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Genetic control of frost tolerance in wheat (*Triticum aestivum* L.)

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Summary

The frost tolerance of winter wheat is one component of winter hardiness. If seedlings are frost resistant, it means that they can survive the frost effect without any considerable damage. To study the genetic control of frost tolerance, an artificial freezing test was used. Frost tolerance is controlled by an additive-dominance system. The results of diallel analyses indicate the importance of both additive and non-additive gene action in the inheritance of this character. The dominant genes act in the direction of lower frost tolerance and the recessive genes in the direction of a higher level of frost tolerance. The results of monosomic and substitution analyses show that at least 10 of the 21 pairs of chromosomes are involved in the control of frost tolerance and winter hardiness. Chromosomes 5A and 5D have been implicated most frequently. The gene *Fr1* (Frost 1) was located on the long arm of chromosome 5A. Crosses between cultivars, chromosome manipulation and the induction of somaclonal variation may be suitable methods for broadening the gene pool for frost tolerance.

Introduction

In Europe winter wheat is predominantly grown, giving yields 30 to 40% higher than spring wheat, provided frosts in snowless winters to early spring do not damage the crop. Under Hungarian conditions the wheat yield is influenced not only by the genetic yield potential and disease tolerance of the cultivars but also to a great extent by their winter hardiness. The winter exposes young wheat seedlings to many kinds of stresses: direct frost effect, cold winds, snow cover, intense freezing and glaciation of the soil, frost lifting in spring and various diseases which thrive in or can withstand the cold.

Frost tolerance is one component of winter hardiness. If seedlings are frost resistant, it means that they can survive the frost effect without any considerable damage.

Methods

Over the last 50 years many methods have been devised for studying frost tolerance. According to the method developed in the Martonvásár phytotron for the testing of genetic material, the growth and hardening of the plants are carried out in autumn-winter type plant growth units (Sutka, 1981). The growth period lasts for 5 weeks with decreasing temperature and illumination. During the 6th week hardening is carried out at a day temperature of + 2 °C and a night temperature of 0 °C with 20 h illumination. After hardening the boxes are transferred to the frost tolerance testing chamber, where the temperature is reduced by 1 °C/h to a value of − 4 °C. Hardening is continued in this chamber for another 2 days in the dark, after which the frost treatment is carried out at various temperatures depending on the genetic material. After 24 hours of freezing without illumination, the temperature is raised by 2 °C an hour to + 1 °C and the plants are kept at this temperature for 15 hours. The boxes are then transferred to a growth bench (GB) unit for recovery at a

Table 1. Analysis of variance for combining ability and reciprocal differences of parents and F_1 hybrids for frost tolerance from a six-parental diallel

Source of variation	df	SS	MS	F
General combining ability (GCA)	5	37.4	7.473	202.76*
Specific combining ability (SCA)	15	7.6	0.5066	13.74*
General reciprocal effect (GRE)	5	0.4	0.08901	2.41
Specific reciprocal effect (SRE)	10	0.2	0.03686	
Error	35	1.3	0.03686	

*Significant at P = 0.001

day temperature of 16 °C and a night temperature of 15 °C with a 14 hour day for 18 days.

To study the relationship between hardening period and the expression of frost tolerance plants were kept during two weeks of preliminary growth at day and night temperatures of 15 °C and 10 °C, respectively, with a 12 hour day. After this, the temperature was maintained at 2 °C continuously for 90 days, again with a 12 hour day. During the 90-day hardening period frost tests were carried out every 10 days in the freezing chamber. In this plant growth unit, plants were further hardened for 2 days at − 4 °C after which the temperature was lowered by 2 °C per hour to − 11 °C. The intensity of illumination during the growth and hardening of the plants is Q = 260 μEs^{-1} m^{-2} (15 klx), using Sylvania Gro-Lux/WS fluorescent tubes.

After freezing, the leaves are cut off with scissors a few centimetres above the soil. This is in order that regrowth can be more accurately evaluated, and to avoid the risk of infection by fungal diseases. Frost tolerance is assessed in terms of regrowth on a 0 (dead) to 5 (undamaged) scale and also as percentage survival.

Results

Genetic determination and gene interaction

The genetics of frost tolerance was studied in detail in winter wheat with a method using complete diallel crosses (Gullord, 1974; Puchkov & Zhirov, 1978; Parodi et al., 1983). Their data showed that frost tolerance is controlled by an additive-dominance system.

In our experiments two diallel crosses involving six and ten wheat varieties were tested for frost tolerance under controlled freezing conditions (Sutka, 1981, 1984). In the analysis of variance for combining ability, variance due to the general combining ability (GCA) and specific combining ability was significant (Table 1).

This indicates the importance of both additive and non-additive gene action in the inheritance of frost tolerance. The high GCA:SCA ratio (14:6) revealed a preponderance of additive genetic variance. No significant average maternal differences or other reciprocal differences were found between the reciprocal crosses.

The variance (V_r) and covariance (W_r) were calculated for the freezing test, averaged over the reciprocal crosses. The regression coefficient is significantly different from zero but not significantly different from unity. This indicates that non-additive genetic variation is present as dominance only. The dominant genes acted in the direction of lower frost tolerance and the recessive genes in the direction of a higher level of frost tolerance. In our experiments the values of narrow and broad heritability are 81.10% and 97.55%, respectively. From these data one can expect successful selection for frost tolerance.

In our experiments with one exception the cytoplasm has no significant effect on frost tolerance in varietal reciprocal F_1 hybrids. The survival of alien alloplasmic lines are presented in Table 2. In this experiment *Ae. umbellulata* cytoplasm combined with a nuclear background of Chinese Spring or Cappelle Desprez significantly reduced frost tolerance in comparison with *T. aestivum* cytoplasm. This could probably be explained by the *Ae. umbellulata* cytoplasm causing a decrease in plant vigour and in the case of the variety Cappelle Desprez interacting to produce winter variegation (Worland et al., 1987).

Location of genes on chromosomes

Several cytogenetic studies have been conducted in wheat using monosomic and substitution analyses

Table 2. Effect of *Aegilops* cytoplasm on the frost tolerance (%) of *T. aestivum* L.

Cytoplasmic background	Nuclear background			
	Chinese Spring		Cappelle Desprez	
	−12 °C	−14 °C	−12 °C	−14 °C
Ae. mutica Boiss.	62.0	4.0	100.0	92.0[a]
Ae. squarrosa L.	66.0	12.0	92.0	82.0
Ae. umbellulata Zhuk.	32.0[b]	6.0	80.0	12.0[c]
Ae. variabilis Eig.	72.0	4.0	96.0	68.0
T. aestivum L.	72.0	8.0	84.0	68.0

[a], [b], [c]Significant at the 0.05, 0.01 and 0.001 probability levels, respectively, in comparison with the *T. aestivum* cytoplasmic background

Table 3. Frost tolerance of F_3 lines, homozygous for chromosome 5A of Chinese Spring (CS) or Rannyaya 12 (Ran.12) or Mironovskaya 808 (Mir.808), derived from the reciprocal crosses

Hybrid background	Source of chromosome 5A	−12 °C		−14°C	
		Average rating	Survival %	Average rating	Survival (%)
CSxMir.808	Mir. 808	2.32	92	1.47	78
Mir.808xCS	CS	0.46	32	0.07	7
Difference		1.86*	60*	1.40*	71*
CSxRan.12	Ran. 12	1.70	81	0.82	54
Ran.12xCS	CS	0.59	36	0.24	16
Difference		1.11*	45*	0.58*	38*

*P = 0.001

which have allowed the chromosomal locations of the quantitative trait loci (QTL) to be established for frost tolerance (Goujon et al., 1968; Puchkov & Zhirov, 1978; Sutka & Rajki, 1979; Rigin & Barashkova, 1984). The results of monosomic analysis suggest that considerable variation exists between the effects of different chromosomes. This difficulty can be overcome by using reciprocal monosomic analysis (Sutka & Kovács, 1985). To demonstrate this idea the monosomic lines for chromosome 5A of the wheat varieties Chinese Spring, Mironovskaya 808 and Rannyaya 12 were used as parents. In the F_1 the 5A monosomics were selected and then self-pollinated. In the F_2 generation the disomics were selected and selfed. Seedlings of F_3 disomic lines were used for the freezing test. The average rating and percentage survival at freezing temperatures of − 12 °C and − 14 °C reveal a significant difference between 5A chromosomes from different varieties (Table 3). For the given hybrid backgrounds the 5A chromosomes of Mironovskaya 808 and Rannyaya 12 have a greater effect on frost tolerance than that of Chinese Spring.

Intervarietal chromosome substitutions provide one of the best means of studying the cytogenetic control of frost tolerance. The survival of Chinese Spring (Cheyenne) substitutions was tested under artificial conditions (Sutka, 1981). In each of the substitution lines one pair of chromosomes from Chinese Spring was replaced by the corresponding homologues from the frost resistant variety Cheyenne. Freezing tests in the Martonvásár phytotron confirmed earlier observations which indicated that the chromosomes of homoeologous group 5 of Cheyenne carry major factors controlling frost hardiness (Jenkins, 1971; Cahalan & Law, 1979). When comparing phytotron frost testing and nursery winter hardiness it can be seen that chromosomes of homoeologous group 5 and the 2B and 4B chromosomes play an important role in both environments, but considerable differences were also observed (Table 4).

Table 4. Frost tolerance and winter hardiness of Chinese Spring/Cheyenne chromosome substitutions under phytotronic and nursery conditions

Chromosome substitution	Phytotron	Nursery
1A	59	17
2A	45	12
3A	39	25
4A	58	25
5A	94[c]	71[c]
6A	31[a]	56[c]
7A	75[c]	22
1B	63	19
2B	74[c]	31[a]
3B	62	19
4B	74[c]	40[c]
5B	73[b]	47[c]
6B	65[a]	30
7B	48	19
1D	61	21
2D	43	28
3D	36	23
4D	75[c]	17
5D	93[c]	41[c]
6D	59	31[a]
7D	37	22
Chinese Spring (recipient)	49	19
Cheyenne (donor)	100[c]	70[c]

Significance of differences compared to Chinese Spring. [a], [b], [c] P = 0.05–0.01, P = 0.01–0.001 and P = 0.001, respectively

Table 5. Survival percentage of varietal chromosome substitution 5A into wheat variety Saratovskaya 29 (S 29)

Genotypes	Freezing temperatures		
	− 12°C	− 14°C	− 16°C
Recipient			
Saratovskaya 29	0	0	0
Donors			
Mironovskaya 808	100	100	95
Albidum 11	100	100	100
Ulyanovka	95	100	100
Lutescens 230	100	100	100
Substitutions			
S29/Mironovskaya 808 5A	81	7	0
S29/Albidum 11 5A	81	40	3
S29/Ulyanovka 5A	81	23	2
S29/Lutescens 230 5A	80	13	5

A number of other studies have been conducted to determine which wheat chromosomes contain genes that affect frost tolerance (Roberts, 1986; Sutka, 1989). The results are consistent with the notion that frost tolerance is a complex character, at least 10 of the 21 pairs of chromosomes are involved in the control of frost tolerance and winter hardiness. Chromosome 5A and 5D have been implicated most frequently and they appear to carry major genes.

The location of the gene(s) responsible for frost tolerance on chromosome 5A was studied using chromosome recombinant lines from a cross between the substitution line Hobbit (*Triticum spelta* 5A) and Hobbit. In this sample of recombinant lines the locus for frost tolerance, designated *Fr1* (Frost 1) was completely linked to the locus *Vrn1* controlling the vernalisation requirement. The results can be explained by a pleiotropic action of the *Vrn1* locus or by close genetic linkage between *Vrn1* and *Fr1* (Sutka & Snape, 1989). The locus involved in the control of cold hardiness on chromosome 5A of wheat was also identified by Roberts (1990).

There was a substantial difference between Chinese Spring (CS) and Cheyenne (Ch) not only in the level of frost tolerance, but also in the dynamics of tolerance, making it possible to study the effects exerted by the individual chromosomes in the course of the hardening period. The substitution line 5A reached the maximum level of hardiness on the 40th day of hardening. Substitution line 7A did not increase frost tolerance during the initial stages of hardening, but caused a significantly higher rate of survival from the 50th to the 80th day than in the variety Chinese Spring (Fig. 1). This confirmed that the frost tolerance gene on chromosome 7A became activated during the second half of the hardening period, thus providing reliable protection against late frosts.

Increase in genetic variation

Several methods exist for the expansion of the gene pool. By means of interspecific crosses, transgressive segregants can be selected from progeny generations with frost tolerance exceeding that of the parents. A

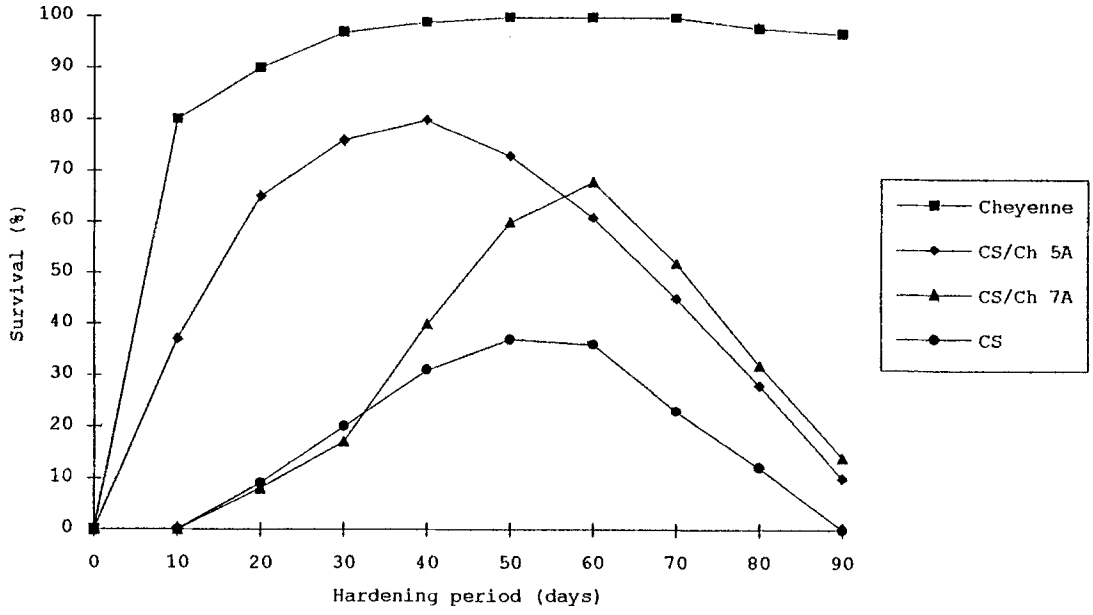

Fig. 1. Mean % plant survival of the parents and various Chinese Spring/Cheyenne chromosome substitution lines following increasing periods of hardening at 2°C and freezing treatment at −11°C

further possibility is to improve the frost tolerance of frost sensitive wheat varieties by substituting chromosomes from highly frost resistant varieties into the frost sensitive wheat. The frost tolerance of the very frost sensitive spring wheat variety Saratovskaya 29 can be improved to such an extent by substituting the 5A chromosome with that of the extremely frost resistant varieties Albidum 11, Ulyanovka or Lutescens 230, that it becomes capable of surviving freezing at −14 °C (Table 5).

Wild species related to cultivated wheat are extremely promising sources of increased genetic variation. *Aegilops cylindrica*, *Agropyron glaucum* (*intermedium*) and *Agropyron elongatum* have proved to be very resistant to frost. The addition of *Agropyron glaucum* chromosomes to the chromosome set of cultivated wheat led to a pronounced increase in frost tolerance; disomic additions are able to survive freezing to a temperature as low as −18 °C (Table 6).

Tissue culture techniques now make it possible to initiate callus cultures from immature embryos and to regenerate plants from the calli. Regenerants known as somaclones, produced from calli of various wheat varieties, including GK Csongor, were propagated for three generations. When the frost tolerance of the somaclonal plants was tested in the SC_4 generation it was weaker than that of the control, which consisted of GK Csongor wheat plants of non tissue culture origin (Table 7). The tolerance of 6 somaclones tended to be better than that of the control, but only somaclone No. 4 proved to be significantly better and thus of practical importance (Galiba & Sutka, 1989).

Table 6. Survival percentage of wheat cultivars, species and alien chromosome addition lines

Genotypes	Chromosome number	Freezing temperature	
		− 16 °C	− 18 °C
Chinese Spring	42	0	0
Martonvásári 8	42	93	50
Martonvásári 14	42	23	10
Cheyenne	42	100	90
Aegilops cylindrica	28	90	87
Aegilops squarrosa	14	17	0
Agropyron glaucum	42	100	100
Agropyron elongatum	14	100	100
Amphidiploid of *T. aestivum/Ag. glaucum* (PPG 829)	84	100	100
Disomic additions of *T.aestivum/Ag.glaucum* (I–VII)	44	100	100

In summary it can be stated that interspecific crossing, chromosome manipulation and the induction of

Table 7. Survival percentages of 'GK Csongor' SC_4 somaclones at a freezing temperature of −13 °C

SC_4 families	Survival (%)	Difference from control
1	86	12
2	76	2
3	46	-28^b
4	94	20^a
5	48	-26^b
6	44	-30^b
7	36	-38^c
8	60	−14
9	90	16
10	50	-24^a
11	66	− 8
12	48	-26^b
13	66	− 8
14	52	-22^a
15	74	0
16	58	−16
17	46	-28^b
18	26	-48^c
19	42	−32
20	86	12
21	60	−14
22	40	-34^c
23	56	−18
24	70	− 4
25	44	-30^b
26	56	−18
27	54	-20^a
28	88	14
29	54	-20^a
30	92	18
31	40	-34^b
Control	74	

^{a}, ^{b}, ^{c}Significant at the 0.05, 0.01 and 0.001 probability levels, respectively

somaclonal variation may be suitable methods for increasing the genetic variation of frost tolerance.

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