

43. T. I. Gerasimova, L. Yu. Mizrokhi, and G. P. Georgiev, "Transposition bursts in genetically destabilized lines of Drosophila melanogaster," Dokl. Akad. Nauk SSSR, 274, No. 6, 1473 (1984).
44. M. P. Snyder, D. Kimbrell, M. HanKapiller, R. Mill, J. Finstrom, and N. Davidson, "A transposable element that split the promoter region inactivates a Drosophila culture protein gene," Proc. Natl. Acad. Sci. USA, 79, 7430 (1982).
45. Yu. P. Altukhov, Genetic Processes in Populations [in Russian], Nauka, Moscow (1983).
46. L. I. Korochkin, "The evolutionary significance of genetic mobile elements. A hypothesis," Tsitol. Genet., No. 4, 67 (1983).
47. L. E. Orgel and F. H. C. Crick, "Selfish DNA: the ultimate parasite," Nature, 284, 604 (1980).

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THE PROBLEMS OF FROST-_Δ AND WINTER-
HARDINESS IN GENETIC STUDIES OF
WINTER WHEAT

A. P. Orlyuk

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This survey summarizes the main findings of research into the genetic control, the inheritance, and the variability of frost- and winterhardiness of winter wheat, and examines the latest ideas concerning the possibilities of combining winterhardiness with other valuable economic characters.

Frost resistance and resistance to sharp fluctuations of temperature during the winter-spring period are the principal factors in the winterhardiness of winter wheat in most regions of the Soviet Union. The improvement of these important economic and biological properties has always been a prime task of the breeder and geneticist, but while studies of the frost- and winterhardiness of winter wheat are being pursued in various directions [1-5], the genetics of these properties has clearly not been investigated to a degree sufficient to enable the practical problems of selection to be successfully resolved on a level with current requirements.

In this paper we review the most important publications on winterhardiness in connection with the selection of intensive varieties of winter wheat.

GENETIC CONTROL

The polyploid nature of bread wheat (Triticum aestivum) creates considerable difficulties for its genetic analysis. On the other hand, the fact that bread wheat is trigenomic makes for a high degree of tolerance for varying degrees of aneuploidy and makes it possible to use cytogenetic methods in unraveling the genetic nature of many characters, including frost resistance.

According to generalized data [5], frost resistance in wheat is controlled by genes located on four chromosomes: 7A, 1B, 4D, and 5D. According to other findings [6], the genetic control of frosthardeness is carried out by loci of seven chromosomes: 5A, 7A, 2B, 4B, 5B, 4D, and 5D. It has been stated [7] that chromosome 5A of the variety Odesskaya 16 determines the especially high resistance of this wheat to low temperatures, but other authorities do not single out this chromosome.

Monosomics of chromosomes 3A, 3B, and 6D have shown a lowered resistance to low temperatures [6]. There are reports to the effect that no less than eight chromosomes are responsible for frost resistance [8, 9].

Different chromosomes may have different sets of frosthardeness genes, which function variously in dependence on the phase of development of the plants and on the freezing regimes [10]. Thus, the genes determining frost resistance in the coleoptile phase are linked with the genes of winter growth and are localized in some varieties on chromosome 5A and in others on chromosome 2D. Chromosome 5D not only contains the

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genes of frost resistance but also controls the mode of life of the plant and the responsiveness to jarovization. This explains the facts emanating from selection and genetic studies to the effect that there is a mutual relationship between enhanced frosthardeness and the winter character of plants. On the other hand, the cold-resistance genes located on chromosomes 7A and 1B apparently have no genetic linkage with the genes of winter cropping.

A link has been established between the period of jarovization and winterhardness [8]. Genotypes with a longer jarovization period generally possess a better winterhardness too. This relationship is highly complex genetically and has not been fully elucidated. The jarovization period is governed by many genes localized on chromosomes 4A, 5A, 3B, 5B, 6B, 1B, 1D, 2D, 5D, and 7D [11], but it is not clear which of these plays the leading role. It appears that the genes of chromosome 5A are mainly responsible for determining the degree of the connection between the jarovization period and frost resistance.

INHERITANCE AND VARIABILITY

A brief overview of the data on the genetic control of frost- and winterhardness of winter wheat attests to the fact that a clearly expressed polygenic nature of determination is intrinsic to these properties. Confirmation of this is seen in the data of hybridological analyses, which have shown an intermediate type of inheritance or dominance of greater winterhardness [12-15].

Characteristic for the winterhardness of the intraspecific hybrids of winter wheat are the following types of inheritance: overdominance, or heterosis, dominance of more winterhardy parents (positive dominance), intermediate inheritance, and dominance of less winterhardy parents [16]. The proportion of hybrids with dominance of the more winterhardy parents amounts to 40% or more, while the proportion of hybrids with an intermediate inheritance comes to 30-37% of the combinations studied.

The type of inheritance of winterhardness depends to a marked extent on the strength of influence and the intensity of the damaging winter factors [10]. According to our data [16], dominance of good winterhardness manifests itself more distinctly in years with lower temperatures and in hybrids from crosses of varieties differing for the property being analyzed. This is especially characteristic for combinations one of whose components is a winterhardy variety of a steppe or forest-steppe ecotype (Odesskaya 16, Odesskaya 51, Mironovskaya 808, Krasnodarskaya 39, and others). Dominance of poor winterhardness is a relatively rare type of inheritance, with an incidence of 10.4-14.1%.

When creating winterhardy selection material, researchers [17-22] attach great importance to the selection of maternal forms for crossing. According to their data, in reciprocal crossings the winterhardness of the maternal form often dominates, although there are cases of inheritance through the paternal line.

Our studies show that the effects of dominance of the resistance of the maternal or paternal form manifests itself mainly in the F_1 hybrids and is leveled out in subsequent generations.

So far there are no convincing explanations for the manifestation of a maternal (or paternal) inheritance of winterhardness in winter wheat. It has been found [23] that the cytoplasm of the species Triticum timopheevi, T. timonovum, and T. ovata, which is not akin to that of T. aestivum, does not produce appreciable changes in frost resistance when combined with the genome of bread wheat. On the basis of these and other data it is concluded that frost resistance in winter wheat is determined mainly by nuclear factors of inheritance. It is assumed [24] that the effect of cytoplasmic factors on the type of inheritance of the winterhardness property in winter wheat is induced as a result of the reception of environmental factors by extrachromosomal structures and the activation, at certain moments, of the genetic factors of the nucleus which control this property. However, for the time being there is no experimental confirmation of this hypothesis.

In order to improve the frost- and winterhardness of selection material and especially to raise the frequency of recombination of resistance with other valuable economic and biological characteristics, breeders often perform saturating and complex multistage inbreedings [19, 25-27]. Most authorities note the great effectiveness of these measures for yielding valuable selection material. However, it must be pointed out that the characteristics of the inheritance and variability of winterhardness in saturating inbreedings have been very sparsely studied, a fact which limits the methodological significance of the studies which have been carried out.

According to our studies (Table 1), one saturating inbreeding is enough to raise the winterhardness of hybrids to the level of the more resistant recurrent parents (Odesskaya 51, Odesskaya semidwarf). When more than two parental forms are involved in the hybridization, great attention must be paid to the choice of

TABLE 1. Inheritance and Variability of Winterhardiness in F₃ Hybrids from Saturating Inbreedings of Winter Wheat

Hybrid, variety	$\bar{x} \pm S_x$	Cv	H ²
Odesskaya 51	95,3±1,1	5,5	—
Odesskaya 51 × Rusalka	77,3±3,7	25,4	82,3
Odesskaya 51 BC ₁ × Rusalka	94,7±2,5	12,6	71,4
Odesskaya 51 BC ₂ × Rusalka	96,1±1,9	9,3	50,5
Odesskaya 51 × Rusalka BC ₁	60,2±3,1	27,7	81,7
Odesskaya 51 × Rusalka BC ₂	45,4±3,2	22,5	72,3
Rusalka	42,1±1,5	7,7	—
Odesskaya semidwarf	94,3±1,1	5,3	—
Odesskaya semidwarf × Saniya	72,5±3,8	24,4	85,3
Odesskaya semidwarf BC ₁ × Saniya	90,4±2,6	14,7	74,3
Odesskaya semidwarf × Saniya BC ₁	54,4±2,9	23,3	84,6
Saniya	42,7±1,2	7,4	—

the last crossing component, since it will impart to the hybrid organism ~50% of the hereditary factors and must therefore be as frost- and winterhardy as possible. Dominant winterhardiness is exhibited by the varieties Odesskaya 51, Odesskaya semidwarf, Il'ichevka, Mironovskaya 808, Krasnodarskaya 39, and Severodonskaya, which are valuable donors of this property.

The variability parameters of winterhardiness as a polyfactorial character depend heavily on the genetic structure of the population of plants. In constant varieties, even those differing markedly in general winterhardiness (Odesskaya 51, Odesskaya semidwarf, Rusalka, and Saniya), the coefficient of variability is usually not high: 5-8% (see Table 1). In heterogeneous hybrid populations - Odesskaya 51 × Rusalka or Odesskaya semidwarf × Saniya - the degree of variability is considerably higher: 25.4 and 24.4%, respectively. With increasing saturation of a hybrid with the hereditary factors of the more winterhardy variety the variability of the property being analyzed diminishes and approaches the values of the recurrent parent. Lower variability values are also found for simple, but highly resistant hybrids of the types Odesskaya 16 × Mironovskaya 808 and Odesskaya 51 × Mironovskaya yubileinaya [16]. In the case of a low mean-populational winterhardiness of hybrids created by crossing markedly differing varieties, the variability of their strains will be markedly greater. In our experiments it attained 30-39%.

Some publications [12, 16, 28] offer data on the heritability of frost- and winterhardiness. The indexes given vary. According to our findings [16], the heritability of winterhardiness in the broad sense (H²) ranges from 30.7 to 84.4% and in some hybrids is as high as 85.3% (Table 1). Heritability depends markedly on the genetic origin of the hybrids and on the overwintering conditions. For instance, relatively mild winters do not cause a great differentiation of strains of the same origin, and the indexes of their genotypic variability can be comparatively low. Under such conditions the heritability of winterhardiness will have low values too. And vice versa, in years with heavy frosts the differences in winterhardiness between strains will manifest themselves more strongly and the index of genetic variance will rise, which ultimately leads to a rise in the heritability index.

Of course, the heritability indexes are merely a mathematical reflection of the genetic structure of a hybrid population. When varieties which differ in frost- and winterhardiness are crossed, the heritability of the properties in question increases in the hybrids, but this comes out much more clearly in years with lower temperatures, and the genetic effect of the selection of resistant forms waxes strongly in such years [29].

In selection-genetic studies of winterhardiness scant attention has been paid to the phenomenon of transgressive variability [4, 30], although instances of the appearance of outstandingly winterhardy biotypes in segregating hybrid populations have been mentioned by numerous authorities [15, 17, 23, 31-34]. The available data point to the fact that transgressive forms can be obtained with both intraspecific and interspecific crossings.

Barashkova [34] thinks that the success achieved with the selection of *T. aestivum* for frost resistance is due to the D genome. However, many samples of [wild] *Aegilops tauschii* bearing the D genome have poor frost resistance. The initial increase in frost resistance in bread wheat is due to the transgression occurring when the genetic material of the tetraploid (A^uB) is combined with the D genome following spontaneous hybridization [35].

It has been noted [23, 30, 33] that it is a difficult matter to obtain positive transgressions in hybrids created with the participation of winterhardy varieties. Hybrid populations obtained from crosses of such varieties as Odesskaya 16, Odesskaya 51, Mironovskaya 808, and Al'bidum 114 have good winterhardness, but during the process of segregation biotypes which surpass their parents hardly ever appear. It seems that highly frost-resistant varieties with the bounds of a specific ecotype have reached a certain limit in the manifestation of this property, that is, during natural and artificial selection they have accumulated coadapted blocks of genes. In the majority of cases these blocks create a specific genetic system with a dominant determination of frost resistance and winterhardness. We believe many of the highly winterhardy varieties to be positive transgressions, reinforced by deliberate selection. To create on their genetic basis still more resistant genotypes will be very difficult without knowing the genotypic structure of the frosthardness property.

Another very important and complicated problem is that of predicting transgressive variability for frost resistance. Instances of the appearance of highly resistant forms for crosses of varieties of medium winterhardness [17, 30, 31, 36, 37] or for the use of spring forms in hybridization [23, 38] attest merely to the possibility of selection in that direction, but do not exclude elements of empiricism and intuition on the part of the breeder. It should be pointed out, however, that the frequency of positively winterhardy forms rises in the case of crosses of varieties with different rhythms of development during the fall period [31, 39]. Didus' [39] believes that winterhardy-positive plants appear when one crosses varieties in whose pedigrees there are already genotypes with an enhanced or a high winterhardness. This working hypothesis is also corroborated by our selection-genetics work.

Reports of an increased frequency of transgression in hybrids from crosses of remote ecological and geographic forms [17, 37] can be attributed to complementary interactions of the genetic systems of the hybridization components. In many of the varieties we know to be highly resistant to low temperatures there are "frost resistance" alleles with a weak functional effect. When two such varieties are crossed, as a result of crossing-over with the subsequent complementation of alleles favoring the enhancement of winterhardness, a block of genes is created which causes an enhanced resistance to subzero temperatures. The crux of the matter here lies not in the geographic remoteness of the parental forms, but rather in the genetic characteristics of individual loci that are capable of entering into complementary interaction and of thereby producing a transgressive effect.

To bear this out we can cite our data on the winterhardness of grain obtained from crossing the two closely related strains L1202 and L1099, which were themselves created by the method of intravarietal selection from the semidwarf form Khersonskaya yubileinaya (Table 2). It turned out that in the L1022 × L1099 hybrid forms in which winterhardness was markedly higher than in the superior parent segregated out with a frequency of 12.3%. Individual strains were comparable to the highly resistant variety Odesskaya 51 in their degree of winterhardness.

Quite a high frequency of segregation of forms with enhanced winterhardness has also been observed in other hybrids (Table 2). The winterhardness of their parental forms is not high and is apparently controlled by alleles with a weak genetic effect. The complementary interaction of these alleles as a result of hybridization leads to a marked increase in winterhardness in individual biotypes.

TABLE 2. Positive Transgression for Winterhardness in F₃-F₄ Hybrids of Winter Wheat

Hybrid	Winterhardness of grain, % of surviving plants		Frequency of transgression, %
	of superior parent	of hybrid	
L1202 × L1099	65-71 68,3	70-93 90,2	12,3
Bezostaya 1 × Khersonskaya yubileinaya	75-81 79,4	79-94 90,4	15,2
Khersonskaya yubileinaya × Khersonskaya 170	73-78 75,3	80-92 89,4	10,4
KMB-1 × Mutant 48	57-62 60,4	60-84 76,6	14,7

Note. The numerator gives the boundaries of manifestation of the character, the denominator the mean values.

Research using biochemical testers of frosthardiness in winter wheat is currently underway and is proving to be of importance for the theory and practice of selection work. It has been found [40, 41] that certain fractions of gliadin proteins can serve as biochemical markers of frost resistance.

Thanks to the development of studies in experimental mutagenesis, there are real possibilities of obtaining winterhardy forms of winter wheat under the influence of diverse mutagenic factors [42-44], Litvinenko et al. [44] report that chemical mutagens cause an increase in the variance for winterhardiness. A greater variability is observed in varieties with poorer resistance, but in absolute terms the winterhardiness of the mutants is higher in those cases where they are obtained on the basis of more resistant varieties [42, 44].

In selection and genetic studies of winter wheat considerable attention is being paid to the problem of combining high yield and winterhardiness in one variety [17, 20, 21, 26, 27, 33, 37]. Most authors note that these complex properties are rarely encountered together in one genotype, since the elementary characters responsible for them often develop in different directions in the organism. Good winterhardy biotypes exhibit slower growth processes, a finely cellular structure of the tissues, small leaves, ears, and grain, and thin straw. A set of such genetically determined traits cannot make for high yield [33]. Selection workers must therefore create and work with large volumes of breeding material and use in the future those rarely encountered forms which exhibit winterhardiness and good yield at the maximum level possible. The theoretical groundwork for creating winterhardy selection material still needs to be developed. A universally recognized principle is that of hybridizing highly resistant varieties with high-yielding ones [37]. It is precisely according to this principle that the valuable varieties Krasnodarskaya 39, Donskaya ostistaya ostistaya, Zaporozhskaya ostistaya, Odesskaya 51, Odesskaya semidwarf, and others were created.

The frequency of recombination of winterhardiness and high yield depends to a great extent on the genetic structure of the genotypes involved in hybridization. There is a marked increase in the yield of valuable recombinations in the system of complex saturating or multistage inbreedings [15, 19, 27, 33].

A very acute problem in the selection of intensive lodging-resistant varieties of winter wheat is that of combining in one genotype winterhardiness and short-stemmedness [43-47]. The genes determining low growth in winter wheat frequently lower its frosthardiness, and this creates great difficulties for selection. However, these properties are controlled by nonequivalent genetic systems in different genotypes [8] and their phenotypic expression varies widely, all of which gives hope for successful recombination.

Among the low-growing forms of winter wheat there are plants with a short epicotyl (underground internode). In such morphobiotypes the tillering node lies deeper than in tall-growing plants, and this plays a positive role in enhancing winterhardiness [47, 48]. It is just this type of resistance to adverse winter factors that is characteristic of our high-yielding semidwarf varieties Khersonskaya 170, Ostistaya 3, and Ostistaya 5. They have a short epicotyl (1.9-2.3 cm), so that the tillering node lies at a depth of 6-7 cm, which is a guarantee of good overwintering (usually by spring at least 90% of the plants have survived).

The nature of the variability of frost resistance in hybrid plants can depend significantly on the freezing regimes [45]. In a less harsh regime biotypes with straw of varying length will survive, while if the temperatures plunge, only the taller forms will survive. We believe that at present the gene pool of winter wheat enables varieties of the semidwarf type to be created with a winterhardiness at the level of the widely distributed resistant varieties Odesskaya 51, Mironovskaya 808, and others.

CONCLUSION

Frost- and winterhardiness are polyfactorial properties with a so-called threshold effect of manifestation [49]. This means that after the low temperatures have wrought their effect, the experimenter is left with only the surviving plants. Among those which have perished (just as among those surviving) there are biotypes with varying degrees of resistance, and it may be that they can satisfy the selection worker in many of their properties.

At the same time, the polyfactorial genetic control of resistance lays a basis for the complementation of alleles during hybridization and crossing-over, as a result of which transgressive forms with positive variations appear. It is still hard to predict the parameters of transgressive variability [30], since the genetic structure of the properties of frost- and winterhardiness has been insufficiently studied. We believe that real help in this direction will come from seeking and researching various biochemical and physiological testers with a view to expanding positive transgressive variability for these properties.

Also of great practical value are the methods of testing resistance according to various biophysical and morphophysiological indicators [22].

Low growth and high yield are hard to combine with frost resistance and winterhardiness in winter wheat. Higher parameters of recombination of these properties are achieved in cases where winterhardy forms are crossed with low-growing high-yielding ones. Here the degree of frosthardiness of the short-stemmed donors is of great importance: the higher it is, the higher the frequency of recombination. Another effective method is to induce low-growth mutations in good winterhardy varieties by means of mutagenic factors.

LITERATURE CITED

1. Ch. Olaien, "Physicochemical basis of frosthardiness in winter crops," *Sel. Khoz. Rubezhom*, No. 9, 31 (1968).
2. D. F. Protsenko, V. N. Remeslo, and V. N. Musich, Winterhardiness of the Root Systems of Winter Wheat [in Russian], *Izd. Kiev. Gos. Univ.*, Kiev (1971), p. 136.
3. P. Mazur, "Frost damage to plants," *Sel. Khoz. Rubezhom*, No. 1 (1971).
4. G. V. Gulyaev, P. F. Magurov, and A. N. Berezkin, "Selection and genetic aspects of enhancing the winterhardiness of winter wheat," *Genetika*, 9, No. 4, 132 (1973).
5. V. G. Rigin and É. A. Barashkova, "Some aspects of the frosthardiness genetics of bread wheat," in: *Methods and Techniques of Increasing Winterhardiness in Winter Grain Crops* [in Russian], Kolos, Moscow (1975), p. 119.
6. J. Sutka, "Genetic studies of frost resistance in wheat," *Theor. Appl. Genet.*, 59, No. 3, 145 (1981).
7. G. P. Bondar', "Results of a study of winterhardiness of wheat of the variety Odesskaya 16 in a system of monosome analysis," in: *Ecology and Genetics of Plants and Animals* [in Russian], Synopses of Reports, Part II, Shtiintsa, Kishinev (1981), p. 547.
8. J. Lelley, *Wheat Breeding. Theory and Practice*, Kaido, Hungary (1976).
9. V. N. Musin and G. P. Bondar', "A study of frost resistance of the winter wheat Odesskaya 16 in a system of monosome analysis," in: *Biological Aspects of the Study and Rational Use of the Animal and Plant World* [in Russian], Zinatne, Riga (1981), p. 70.
10. L. I. Surkova, "Genetic control of frost- and winterhardiness of winter grain crops," *Sel. Khoz. Rubezhom*, No. 7, 18 (1978).
11. R. Morris, "Chromosomal locations of genes for wheat characters," *Ann. Wheat Newslett.*, 9-19, (1962-1973).
12. M. V. Kir'yan and É. A. Barashkova, "Evaluation of winter wheat hybrids of the first and second generation in terms of winter- and frosthardiness," *Byull. Vses. Nauchn.-Issled. Inst. Rasteniievodstva*, No. 94, 6 (1979).
13. V. N. Musich, A. V. Nefedov, and G. G. Maistrenko, "Frost resistance of early generations of winter wheat hybrids," in: *Selection and Varietal Agrotechnics of Winter Wheat* [in Russian], Kolos, Moscow (1979), p. 143.
14. V. N. Musich, "Inheritance of frosthardiness in winter wheat hybrids," in: *Selection of Wheat in the Southern Ukraine* [in Russian], *Vses. Selektiv.-Genet. Inst.*, Odessa (1980), p. 92.
15. V. V. Shelepov, O. A. Kul'chitskaya, and V. I. Shelepova, "Selection for winterhardiness of winter wheat under the conditions in the Southern Ukraine," in: *Selection and Seed Production* [in Russian], Urozhai, Kiev (1972), No. 42, p. 12.
16. A. P. Orlyuk, "Some genetic aspects of the selection of winter wheat for winterhardiness," in: *Methods and Techniques of Increasing Winterhardiness in Winter Grain Crops* [in Russian], Kolos, Moscow (1975), p. 125.
17. F. G. Kirpichenko, "Methods of creating winterhardy varieties of winter wheat with a steppe ecology," *ibid.*, p. 58.
18. K. D. Gotsov and A. S. Tsenov, "Frost resistance of wheat hybrids in the first generation," *Sel'sk. Biol.*, 4, No. 4, 628 (1969).
19. V. P. Laskin and É. N. Maslovskaya, "Inheritance of flour strength and of frost resistance in complex hybrids of winter wheat," in: *Selection and Varietal Agrotechnics of Winter Wheat*, [in Russian], Kolos, Moscow (1971), p. 133.
20. A. F. Sukhorukov and V. F. Ivannikov, "Winterhardiness and yield of winter wheat hybrid populations," *Sel. Semenovod.*, No. 4, 23 (1974).
21. V. N. Remeslo, Yu. P. Shalin, and L. A. Zhivotkov, "Effect of parental forms on the frost resistance of selection material," *Sel. Semenovod.*, No. 1, 25 (1977).

22. G. V. Gulyaev, D. A. Dzhanumov, and A. E. Rodionova, "Biophysical express method of estimating the frost resistance of hybrid material," *Sel. Semenovod.*, No. 3, 8 (1982).
23. S. F. Lyfenko, "Inheritance of frosthardiness in winter bread wheat hybrids," *Nauchn. Tr. Vses. Sel.-Genet. Inst.*, No. 14, 71 (1976).
24. R. M. Karamyshev, "Effect of the cytoplasm on the nature of quantitative inheritance in bread wheat," in: *Problems of Developmental Genetics [in Russian]*, Kolos, Moscow (1972), p. 97.
25. V. V. Ivannikov and N. P. Mironova, "On selection of pairs for crossing in winter wheat selection," in: *Selection and Varietal Agrotechnics of Winter Wheat, [in Russian]*, Kolos, Moscow (1971), p. 170;
26. R. U. Urazaliev, "An effective method of creating winterhardy forms of winter wheat," *Sel. Semenovod.*, No. 3, 19 (1977).
27. I. G. Kalinenko, "Methods of creating and introducing winter wheat varieties into production," *Sel. Semenovod.*, No. 9, 7 (1980).
28. M. V. Kir'yan, "Variability and heritability of the character of winterhardiness in intervarietal hybrids of winter wheat," in: *Scientific Proceedings of the Mironov Research Institute of Wheat Selection and Seed Production [in Russian]*, No. 5 (1979), p. 25.
29. A. A. Gorlach, "Selection of winter wheat at the Belotserkovskaya Experimental Selection Station," in: *Selection and Varietal Agrotechnics of Winter Wheat [in Russian]*, Kolos, Moscow (1971), p. 218.
30. A. P. Orlyuk, "Transgressive variability in winter wheat and its use in selection," *Genetika*, 12, No. 2, 15 (1976).
31. A. A. Gorlach, "On selection of pairs in breeding winter wheat for winterhardiness," *Sel'sk. Biol.*, 3, No. 6, 830 (1968).
32. F. G. Kirichenko, A. V. Nefedov, and N. A. Litvinenko, "Results and ways of creating winterhardy varieties of winter wheat in the Ukrainian steppe," *Nauchn.-Tekh. Byull. Vses. Sel.-Genet. Inst.*, No. 29, 3 (1976).
33. A. F. Shulyndin, "Evolutionary-genetic principles for increasing the winterhardiness of wheat-type plants," in: *Methods and Techniques of Increasing Winterhardiness in Winter Grain Crops [in Russian]* Kolos, Moscow (1975), p. 79.
34. É. A. Barashkova, "Role of the D genome in enhancing the frost resistance of winter wheat," in: *Genetic Resources and Selection of Plants for Resistance (9th "EUKARPIYA" Congress) [in Russian]*, Leningrad (1980), p. 43.
35. É. A. Barashkova, E. N. Alekseeva and É. D. Migushova, "The connection between frost resistance and the genomic composition of wheat," *Genetika*, 13, No. 10, 1694 (1977).
36. A. A. Gorlach, "Genetic aspects of the selection of winter wheat for winterhardiness," in: *Methods and Techniques of Increasing Winterhardiness in Winter Grain Crops [in Russian]*, Kolos, Moscow (1975), p. 98.
37. P. P. Luk'yanenko and Yu. M. Puchkov, "Selection of winterhardy varieties of winter wheat," *ibid.*, p. 9.
38. S. Ya. Skatova, "Toward the selection of low-growing and winterhardy forms of winter wheat," *Sel. Semenovod.*, No. 5, 21 (1979).
39. V. I. Didus', "Selection of winter wheat for winterhardiness and yield," in: *Methods and Techniques of Increasing Winterhardiness in Winter Grain Crops [in Russian]*, Kolos, Moscow (1975), p. 30.
40. A. A. Sozinov and F. A. Poperelya, "Polymorphism of prolamines and selection," *Vestn. Sel'sk. Nauk*, No. 10, 21 (1979).
41. J. Cerny and A. Harisova, "Vyuziti gliadinovych markern k Rodnoceni alechtitelskeho materialu pšenice," *Sb. UVTIZ-Genet. Sel.*, 16, No. 2, 1 (1980).
42. A. P. Orlyuk, "Manifestation of economic and biological characters in winter wheat mutants under irrigated conditions," in: *Chemical Supermutagens in Selection [in Russian]*, Nauka, Moscow (1975), p. 198.
43. G. N. Okhremenko, V. S. Mozhaeva, and S. Ya. Kraevoi, "The use of induced mutagenesis for enhancing recombination for characters of short-stemmedness and frost resistance in winter wheat hybrids," in: *Adaptation and Recombinogenesis in Cultivated Plants [in Russian]*, Shtiintsa, Kishinev (1979), p. 38.
44. N. A. Litvinenko, V. M. Pyl'nev, and A. V. Nefedov, "A method of increasing the winterhardiness of wheat varieties," *Sel. Semenovod.*, No. 1, 13 (1981).
45. A. P. Orlyuk and V. V. Bazalii, "Frosthardiness of plants of hybrid populations of winter wheat as a function of the original forms," *Tsitol. Genet.*, No. 6, 507 (1976).
46. S. F. Lyfenko, N. V. Tupitsyn, T. G. Kotova, and V. P. Fedchenko, "The connection between the tall-stem character of winter wheat and frost resistance," *Nauchn.-Tekh. Byull. Vses. Sel.-Genet. Inst.*, No. 3 (37), 6 (1980).

47. A. P. Orlyuk, "Relationship between length of coleoptile and epicotyl of winter wheat hybrids and the winterhardiness of the plants and germination capacity of the seeds," in: Sel. Semenovod., Urozhai, Kiev (1981), No. 47, p. 24.
48. J. W. Taylor and M. A. McCall, "Influence of temperature and other factors on the morphology of wheat seedlings," J. Agric. Res., 52, 569 (1963).
49. J. L. Brewbaker, Agricultural Genetics, Prentice-Hall (1964).

USE OF SPECIFIC DNA PROBES FOR THE
MAPPING OF THE CERULOPLASMIN GENE ON
RAT CHROMOSOMES BY DIRECT HYBRIDIZATION
IN SITU

V. S. Baranov, A. L. Schwartzman,
V. N. Gorbunova, V. S. Gaitskhoki,
and S. A. Neifakh

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Using specific DNA probes representing fragments of the natural rat ceruloplasmin (CP) gene, λ RCP-1, λ RCP-2, and λ RCP-3 and the cDNA transcripts of the CP mRNA within the DNA of plasmid pBR-322 (pRC_p-2), labelled in the reaction of nick translation with ¹²⁵I dCTP, we carried out mapping of the gene CP-protein of the α_2 globulin fraction of blood plasma by hybridization in situ. The specific activity of the probes was within the range 1.5×10^7 to 3.4×10^8 decay/min \cdot μ g DNA. We calculated the number of silver grains and analyzed their distribution along differentially-stained chromosomes on 653 metaphase plates from bone marrow cells of laboratory white rats. The results of in situ hybridization of all four probes show good agreement and show that the most likely location of gene CP is in the q13-q22 segment of chromosome 7. The possible reasons for the local concentration of the radioactive label above the other chromosomes is discussed.

The location of eukaryote genes by direct hybridization in situ, i.e., directly on the preparations of metaphase chromosomes, has a number of obvious advantages over other methods for the mapping of chromosomes of mammals, including humans. This method not only allows one to assess the required chromosome in the karyotype relatively quickly but also, which is particularly important, to locate the gene under investigation within the chromosome, i.e., to determine its position in relation to the drawing of the differential diagram, and in this way to detect the segment of the chromosome containing this gene. The method of hybridization in situ, used successfully for the mapping on chromosomes of higher eukaryotes of heterochromatin blocks and multiply repeated structural genes (histone, ribosomal, and others) [1-4], has been used increasingly in recent years also for the mapping of unique genes, represented by single copies per haploid genome. The development of newly developed techniques for the preparation of specific molecular probes (DNA fragments of natural genes and double strand cDNA copies of the corresponding mRNAs), which are strongly labelled in the nick translation reaction [5], and a significant improvement in the technique of hybridization in situ, increasing 100-fold the resolution of the method [6], are essential preconditions for such studies. The main possibility of the mapping of unique mammalian genes by hybridization in situ using specific DNA probes was demonstrated independently and simultaneously in two different laboratories [7, 8]. Later the method was used successfully for the mapping of the genes of insulin [9], α -globin [7, 10], the family of interferon genes [11], and the genes of procollagens of type I [12].

Using a similar method, in this study we have carried out mapping on rat chromosomes of the unique structural gene of ceruloplasmin (CP), a glycoprotein of the α_2 -globulin fraction of blood plasma, which plays an important role in processes of transport, intracellular exchange, and the elimination of copper from the

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