ROLE OF THE CHROMOSOME 4B IN SUPPRESSING FROST RESISTANCE IN WINTER WHEAT (*Triticum aestivum* L.)

Ottó Veisz and József Sutka

Agricultural Research Institute of the Hungarian Academy of Sciences, H-2462 Martonvásár, Hungary

SUMMARY

Frost resistance of different monosomic lines were investigated under controlled environment conditions. In the case of Cheyenne, Mironovskaya 808 and Rannyaya 12 a reduction in the degree of frost resistance was observed when chromosomes 5A, 3B, 3A, 5B or 7A were missing. In two of the monosomic series there was also a drop in the level of frost resistance in monosomic lines 5D and 7B. The frost resistance of monosomic line 4B, on the other hand, was significantly better than that of the disome in two of the three cultivars. In previous experiments using substitution lines, the presence of a 4B chromosome originating from a variety with better frost resistance also led to an increase in frost resistance. The results suggest that a gene suppressor for frost resistance may be located on chromosome 4B.

Index words: *Triticum aestivum*, monosomic series, frost resistance, hardening, gene suppressor

INTRODUCTION

Under the climatic conditions of the Carpathian Basin the winter wheat yield is influenced not only by genes directly concerned with yield and for disease tolerance, but also to a great extent by genes for winter hardiness (Lelley, 1956; Rajki, 1980). During the winter, young wheat seedlings are exposed to many kinds of stress: direct frost effects, 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 of the most important components of winter hardiness. If seedlings are frost resistant, it means that they can survive the frost effect without any considerable damage (Lelley and Rajháthy, 1955).

Several genetic studies have been conducted to determine the gene action controlling the expression of frost resistance in wheat. Valuable results concerning the frost resistance of hexaploid wheat have been obtained not only through the analysis of hybrid generations, but also by studying aneuploids. Monosomic and substitution analysis have made it possible to identify chromosomes determining cold resistance (Jenkins, 1971; Cahalan and Law, 1979). Despite the difficulties encountered when using monosomics, many researchers have used monosomic analysis in the study of cold tolerance. Goujon et al. (1968) tested F_2 monosomic hybrids derived from the Chinese Spring monosomics crossed to six winter and spring wheat varieties for cold tolerance at the coleoptile stage. Chromosomes 5A, 2D and 5D were found to carry genes responsible for cold tolerance, while chromosomes 7A, 2D

and 5D were more sensitive to frost. These results were later confirmed by Sutka and Rajki (1979), who tested F_2 monosomic series of Chinese Spring x Mironovskaya 808 and Chinese Spring x Rannyaya 12. During the F_2 monosomic analysis of the variety Arthur, Sutka (1981) showed that chromosomes 5A, 2B, 4B and 5D increased frost resistance, while chromosomes 3A, 3B and 6D had the reverse effect.

The use of intervarietal chromosome substitution series has also proved to be a reliable cytogenetic method. Various authors have reported that at least 10 of the 21 wheat chromosomes are involved in the control of frost resistance and winter hardiness (Goujon et al., 1968; Law and Jenkins, 1970; Puchkov and Zhirov, 1978; Cahalan and Law, 1979; Rigin and Barashkova, 1984; Zemetra and Morris, 1988). Throughout, chromosomes 5A and 5D have been implicated most frequently and they appear to have the major effect on this character. Their effects can also be detected in the survival of wheat tissue cultures exposed to freezing (Sutka et al., 1986).

In a study on the frost resistance of Chinese Spring ditelosomics, both the short and long arms of chromosome 7A and the long arms of chromosomes 5A, 3B, 5B, 7B and 5D were found to have an effect (Veisz and Sutka, 1993). This suggests that the major effects of chromosomes 5A and 5D are most likely due to genes located on the long arms of these chromosomes. This has been confirmed, in the only detailed investigation so far of the genes responsible for frost resistance where it was found that a locus *Fr1* (Frost 1) was located on the long arm of chromosome 5A, tightly linked to the locus *Vrn 1* controlling vernalisation requirement (Sutka and Snape 1989).

This paper reports the behaviour of three sets of monosomics in the varieties Cheyenne, Mironovskaya 808 and Rannyaya 12 to freezing and the detection of a gene suppressor for frost resistance on chromosome 4B.

MATERIALS AND METHODS

The experiments were carried out under controlled conditions in the phytotron of the Agricultural Research Institute of the Hungarian Academy of Sciences. The monosomic series of Cheyenne, Mironovskaya 808 and Rannyaya 12 were used in the experiments. The Cheyenne monosomic series was developed by R. Morris, University of Nebraska and obtained from C.N. Law of the former Plant Breeding Institute, Cambridge, UK, while those of Rannyaya 12 and Mironovskaya 808 were developed in the Genetics Department of the Martonvásár Institute.

The chromosome numbers of plants were counted in somatic and meiotic cells, so the progeny of monosomic plants was only planted in boxes. This means that approximately 73 percent of the progeny of selfed monosomic was expected to be monosomic. In this case the results come from hemizygous ineffective loci.

The monosomic lines were grown in a randomised design in wooden boxes, the internal dimensions of which were 38x26x11 cm. Thirty lines were sown in each box, with 5 plants per line. Eighteen replications were used in the experiment, so that 90 plants were evaluated from each monosomic line. Seeds were germinated for 2 days in petri dishes and then transfered to the wooden boxes containing a 4:1 mixture of good quality garden soil and

sand (Tischner et al., 1997b) and raised for six weeks. At first the plants were watered with tap water, then from the third week onwards with Volldunger nutrient solution. During the one-week hardening period (6th week), the plants were given less nutrient solution, and during freezing they were not watered at all. Following freezing they were again given the optimum quantity of nutrient required for regrowth. The plants were raised and hardened in Conviron PGV-36 plant growth chambers, frozen in C-812 frost rooms and placed on GB-48 growth benches (Tischner et al., 1997a) for recovery.

Before freezing, the seedlings were subjected to gradually decreasing temperatures and daylength (Table 1). The intensity of illumination during both these periods of treatment was 15,000 lux, Q=260μEs^{-1}m^{-2} using Sylvania Gro-Lux-WS fluorescent tubes, with a 14 hours day (Tischner et al., 1997). Due to the relatively low temperature during growth and hardening none of the lines reached floral initiation. After hardening the boxes were transferred to the frost testing chamber, where the temperature was reduced by 1°C per hour from 0°C to -4°C. The plants were hardened for another 2 days at -4°C, then the temperature was further reduced, depending upon the experiment, either to -15°C, -16°C or -17°C. After 24 hours of freezing without illumination the temperature was raised by 1°C per hour to +1°C, and the plants were kept at this temperature for 15 hours. After this the boxes were transferred to a growth bench for recovery at a day temperature of 16°C and a night temperature of 15°C for 21 days. After freezing, the leaves were cut off with scissors a few centimetres above the soil, so that regrowth could be more accurately evaluated. After 21 days, plants which had survived freezing and showed regrowth could be clearly distinguished from those which had died. A 0-5 scale was used to score each plant (those which had died off were scored as 0, while well-developed, tillering plants were given 5 points).

Table 1

The FDA climatic programme

	Weeks					
	1	2	3	4	5	6
	Temperature (°C)					
Day	15.0	10.0	10.0	5.0	5.0	2.0
Night	10.0	5.0	5.0	0.0	0.0	0.0
	Relative humidity (%)					
Day	70	75	75	*	*	*
Night	75	80	80	*	*	*

* At temperatures below 5°C the humidity is not regulated to avoid frost damage to the equipment

The evaluation of the experimental data was carried out using single factor analysis of variance. Differences between means were tested by least significant differences (LSD) (P = 0.05, 0.01 and 0,001).

RESULTS

The frost testing of each of the monosomic series of Mironovskaya 808, Rannyaya 12 and Cheyenne was carried out at the freezing temperatures given in Table 2.

Table 2

Frost resistance of the lines of monosomic series

Monosomic series	Cheyenne		Mironovskaya 808		Rannyaya 12	
Monosomic lines	Score	Deviation from the variety	Score	Deviation from the variety	Score	Deviation from the variety
1A	1.24	-0.02	1.40	-0.47	1.51	-0.33
2A	1.60	0.42	2.02	0.15	1.84	0.00
3A	1.00	-0.26	1.22	-0.65*	0.88	-0.96***
4A	1.20	-0.06	1.77	-0.10	1.92	0.08
5A	0.74	-0.52*	0.92	-0.95***	0.95	-0.89***
6A	0.56	-0.70**	1.90	0.03	2.30	0.46
7A	0.86	-0.40	0.96	-0.91***	1.38	-0.46
1B	0.90	-0.36	1.58	-0.29	1.78	-0.06
2B	1.44	0.18	-	-	2.44	0.60
3B	0.16	-1.10***	1.20	-0.67*	1.00	-0.84***
4B	1.86	0.60**	2.18	0.31	2.74	0.90***
5B	0.72	-0.54*	1.47	-0.40	1.60	-0.24
6B	1.32	0.06	1.66	-0.21	2.06	0.22
7B	0.64	-0.62**	2.24	0.37	1.12	-0.72**
1D	1.28	0.02	1.96	0.09	1.62	-0.22
2D	1.58	0.32	1.50	-0.37	2.06	0.22
3D	1.22	-0.04	1.93	0.06	1.48	-0.36
4D	1.18	-0.08	1.54	-0.33	2.15	0.31
5D	0.58	-0.68**	1.12	-0.75**	1.98	0.14
6D	1.10	-0.16	1.92	0.05	2.65	0.80**
7D	1.26	0.00	2.26	0.39	1.74	-0.10
Disome	1.26		1.87		1.84	
Freezing temperature	-17°C		-16°C		-15°C	

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively

The development of the 2B monosomic line of Mironovskaya 808 has not yet been completed, so this line could not be tested. The various freezing temperatures and the frost resistance values achieved at these temperatures also express the frost sensitivity levels of the individual monosomic series. The lack of chromosomes 5A and 3B caused a large drop in frost resistance in all three monosomic series. The frost resistance of monosomic lines 3A, 7A and 5B also decreased, but the difference from the original variety was not significant for all three series. In two series the loss of chromosomes 5B and 7B caused a significant reduction. The frost resistance of the 4B monosomic lines, however, was better

than that of the disomes. The 6A monosomic lines of the three varieties behaved in different ways: in Cheyenne the loss of 6A led to a significant reduction in frost resistance, which was increased in the 6A monosomic of Rannyaya 12. The frost resistance score of the Mironovskaya 808 6A monosomic was the same as that of the variety. The monosomic 6D line of Rannyaya 12 had better frost resistance than that of the disomics.

DISCUSSION

In the present experiments, when monosomic lines were frozen the frost resistance was found to increase when chromosome 4B was missing. At the same time, in F_2 monosomic analysis (Sutka and Rajki, 1979) and in tests on substitution lines (Sutka et al., 1986) the presence of the 4B chromosome from a variety (Cheyenne) with better frost resistance also increased the percentage survival. It is probable that a suppressor gene is located on chromosome 4B, which in certain cases inhibits the manifestation of genes on other chromosomes. Its functioning can be compared with that of the gene on chromosome 5B, which inhibits homoeologous pairing (Feldman, 1968), or the genes identified by Stracke et al. (1995) on chromosomes 2D, 3D, 4A, 4B and 5A, which suppress mildew resistance.

The results of tests on various monosomic and substitution series confirm the hypothesis that genes responsible for frost resistance are to be found on certain chromosomes. On the basis of tests on monosomic and ditelosomic series (Veisz and Sutka, 1993) and F_2 monosomic and substitution analysis (Sutka et al., 1986) chromosomes 5A, 5D and 5B carry genes for frost resistance, as reported by Law and Jenkins (1970), Cahalan and Law (1979) and Poysa (1984). Chromosomes 3A, 3B, 6A and 6D carry genes for frost susceptibility in the varieties Rannyaya 12 and Arthur (Sutka and Rajki, 1979; Sutka, 1981).

In the monosomic lines the functioning of frost resistance genes on other chromosomes was not suppressed when chromosome 4B was absent. It may be that in the course of F_2 monosomic and substitution analysis this inhibitory effect was not expressed due to changes in the genetic background, but it is also possible that the substituted 4B chromosome did not have a frost resistance-increasing effect, but simply had no inhibitory effect. According to another hypothesis, there could be a gene or genes on chromosome 4B for increased susceptibility to freezing. It is difficult to distinguish between these two possibilities. There is also no unequivocal explanation for the effect of the gene on the 6D chromosome of Rannyaya 12, but it is clear from the present and earlier research that this chromosome plays some role in the development of frost resistance (Sutka and Rajki, 1979).

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GENETIC ANALYSIS

N. Mladenov [1], S. Denč
[1]Institute of Field an
[2]Faculty of

Tolerance to low tempera
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Key words: wheat, low temperatu

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