan, and Barnaul) and Kazakhstan was 53.9–55.1% that of the Saratov cultivars. Grain yield of Moscow Region's cultivars made up only 51.1%, whereas that of cultivars developed in the relatively moist regions of Germany and Belarus comprised 36.5% that of the Saratov cultivars (Table 3).

Table 3. Yield capacity of spring bread wheat cultivars produced by different wheat breeding centers in 2009–10.

Region where the cultivar was created	Grain yield capacity	
	t/ha	%
Saratov (modern cultivars)	1.78	100.0
Saratov (historically developed cultivars)	0.78	43.8
Samara	1.19	66.9
Ufa, Orenburg	0.98	55.1
Kurgan, Barnaul, Kazakhstan	0.96	53.9
Moscow	0.91	51.1
Germany, Belarus	0.65	36.5
LSD05	0.39	—

These data demonstrate that the bioclimatic potential of Saratov Region is most fully used by Saratov spring bread wheat cultivars. The cultivars created in other regions are less adaptive. The farther they are in their origin in time or space from the modern Saratov cultivars, the lower the yield capacity. To get optimal use from the bioclimatic potential of the region, the reach of regional breeding centers should be created and developed. The distance between them will depend on the agro-climatic differences between the regions. An increase of 10–15% of the modern local cultivars yiel capacity over those developed in neighboring regions may be used as an indicator of the working efficiency of any regional breeding center.

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Joint inheritance of resistance to leaf rust, spike productivity, and stem length in hybrid soft wheat plants.

V.G. Kyzlasov.

The soft wheat winter cultivar Moscovskaya 39 is characterized by a complex of valuable agronomic features. The cultivar is high-yielding and winter-hardy, and its grain quality is very good. However,Moscovskaya 39 is not resistant to leaf rust. Our aim was to provide resistance to leaf rust from a disomic substitution (2n = 42) wheat–*Aegilops* line (DSL) using a backcrossing technique. The disomic substitution line was selected from a hybrid population '*T. aestivum*/*Ae. speltoides*' (Kyzlasov et al. 2004) that is resistant to leaf rust.

The 'Moscovskaya 39/DSL' hybrid F_1, as well as the DSL itself, proved to be completely resistant to leaf rust. Resistance in the DSL is dominant. The 'Moscovskaya 39/DSL//Moscovskaya 39' F_1 hybrid segregated for resistance

to leaf rust in a ratio of 104 resistant plants : 115 affected plants ≈ 1 : 1. The experiment demonstrated that the resistant plants, in comparison with susceptible plants, had less productive spikes and longer stems (Table 1). The resistant plants had small caryopses, thin stems, narrow laminas, and longer glumes and lemmas.

When joining the features of high spike productivity and short stem with resistance to leaf rust in the same genotype, the author faced some problems because of their linked pattern of inheritance.

Table 1. Spike productivity and stem length in plants susceptible and resistant to leaf rust from a cross 'Moscovskaya 39/DSL//Moscovskaya 39'.

Leaf rust reaction	Spike productivity	Stem length (cm)
Resistant	1.8	119
Susceptible	2.1	101
Significance limit (0.05)	0.2	14

Reference.

Kyzlasov VG, Yatchevskaya GL, and Lazareva HN. 2004. Selection of soft wheat lines disomically substituted by chromosomes of *Ae. speltoides*. Ann Wheat Newslet 50:104-105.

Rye apomixis nonheritable by homozygous offspring.

V.G. Kyzlasov.

This report continues Kyzlasov (2010), which presents the results of a study of rye offspring obtained with no paternal parental participation. A reasonable opportunity for the creation of obligatory apomicts using polyploidization and duplication of homologous chromosomes of heterozygous genotypes is reported.

The germination capacity of the apomictic progenies studied was lower than that of normal rye by 20–30%. Sprouting was observed 2–5 days later than in the control group. Stooling was late as well. Slower plant growth was noted. Most plants died in the winter. A mere 7% of the progenies had survived by harvest time. The plants differed dramatically in their productive capacity, number of shoots/plant (1–30), stem length (30–120 cm), and spike and lamina size. Generally, strong inbreeding depression was manifested in the development of quantitative features, which means that the initial maternal plants, which produced apomictic offspring without pollination, had been heterozygous.

Surprisingly, recombinant plants with normally developed anthers and pollen, appeared among the apomictic progenies. Plants of spring type were found. The initial apomictic maternal plants had sterile pollen and all were winter type. These facts defy explanation, because spring type and pollen fertility are dominant features, whereas winter type and pollen sterility are recessive. As a result of reproducing plants with recessive features, no progeny with dominant features can appear. In the population studies, plants resistant to oidium and unable to produce stems, were found.

Before flowering, stamens were removed from 58 spikes of apomictic origin. One-half of the plants had sterile pollen in the F_3, the other half had fertile pollen. Emasculated spikes were covered with paper cages. Without pollination, practically no seed set in the emasculated flowers. Of 3,550 emasculated flowers, only three produced caryopses in the absence of pollination. Sucha negligibly low frequency of a feature development is statistically insignificant. The studied progenies did not inherit the apomictic reproduction pattern of the maternal plants. A noninheritable apomixis type was earlier described in soft wheat (Kyzlasov 2008).

Normal rye plants are always heterozygous. The studied offspring's failure to inherit apomictic reproduction pattern of their maternal plants can be explained by a transfer of the apomixis genes to homozygous state. We assumed (Maheshwari 1954) that the embryo sac oocyte without pollination can give rise to a diploid embryo due to chromosome endoduplication. The resulting progenies will be, in this case, fully homozygous. There are no reports about homozygous apomicts in the literature. Haploid organisms are fully homozygous. They also are unable to reproduce themselves via apomixis. The apomixis pattern described by the author in winter rye can be an effect of interaction of apomixis genes located in homologous chromosomes of heterozygous plants. Therefore, it is not inherited by homozygous progenies produced due to apomictic reproduction. Apomixis of this type is manifested in the phenotype of heterozygotes only. In homozygous plants, it disappears like the heterosis effect. Kyzlasov (2010) observed in apomictic reproduction, that apomictic progenies have to inherit their maternal plants ability to reproduce themselves via apomixis. However, they were not found to.

In another experiment, the formation of apomictic progenies was repeated in a hybrid population of 'F_2 winter rye R-1 with sterile pollen / spring rye R-2'. Apparently, there are carriers of pollen sterility genes in the population of spring rye R-2. Therefore, pollen appeared to be sterile in approximately 6% of the F_1 hybrid plants obtained. Without pollination, no seed formation was observed in the flowers of these plants. The other plants had fertile pollen. The second generation hybrid population had a segregation ratio by pollen viability of 118 plants with fertile pollen : 41 plants with sterile pollen ≈ 3 : 1 (Table 2). Formation of apomictic caryopses was revealed in the plants with sterile pollen without pollination. Their rate was approximately 10% of the total number of flowers in the spike

Table 2. Segregation pattern of an AaBb rye hybrid in F_2 for pollen sterility (+ = plants with fertile pollen, – = plants with sterile pollen).

	AB	Ab	aB	ab
AB	+	+	+	+
Ab	+	+	+	+
aB	+	+	–	–
ab	+	+	–	–

One-half of the apomictic progenies in the F_3 demonstrated fertile pollen, and the other half had sterile pollen. In the absence of flower pollination, 68 apomictic progenies in the F_3 produced no seeds, in the same manner as in the experiments of prior years. A model of formation of rye apomictic progenies with sterile pollen (aaBb) can be imagined as a result of allelic interaction between 'B' and 'b' genes in heterozygous state (B – b).

This apomictic reproduction type, revealed in rye, is supposed to be inherent to heterozygous plants only. A possibility of apomict formation through the interaction of genes located on homologous chromosomes of heterozygous organisms, is reported for the first time. In such cases, obligate apomicts can appear as a result of chromosome set doubling in genotypes heterozygous by apomixis genes, or unequal crossingover in heterozygous plants in meiosis, or a duplication of homologous chromosomes of heterozygous plants. Possibly, that is why the apomicts found in nature are usually polyploids or aneuploids with unbalanced chromosome number. In sexual reproduction also, flowering plants are known produce seeds entirely as a result of interaction of genes located in homologous chromosomes. If without the flower pollination, no seeds will appear.

These results are consistent with the hypotheses of apomictic plant species formation by means of hybridization and polyploidy (Strasburger 1905; Ernst 1918; Winkler 1908; Powers 1945). The data obtained substantiate the principles of the theory, now universally recognized, that apomixis is determined by the action of genetic factors (Petrov 1988).

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Research of the potassium maintenance in leaves of triticale seedlings in the presence of aluminum toxicity by means of ion-selective electrodes.

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Reaction of wheat and triticale plants to aluminum ions in a soil solution has been observed at very low aluminum concentrations. Some plants are able to grow in acclimation to aluminum toxicity, but is the growth of such plants accompanied by increased soil ion absorption, in particular potassium? Research of potassium movement in vegetation by ardent photometrics is expensive and labor-consuming and, because, of it its use is limited.

A fast and complete use of potassium ions in plant biomass by means of ionometry has been developed. The essence of the method consisted of the allocation of potassium from plants in two steps: first, the cut sheet within an hour per a solution of $CaCl_2$ (0.01 M, potassium of the apoplast), and second, after boiling the fabric sheet within 3 min in the same solution. After each stage, potassium ions were measured using a potash electrode (ELIT-031) on a Ekoniks EXPERT 001. This device allows to defining potassium maintenance in mg/L, and also to construct a model of dependence EMF from the concentration of potassium, on a preliminary constructed scale in a range of concentrations. After boiling, data of potassium level in the leaves was obtained. The triticale cultivar Legalo, after the addition of aluminun ions in the soil, was studied for the reaction of plants with the following scheme: control (0 mg/Al), Al4 ($AlCl_3$/100 g soil), K10 (KCl/100 g soil), and K10+Al4 (4 мg Al + 10 мg soil K/100 g). After 14 days, the plants were measured for maintenance of potassium before (potassium of apoplast) and after (general potassium) boiling.

The presence of aluminun ions in the soil sharply reduces apoplast potassium, whereas dependent simplast potassium fluctuates slightly (Fig. 1). The addition of potash salts to the soil does not activate potassium accumulation in the leaves of triticale seedlings. With the simultaneous addition to the soil of potash and aluminum salts, a decrease in the maintenance of potassium in the simplast is observed, which essentially stops the absorption of potassium by means of potash pumps and a strengthening of potassium in apoplast. The mechanism for the decrease in absorption of water and nutrients is the presence of aluminum ions, because aluminum toxicity has a negative influence on root metabolism. In the apoplast, the raised maintenance of potassium is observed. Potassium exit from the intercellular space is observed.

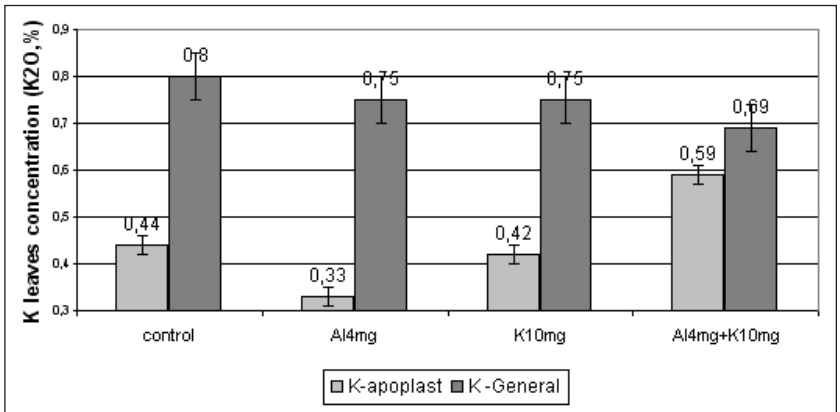

Fig 1. The potassium maintenance (K_2O, %) in seedlings leaves of the triticale cultivar Legalo.

Aluminum has an essential impact on seedling growth in triticale. Aluminum ions activate adaptable seedling growth; the dry weight increased 86%, whereas from potash salt use it was more than 57.6%, and from a potassium application in the presence of aluminum more than 56.4%. Potassium lowers the activation of growth in Legalo triticale. Growth did not caused a raised absorption of potassium ions. The absorption of potassium is dependent on metabolism by roots in the presence of aluminum ions (Poukhalskaya et al. 2008). Earlier, we observed similar growth activation of wheat plants in the presence of aluminum (Poukhalskaya et al. 2006).

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Influence of exogenous phytohormones on the functional activity of apical meristematic cells in wheat seedlings.

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The functioning of plant apical meristems is controlled by the hormonal regulatory system, which operates at all stages of plant ontogenesis. A topical problem is the identification and further study of molecular markers involved in the perception of a hormonal signal and its transmission to the plant cell genome.

Our preliminary work has found that the meristematic cells of the wheat apex are characterized by the presence of a marker protein called the proliferative antigen of initials (PAI), whose content in root and stem meristematic cells correlates with their mitotic index (i.e., it defines the extent of activity of these cells (Evseeva et al. 2009). The suggestion has been made that PAI is associated with the perception of an auxin or cytokinin signal and its transmission to the cell genome. Our aim was to examine the influenceof exogenous auxins and cytokinins on the functional activity of meristematic cells in the seedllings of wheat cultivar Saratovskaya 29.

The root system of 5-day-old seedlings was treated with solutions of indole-3-acetic acid (IAA; 1 and 0.1 mg/L) and 6-benzilaminopurine (6 BAP; 1 and 0.1 mg/L). The activity of meristematic cells was assessed by the results of determination of the cells mitotic index and by comparative immunochemical estimates of PAI content in these cells.

IAA at 1.0 and 0.1 mg/L enhanced the mitotic activity of the root meristematic cells 2- and 2.5-fold, respectively. The PAI content of the apical meristems changed insignificantly. In turn, in response to 6-BAP at 0.1 mg/L, cellular mitotic activity increased 2-fold and PAI content increased 1.2-fold. These results suggest that PAI involved in the perception of signals from gormones of the cytokinin series and their transmission to the plant cell genome.

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The programmed cell death in winter wheat suspension culture at low temperatures.

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Programmed cell death (PCD) is the genetically controlled process of the organized destruction of superfluous or defective cells (Krishnamurthy et al. 2000; Kingston-Smith et al. 2008). The mechanisms of PCD are well known in animals, whereas many features of this process in plants are needed to investigate. PCD in plants plays a crucial role in real-

izing of development program, response to pathogens and different abiotic stress (Heath 1998; Jones 2001; Gao et al. 2008). PCD in plant cells is accompanied by a number morphological and biochemical changes, just as in animal cells, and include chromatin condensation with subsequent nuclei disintegration and DNA fragmentation, concentration and vacuolization of the cytoplasm, protoplast condensation, release of cytochrome c from the mitochondria, activation of endonucleases and caspase-like proteins, generation of reactive oxygen species, and dependence of the death process on ATP level in the cell and protein synthesis de novo (Reape et al. 2008).

The available literature data about possibility of induction and development PCD process under cold conditions are not numerous (Koukalová et al. 1997; Ning et al. 2002). In these works, the possibility of PCD activation is investigated under low temperature treatment. Nothing is known about opportunity of subzero temperatures to cause PCD in plants. The aim of our work was to investigate conditions for PCD activation in a winter wheat suspension culture during treatment with low and subzero temperatures.

Materials and methods. Suspension-cultured cells of *T. aestivum* were grown in the dark at 26°C under continuous shaking in Murashige and Scoog (MS) medium containing sucrose (3%), thiamine (1.0 mg/L), pyridoxine (0.5 mg/L), nicotinic acid (0.5 mg/L), 2.4-D (2.5 mg/L), inositol (0.01%), and sodium dithiocarbamate (0.0005%). Suspensions were subcultured every 14 days using 2:7 dilutions. All treatments were carried out using log-phase cells 8 days after subculture. Suspension-cultured cells were subjected to cold hardening for 7 days at 8°C or 4°C and following short-term treatment (–8°C, 6 hours). After these treatments, suspension cells were moved under the control conditions (26°C) for 3, 6, and 10 days. Evans' blue staining of cell culture was used to determine the number of dead cells and cells with condensed protoplasts (Baker and Mock 1994). At least three independent experiments were performed with more than 500 cells counted per conditions. The quantity of stained cells and cells with condensed protoplasts were calculated using light microscope Axiostar plus (Carl Zeiss, Germany). Images were made using inverted fluorescent microscope AxioObserver Z1 (Carl Zeiss, Germany) with digital monochrome camera AxioCam MRm3 and the AxioVision Rel.4.7.2 software.

Results and discussion. Our experiments showed that reaction of suspension cultures hardened at different temperatures to transferring in the control conditions and following treatment with subzero temperatures differ greatly. About 15% of the cells were dying during culture treatment with 8°C, but during the following 10 days of the experiment at 26°C, cell death stopped (Fig. 1). Furthermore, the subsequent cold shock (the treatment with subzero temperature, CS) did not cause the mass mortality of suspension cells and during our experiment. The decrease in the quantity of living cells in the culture exposed to preliminary hardening at 8°C and then CS was only about 10–15% compared to the control culture (Fig. 1). The control culture treatment with subzero temperature caused the death of 15% of cells during exposure and 45% after transferring the culture in the control conditions (Fig. 1). The defense mechanisms became apparent in the suspension culture exposed preliminary cold hardening at 8°C. They allowed cells to withstand CS.

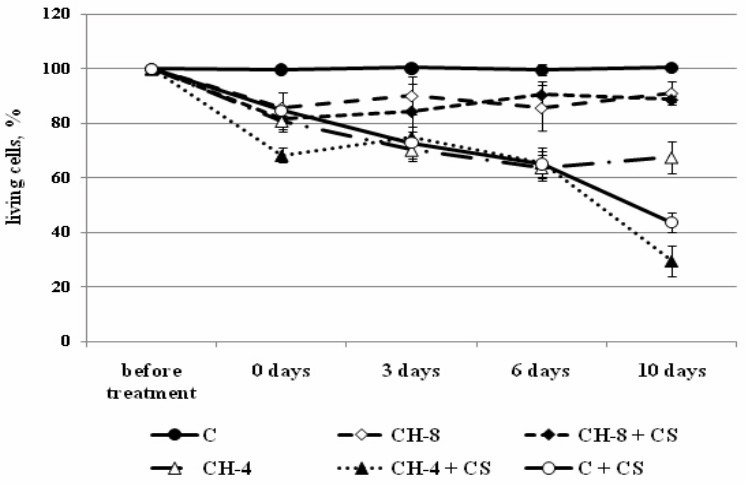

Fig. 1. The influence of subzero temperature (–8ºC, 6 h) on the dynamics of the cell death in the control and hardened suspension culture of winter wheat. C – control suspension culture without any treatment; CH-8 – cold hardening at 8ºC, 7 days; CH-4 – cold hardening at 4ºC, 7 days; CS – cold shock (treatment with subzero temperature) (M ± m; n=3–6).

Other tendency was observed in experiments at 4°C. The quantity of dead cells during this treatment was slightly greater than that in the culture hardened at 8°C (about 5%), but after transferring this suspension culture to the control conditions, the process of cell death continued and 70% of cells dyed during following 10 days of the experiment (Fig. 1). This quantity was greater than that of the respective percent of the dead cells in the control culture after the treatment with subzero temperature. In this connection, it is possible that metabolic state of the cells determined their

further existence (death program or adaptation). The process of PCD on the first stage is reversible, therefore after transferring the culture to the control conditions, those cells in which development of PCD has passed 'the point of no return' were dying during the 6 days of the experiment. At the same time, those cells in which the adaptation mechanisms have been formed or the development of PCD was at the reversible initial stages, returned gradually to the normal vital functions (O'Brien et al. 1998). The process of cell death caused the treatments with low and subzero temperatures to a have gradual, prolonged character; the process developed during several days and not at the same time as the CS treatment, but after it (Fig. 1). This fact allowed us to suppose the active character of the death in suspension culture. Thus, one of the important features characterizing active, genetically programmed cell death became apparent, the development of the process takes a long time. Reape et al. (2008) observed that PCD in plants is slower than in animals and develops during several hours, rarely during one day. In our experiments, PCD was connected with features of stress to low and subzero temperatures.

PCD in plants and animals depends on activity of many enzymes and protein and ATP synthesis (Williams and Dickman 2008). At subzero temperatures or the temperatures near 0°C, many enzymes in the cell denaturate because of a decrease in hydrophobic pressure providing their functional activity, in particular disintegration of the ATP-synthase complex (Finkelstein and Ptitsyn 2002). During cold denaturation of protein, the forming of 'boiling up' of a protein rather than 'molten globule' is observed is significant. Thus, the recovery of disturbed bonds in a spatial pattern takes much time after cold treatment and explains the slow character of the death process in our experiment. Koukalová et al. (1997) have shown the development of PCD under low temperature treatment of tobacco cell culture during 5 weeks.

The shrinkage of the cell and the condensation of the protoplast away from the cell wall is the one of more prominent features of PCD (Reape et al. 2008) and such changes are easy to observe in a light microscope (Fig. 2). The quantity of the cells with condensed protoplasts in the suspension culture hardened at 8°C was equal to that of the control level, whereas after the treatment with 4°C, cell death is accompanied by a 15–18% increase (Fig. 2). Subsequent treatment with subzero temperatures led to protoplast condensation both in the control culture and in the culture preliminary hardened at 4°C (Fig. 2), agreeing with the data of the cell death process after the treatment (Fig. 1, p. 266).

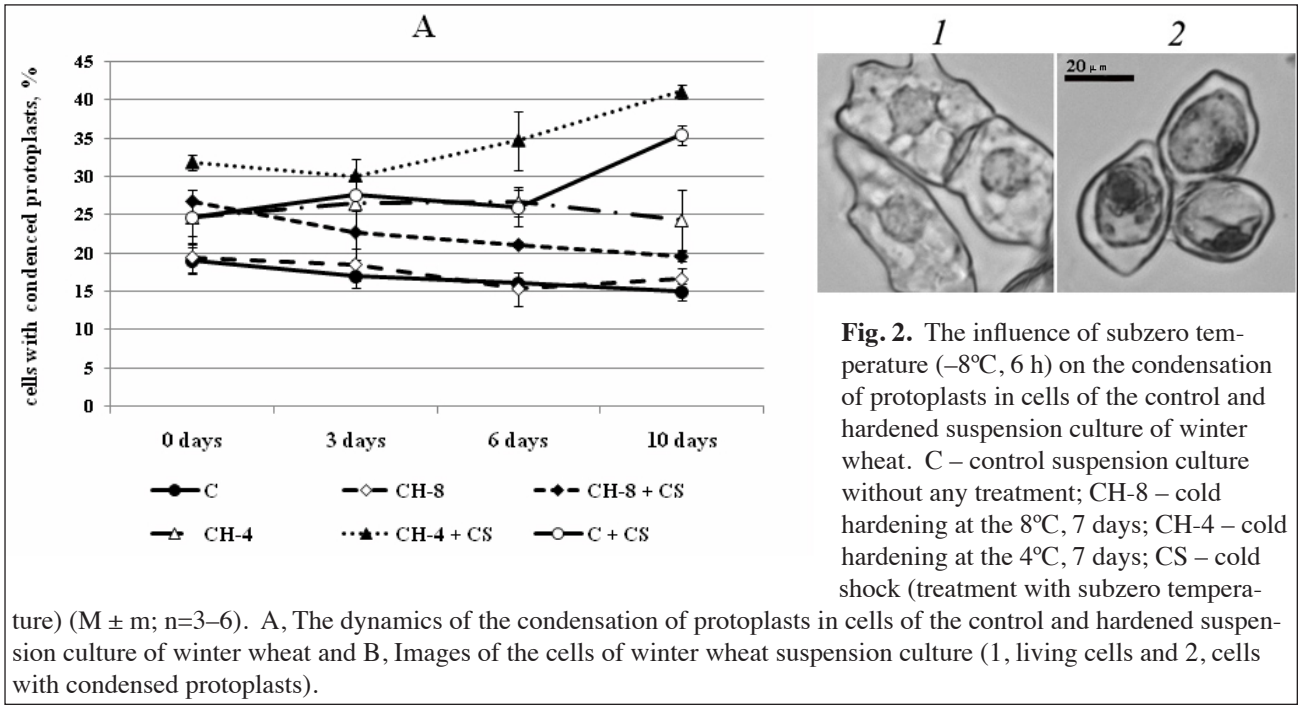

Fig. 2. The influence of subzero temperature (–8ºC, 6 h) on the condensation of protoplasts in cells of the control and hardened suspension culture of winter wheat. C – control suspension culture without any treatment; CH-8 – cold hardening at the 8ºC, 7 days; CH-4 – cold hardening at the 4ºC, 7 days; CS – cold shock (treatment with subzero temperature) (M ± m; n=3–6). A, The dynamics of the condensation of protoplasts in cells of the control and hardened suspension culture of winter wheat and B, Images of the cells of winter wheat suspension culture (1, living cells and 2, cells with condensed protoplasts).

These results allow us to conclude that low temperature may be both necessary for forming of mechanisms of low-temperature adaptation and a factor for PCD activation in suspension culture of winter wheat. At the same time, PCD under low temperature conditions is a slow process, which is accompanied by respective morphological changes.

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The antioxidant function of alternative oxidase and uncoupling proteins in winter wheat mitochondria under cold hardening.

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Cold hardening under low nonfreezing temperatures (first phase of hardening) attacks the ability winter crops to tolerate unfavorable freezing temperature. The acquisition of additional freezing tolerance is the second phase of hardening and takes place when plants are exposed under subzero temperatures (–2–3°C). Cold hardening of winter wheat is known tocause the activation of alternative oxidase (AOX) (one of terminal oxidases of mitochondrial electron transport chain, ETC) (Grabelnych et al. 2003, 2004; Sugie et al. 2006; Mizuno et al. 2008). The ability of alternative pathway (AP) relating to AOX functioning to respond to low-temperature conditions is one of the genetic factors determining cold/frost resistance in winter wheat (Sugie et al. 2006; Mizuno et al. 2008). One function of AOX in plant cells is the decrease of reactive oxygen species (ROS) formation (Popov et al. 1997; Maxwell et al. 1999; Moller 2001) that can be first line of mitochondria protection from oxidative stress (Moller and Kristensen 2004). The uncoupling proteins can carry out similar function in plant mitochondria (Kowaltowski et al. 1999; Considine et al. 2003). But, in contrast to AOX, uncoupling proteins are able to operate under increased ROS content (Rhoads et al. 2006). Sluse et al. (1998) have shown that an increase of free fatty acids (FFA) concentration blocked AOX activity causing activation of uncoupling proteins in vitro. We suppose that the in vivo increase of FFA content in mitochondria along with increase of ROS can regulate AOX and uncoupling proteins activities under stress (particularly, induced by low and subzero temperatures). Our aim was to study of AOX and uncoupling proteins activities under cold hardening in winter wheat seedlings and to detect theirs antioxidant function.

Materials and methods. Three-day-old etiolated seedlings of cold-resistant winter wheat cultivar Irkutskaya ozimaya were germinated on moist paper at 26°C and used as a control. For cold hardening, 2.5-day-old etiolated seedlings germinated at 26°C at 2–3°C for 7 days (first phase) and then placed in an incubator at –2°C for 2 days (second phase). The efficiency of cold hardening was estimated by synthesis of dehydrins. Mitochondria were extracted from shoots by differential centrifugation and purified on Percoll gradient (Pobezhimova et al. 2001). The isolated mitochondria were resuspended in the medium contained 40 mM MOPS-KOH buffer (pH 7.4), 300 mM sucrose, 10 mM KCl, 5 mM EDTA and 1 mM $MgCl_2$. The concentration of mitochondrial protein was analysed by Lowry. Integrity of mitochondrial outer membrane from was calculated on rate of ascorbate-dependent cytochrome-c-induced KCN-sensitive oxygen consumption in presence and absence of 0.04% Triton X-100 and was 92-93%. Mitochondrial activity was recorded polarograph-

ically at 26°C using a closed-type platinum electrode in a 1.4-ml cell. The reaction medium for AOX determination contained 300 mM sucrose, 20 mM MOPS-KOH buffer (pH 7.4), 5 mM $MgCl_2$, 10 mM EDTA, 0.1% bovine serum albumin (BSA) clear free fatty acids, 8 mM succinate (Suc), 5 mM glutamate (Glu), 3 mkM rotenone (Rot), 200 mkM ATP, 1 mM pyruvate, and 5 mM ditiotreitol. The concentrations of inhibitors of respiratory chain were: Rot (3 mkM), antimycin A (A-A) (20 mkM), benzhydroxamic acid (BHAM) (1 mM) and KCN (0.4 mM). The reaction medium for PUMP determination contained 150 mM sucrose, 10 mM Tris-HCl (pH 7.4), 65 mM NaCl, 5 mM EDTA, 0.33 mM EGTA, 8 mM Suc, 5 mM Glu, 3 mkM Rot, 200 mkM ATP, 1 mM BHAM, and 8 mkM linoleic acid (LA). ROS content in isolated mitochondria evaluated by 1 mkM H2DCF-DA (2',7'-dichlorofluorescein diacetate). Fluorescence of DCF was measured by using spectrofluorophotometer SHIMADZU RF-5301PC (Japan) with excitation and emission wavelengths set at 480 nm and 524 nm, respectively. All the experiments were performed on 3–6 separate mitochondrial preparations, arithmetic means and standard error are presented.

Results and discussion. First phase of cold hardening was accompanied by 32-41% decrease of state-3 respiration in winter wheat mitochondria whereas two phases of cold hardening lead to a 65–66% decrease in state-3 respiration. The decrease in the mitochondrial cytochrome pathway (CP) from 77% (control seedlings) to 53% and generation of ROS by mitochondria (a 1.5-fold increase in comparison with control) occurred during first phase of cold hardening (Fig. 3). At the same time, an approximately 1.8-fold activation of AP occured (with 22% to 40%) that was accompanied by synthesis of AOX stress isoforms. Still more ROS generation by mitochondria (2.8-fold) was observed under second phase of cold hardening) (Fig. 3) and at the same time the inhibition of AP (to 17%) and the increase of CP (to 73%) were observed. We suppose that the increase in ROS generation by winter wheat mitochondria under cold hardening is related to signal function of these molecules. AOX is protein of nuclear encoding, transmission of signal from mitochondria into nucleus and induction of nuclear genes consequently of mitochondrial signal pathway realization possible to allow plants to support cell homeostasis in changing environment (Rhoads et al. 2006). Activity of AOX may be able to estimate power of this signal pathway (Vanlerberghe et al. 2009).

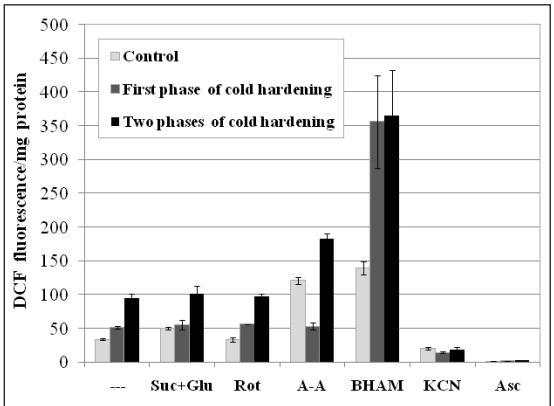

Fig. 3. The ROS content in mitochondria isolated from control and cold hardened seedlings of winter wheat (M±S.E; n=8–10). Control, before cold hardening; first phase of cold hardening, 2–3°C, 7 days; second phase of cold hardening, 2–3°C, 7 days + –2°C, 2 days; ---, without any additions; Suc + Glu, with 8 mM succinate and 5 mM glutamate added; Rot, 3 mkM rotenone added; A-A, 20 mkM antimycin A added; BHAM, 1 mM benzhydroxamic acid added; KCN, 0.4 мM KCN added; and Asc, 60 mM ascorbic acid added.

Succinate and respiratory inhibitors A-A and BHAM increased generation of ROS (1.5-, 3.6-, and 4.1-fold) in mitochondria from control seedlings while KCN inhibited generation of ROS by mitochondria (about 41%) (Fig. 3). These data agree with literature data about ability of A-A and hydroxamic acids to cause an increase of ROS generation by plant mitochondria (Popov et al. 1997). At the same, time suc and A-A did not cause an increase of ROS generation, but BHAM caused a 7-fold the increase in ROS generation in mitochondria from seedlings after first phase of cold hardening (Fig. 3). Taking into consideration that on this stage of cold hardening activation of AP occurs, we may conclude that antioxidant function of AOX is one of cause of the decrease of substrate- and A-A-dependent ROS generation in winter wheat mitochondria and greater ROS generation under addition of BHAM also supports this fact. The second phase of cold hardening also was accompanied the decrease of succinate-dependent ROS generation by mitochondria and lesser ability of A-A to generate ROS in comparison with control mitochondria (a 2-fold), but the effect of BHAM was similar to the control mitochondria (Fig. 3). These data show that antioxidant function of AOX during second phase of cold hardening is carried out in lesser degree than during first. Incubation of winter wheat mitochondria with ascorbic acid leads to full neutralization of ROS and Rot did not influence on ROS production of mitochondria (Fig. 3).

The accumulation of hydrogen peroxide in plant mitochondria during oxidation of suc and BHAM and A-A addition is related to significant increase of superoxide radical anions production (Popov et al. 1997). Superoxide radical anion is known to be an unstable compound and rapidly neutralized to hydrogen peroxide with the participation of superoxide dismutase. Hydrogen peroxide is more stable compound and can diffuse in cell on significant distance that may determine its ability to be a signal molecule.

The decrease of succinate-dependent ROS generation by mitochondria and lesser ability of A-A to produce of ROS during second phase of cold hardening (Fig. 3) may indicate on function of uncoupling proteins. We carried out analysis of state-4 respiration rate, respiration control by Chance-Williams (RC) and ADP/O ratio in winter wheat mitochondria from seedlings subjected to cold hardening. The rate of state-4 respiration in absence of LA was remained constant but the decrease of state-3 respiration rate occurred that was accompanied by the decrease of RC and ADP/O ratio in mitochondria from hardening seedlings. The decrease of ADP/O ratio was maximal under two phases of cold hardening (about 80%). We found that the addition of LA to mitochondria leads to stimulation of state-4 respiration: about 34%, 15%, and 47% in the mitochondria from control, hardened under low, and subzero temperatures seedlings, respectively. The decrease of RC and ADP/O ratio also was most expressed after two phases of cold hardening. The absence of significant stimulation of state-4 respiration in mitochondria after first phase of cold hardening possibly is explained by increase in these conditions of AOX activity. We estimated ROS in the mitochondria of control and hardened seedlings in presence of uncoupling proteins activators and inhibitors. Preliminary results show that activation of uncoupling proteins under incubation of mitochondria with LA effective decreases ROS generation by mitochondria whereas GTP (inhibitor of uncoupling proteins) vice-versa increases ROS.

Thus, first phase of cold hardening leads to inhibition of CP in winter wheat mitochondria, the increase of their ROS content and switch of electrons transport from CP to AP. Under this, likely, ROS carries out the function of signal molecules regulating expression and synthesis of AOX and activation of AP. Antioxidant function of AOX during first phase of cold hardening may present significant component of low-temperature adaptation of winter crops. Under more significant increase of level ROS in mitochondria (not damaging subzero temperatures) uncoupling proteins can replace AOX.

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ITEMS FROM UKRAINE

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New morphological trait in the genus Triticum L.

O.V. Tverdokhleb.

In awned forms of the genus *Triticum*, awns usually are jagged in varying degrees. Smooth awns are relatively rare, they are found only in the cultivated tetraploid wheats *T. turgidum* subsps. *durum* and *turgidum* (Dorofeev 1972) and forms with pubescent awns still have not described in wheat (Tsvelev 1976; Dorofeev 1979). We found such forms in the progeny from a cross '*T. timopheevii* subsp. *timopheevii* / *T. turdigum* subsp. *durum* cultivar Spadshchyna (Fig. 1). In these forms, awn pubescence is a continuation of the pubescence from the top of the lemma and extends to a length of about 2.5 cm, regardless of awn length. In hybrid F_1 plants, the awns were jagged but not pubescent.

Fig. 1. Awn pubscence in the progeny from a cross '*T. timopheevii* subsp. *timopheevii* / *T. turdigum* subsp. *durum* cultivar Spadshchyna. Awn pubescence is a continuation of the pubescence from the top of the lemma and extends to a length of about 2.5 cm, regardless of awn length.

Of the 154 florets of hybrid F_1 plants pollinated with Spadshchyna, 22 seeds were obtained and 12 F_1BC_1 plants were grown. From 12 spikes in the F_2BC_1, four were fertile with seed set from 2.8 to 47.4%. All the spikes had light glumes with light awns and were slightly pubescent. When 25 seeds were sown, plants of F_3BC_1 were obtained, which were divided into five groups.

1. Spikes of dark coffee color, awned, glumes not pubescent, awns dark and pubescent. These five plants were derived from spontaneous pollination of the hybrid by pollen of *T. persicum*. Their fertility was close to zero; only one shriveled seed was found.
2. Spikes light with black pubescent awns, glumes not pubescent. This group included two plants with spike fertility of 18.2–25.0%.
3. Spikes light with black pubescent awns, glumes pubescent. We have assigned to this group five plants with a fertility of 19.2–34.2%.
4. Spikes with light pubescent glumes and awns. To this group were assigned eight plants with fertility from 2.6–3.1%.
5. Spikes light, no pubescence, glumes not pubescent. The group included five plants with a fertility from 2.6–9.4%.