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# HARDINESS OF WINTER WHEAT VARIETIES AS A FUNCTION OF CHANGES IN CERTAIN ENVIRONMENTAL FACTORS

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VEISZ O. and TISCHNER T. Hardiness of winter wheat varieties as a function of changes in certain environmental factors. BIOTRONICS 24, 73-83, 1995. Winter wheat varieties were studied for frost resistance under different artificial conditions. The relationships between important environmental factors, such as temperature, light, water, atmospheric CO2 concentration and hardening period were investigated. Under artificial conditions the development of a state of hardiness in winter wheat varieties depends to a great extent on a gradual decrease in temperature. A gradual rise in temperature, irrespective of whether it was combined with decreasing or increasing daylength and illumination intensity, always had an unfavourable effect. As the result of varying soil water contents, there were significant differences in the average killed plant rate, which dropped in the case of low soil moisture content and rose at a higher moisture content. An increase in the atmospheric  $CO_2$ concentration had a beneficial effect on the cold hardening process. It was found that the wheat varieties achieved a maximum level of hardening on the 50th day of hardening. The % survival gradually increased from the 10th to the 50th day, after which a gradually decreasing tendency was observed. Based on the results described here, it can be seen that frost tolerance is always a relative term. The development, maintenance and termination of frost tolerance are determined by both genetic and environmental factors.

Key words: winter wheat; cold hardiness; environmental factors; growth chamber.

# INTRODUCTION

If reliable winter wheat production is to be achieved, it is essential that the varieties should not only have good yield potential and disease resistance, but should also be resistant to extreme climatic effects, such as low temperature. The first investigations into winter hardiness and frost resistance date back to the second half of the last century (9, 11). During this period field experiments were carried out, while later special nurseries were set up for frost testing (6, 10, 19). It was a great step forward when artificial equipment – at first simple refrigerators and later programmable climatic chambers – became available for the testing of frost resistance (1, 4, 8, 16). Biochemical and genetic methods were also used by more and more scientists

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for the study of frost resistance (7, 17, 20).

Frost resistance is one component of winter hardiness, but is not identical with it. Wheats which are resistant to frost generally have good winter hardiness, which is why conclusions on winter hardiness can be reliably drawn from a knowledge of frost resistance (15).

Hardening plays a very important role in the development of winter hardiness. The cold hardening of a given species depends on two factors:

-the genetically determined adaptability of the species,

-the environmental factors influencing the manifestation of the trait.

Temperature is of key importance in the hardening process. According to Olien (13) the optimum hardening temperature for winter cereals is around 3°C. In plants kept at higher temperatures the degree of hardiness decreased after a time, even if the nutrient and light conditions were optimum (1, 8, 16). Many authors attribute an important role to the length, intensity and spectral energy distribution of the illumination in the development of hardiness in winter wheats (2, 3, 14, 22, 24).

In experiments carried out using various methods on different varieties, Gusta and Fowler (8), Chen and Gusta (5), Tyler et al. (23) and Nass (12) concluded that there was a negative correlation between the killed plant rate and the moisture content of the soil, the plant tissues and the tillering node. In experiments carried out by Andrews et al. (3), the plants reached maximum hardiness after 6-8 weeks at a temperature of  $2-4^{\circ}$ C.

A phytotron offers excellent opportunities for the study of frost resistance under exact conditions. In the Martonvásár phytotron a frost testing method was elaborated (15). The results achieved up to now provide information on the effect of temperature and of the duration and intensity of illumination on the hardening process in winter cereals, but few data are available on the extent to which joint changes in various ecological factors (e. g. temperature and illumination), the length of the hardening period, the atmospheric  $CO_2$ concentration during hardening, the freezing temperature and the soil moisture content influence the frost resistance of various winter cereals.

#### MATERIALS AND METHODS

The work was carried out under controlled conditions in the phytotron of the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár (21). The winter wheat varieties used in the experiments play an important role in Hungarian wheat production and have frost resistance values ranging from poor to excellent according to the scores achieved in previous phytotron tests.

On the basis of the climatic effects, the method employed to determine frost resistance can be divided into 3 phases:

-preliminary growth and hardening,

—freezing,

-further growth and evaluation.

#### Preliminary growth and hardening

For the frost resistance test, germinated wheat seeds were planted. Germination was carried out in complete darkness at a day/night temperature of  $20^{\circ}/12^{\circ}$ C with a 12-hour day. After 3 days of germination, seeds with intact rootlets were planted into a 4:1 mixture of earth and sand in wooden boxes measuring  $42 \times 30 \times 13$  cm. Prior to planting, the boxes were filled with the earth mixture to within 3 cm of the top and were topped up with a further 2 cm when the plants reached the age of 3 weeks. The seeds were planted at a depth of 1.5-2 cm, so the tillering node was at a depth of 3.5-4 cm. Each box consisted of nine rows with 20 plants to a row.

The varieties were planted in four replications, with 20 plants per replication. After planting, the boxes were kept at room temperature for a day, after which they were transferred to an autumn-winter type growth chamber (Conviron PGV-36), where they were kept for 6 weeks. During this period the temperature, light intensity and length of illumination were gradually reduced with a weekly change of programme similar to autumn conditions in the field. Within each weekly programme, the temperature fluctuation, light intensity and daylength were the same each day. The daily temperature fluctuation followed the daily temperature changes experienced in nature.

The preliminary growth stage was followed by 2-phase hardening. The plants were exposed to the first phase in the autumn-winter chamber, where the temperature fluctuated daily between  $+3^{\circ}$ C and  $-3^{\circ}$ C with a 21-hour daylength and a photosynthetic photon flux density (PPFD) of 190  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>. This phase lasted for a week. The second phase of hardening, which lasted for 4 days, took place in a frost resistance testing chamber (Conviron C -812) immediately prior to freezing. The temperature was a constant  $-4^{\circ}$ C with no illumination.

During the preliminary growth period and the first part of the hardening period the plants were irrigated with tap water. The quantity of water was gradually reduced and no irrigation was carried out during the second phase of hardening or during the frost test. The maintenance of the soil moisture content of the boxes at the same level is an important criterion if the frost test is to be successful. Since it was not possible to apply the traditional method for determining soil moisture content, the water content of the soil was deduced from measurements on the electric conductivity of the soil. The measurements were carried out using an OK 102/1 conductometer with 2 electrodes each 5 cm in length.

#### Freezing

Freezing was carried out in the frost resistance testing chamber set up in the phytotron specifically for this purpose. The temperature was gradually decreased. Freezing took place at  $-15^{\circ}$ C for 24 hours. Throughout the frost treatment the plants were kept in the dark. Thawing took place in the freezing chamber at  $+0.5^{\circ}$ C for 2 days.

# Further growth and evaluation

After thawing the boxes were transferred to growth benches (Conviron GB -48) and the plants were cut back to a height of 3 cm. The removal of the dead leaves not only facilitated the development of new shoots, but also simplified evaluation. The plants were grown for a further 3 weeks at a day/ night temperature of  $17^{\circ}/16^{\circ}$ C, with a 14-hour daylength and a photon flux density of  $125 \ \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . At the end of the third week, plants which had survived freezing and had begun to develop were clearly distinguishable from those which had died. The result of freezing is given as a percentage of the plant number prior to freezing.

The testing method described above was used as the starting point and the effect of each ecological component on the hardening process was determined by altering them independently of each other.

#### RESULTS

#### Effect of temperature and illumination on hardening

The effect of joint changes in these two environmental factors, both so important from the point of view of hardening, was studied simultaneously using four different climatic programmes (Table 1).

The survival percentages of plants grown in the first experiment on four different preliminary growth programmes are presented in Table 2. The 1st climatic programme is identical to that described in the Materials and Methods section and generally used for phytotronic frost testing. Over many years of freezing experiments the survival rates obtained using this method have been around 90% for Yubileinaya 50, Bezostaya 1 and Martonvásári 4, 80% for Martonvásári 8 and 50–40% for Bánkúti 1201 and Martonvásári 10. Similar

Climatic programmes during	Climatic programme of week 1				Climatic programme of week 6			
<ul><li>the 6 weeks (week 1 to week</li><li>6) of preliminary growth</li></ul>	Temperature		Illumination		Temperature		Illumination	
	min. ℃	max. ℃	Duration hours	PPFD µmol/m²•s	min. ℃	°℃	Duration hours	PPFD µmol/m²·s
1 decreasing temperature and illumination	7	14	10.5	360	2	4	8.5	200
2 increasing temperature and illumination	2	10	10.5	360	9	18	14.5	520
3 decreasing temperature and increasing illumination	7	14	10.5	360	2	4	14.5	520
4 increasing temperature and decreasing illumination	2	10	10.5	360	9	18	8.5	200

Table 1. Climatic programmes during the 6 weeks of preliminary growth.

VARIETY	CLIMATIC PROGRAMME				
	1	2	3	4	
Yubileinaya 50	86.9	13.2	94.8	28.1	
Bezostaya 1	97.5	2.3	86.0	15.0	
Martonvásári 4	94.7	18.8	96.3	10.8	
Martonvásári 8	71.6	3.8	40.0	5.0	
Bánkúti 1201	54.4	6.3	24.5	0.0	
Martonvásári 10	38.8	1.3	1.3	0.0	
mean	73.9	7.6	57.2	9.8	
freezing temperature:	$-15^{\circ}$ C	LSD <sub>5%</sub> =15.2			

Table 2. Effect of temperature and irradiance on frost resistance during early development (*survival percentage*).

results were obtained in the present experiment. Gradually increasing temperature, daylength and illumination intensity from week to week during the preliminary growth period (Programme 2) led to a significantly lower survival rate at the 0.1% level than for plants raised on Programme 1. In this treatment only Martonvásári 4 had a survival rate significantly higher than all the other varieties except Yubileinaya 50, but even this was less than 20%. In the case of decreasing temperature and increasing daylength and illumination intensity (Programme 3) the survival rates of the three varieties with good frost resistance did not differ from those observed in Programme 1, while those of Martonvásári 8, Martonvásári 10 and Bánkúti 1201 were considerably lower. As the result of increasing temperature and decreasing daylength and illumination intensity (Programme 4) the survival rates were not significantly different from those recorded in Programme 2.

The development of a state of hardiness in winter wheat varieties under artificial conditions depended to a great extent on there being a gradual decrease in temperature. A gradual increase in temperature, whether it was associated with a reduction or increase in daylength and illumination intensity, had an unfavourable effect. The climatic programme applied during the second phase of preliminary growth had a decisive influence on the course of hardening.

#### Effect of hardening period on frost resistance

It is important to know how long is required in any given system of ecological conditions for the genetically determined hardiness of a variety to develop and how long it is able to maintain this level. In practice it is an advantage if the hardening of the winter wheat variety to a satisfactory level takes place within as short a period as possible and can be maintained for a considerable length of time. In this case neither early autumn nor late winter -early spring frosts will cause any great damage. The examinations were



Fig. 1. Frost resistance of winter wheat varieties as a function of hardening period

carried out on varieties of different origins with medium or better frost resistance (Fig. 1). Preliminary growth was carried out for 2 weeks at a day/ night temperature of  $16^{\circ}/15^{\circ}$ C with a photon flux density of  $360 \ \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . The temperature was then lowered to  $+2^{\circ}$ C and maintained at this value day and night for 100 days. During this period frost tests were conducted every 10 days. The arrangement of the plants in the wooden boxes agreed with those described in the Material and Methods section, as did the freezing and further growth conditions.

In general, the wheat varieties reached the maximum degree of hardiness on the 50th day of hardening. The survival rate gradually increased from the 10th to the 50th day, after which it gradually declined. Changes in the level of hardiness were not the same in all varieties.

# Effect of soil moisture content and freezing temperature on the death rate

In the course of both field overwintering and testing in the phytotron the temperature and duration of freezing and the moisture content of the soil, and thus of the plant tissues, play an important role. It is well known from practical observations, that more plants are frozen out on wet, water-logged areas than on drier ground. In the present experiments an exact determination was made of the extent of damage to different varieties in the case of various soil moisture contents (Table 3).

There were significant differences in the mean killed plant rate as the result of different soil water contents, with less damage at lower soil moisture

Varieties ——	Soil Moisture					
	Dry	Normal	Moist			
Martonvásári 4	0.0	10.7	9.1			
Martonvásári 8	7.5	34.1	39.9			
Bánkúti 1201	40.7	56.3	92.5			
NS Rana 2	79.1	88.1	100.0			
Mean	31.8	47.3	60.4			

Table 3. Effect of soil moisture content on the freezing out of wheat (killed plant rate).

Freezing temperature:  $-15^{\circ}$ C

 $LSD_{5\%} = 14.9\%$  between any two combinations  $LSD_{5\%} = 4.9\%$  between the mean values

Soil water content as a % of natural water capacity

Dry = 30% Normal = 45% Moist = 60%

values and greater damage as the water content rose. In the case of varieties with medium or poorer frost resistance (e. g. Bánkúti 1201) the extent of freezing out was sometimes as much as 100% on wet areas. The results indicate, however, that the survival of varieties with excellent frost resistance is not influenced by the soil moisture content.

From the point of view of freezing out, one of the most important factors is low temperature and its duration. The freezing temperature applied for 24 hours in the phytotron is the value measured at the depth at which the tillering node is found. In nature, when the air temperature is -15 or  $-18^{\circ}$ C and there is no snow cover, the temperature 3–5 cm below the soil surface, where the tillering node is to be found in the case of optimum sowing depth, is not lower than -5 or  $-7^{\circ}$ C. It is thus extremely important to make a correct choice of sowing depth. Table 4 illustrates the effect of freezing temperature on a number of winter wheat varieties.

### Effect of atmospheric $CO_2$ concentration on the hardening of cereals

Under phytotronic conditions investigations were also made on the effect of an increasing concentration of atmospheric  $CO_2$  on the hardening process (cold adaptation) of young wheat plants. The experiment was carried out simultaneously in two plant growth chambers (Conviron PGV-36). The growing conditions simulated the mean autumn weather conditions in Hungary and were the same in both chambers except for the  $CO_2$  content of the air. In one unit the  $CO_2$  level was that normal for the environment (nominally 350  $\mu$ mol·mol<sup>-1</sup>), while in the other the atmospheric  $CO_2$  concentration was

Freezing temperature (°C)				
-6	-12	-15	-18	
0.0	0.0	5.6	25.1	
0.0	5.2	13.9	38.3	
2.5	5.3	45.8	73.6	
2.4	68.5	88.1	100.0	
	0.0 0.0 2.5	$ \begin{array}{c ccc} -6 & -12 \\ \hline 0.0 & 0.0 \\ 0.0 & 5.2 \\ 2.5 & 5.3 \\ \end{array} $	$\begin{array}{c cccc} -6 & -12 & -15 \\ \hline 0.0 & 0.0 & 5.6 \\ 0.0 & 5.2 & 13.9 \\ 2.5 & 5.3 & 45.8 \end{array}$	

Table 4. Effect of low temperature on the freezing out of wheat (killed plant rate).

 $LSD_{5\%} = 17.3\%$ 

Table 5. Effect of atmospheric  $CO_2$  concentration on the initial development and frost resistance of cereals.

Varieties		area n²)	Number of plants surviving freezing % CO <sub>2</sub> concentration		
	CO <sub>2</sub> conc	centration			
	$350\mu\mathrm{mol}\cdot\mathrm{mol}^{-1}$	750 $\mu$ mol·mol <sup>-1</sup>	$350\mu\mathrm{mol}\cdot\mathrm{mol}^{-1}$	$750\mu\mathrm{mol}\cdot\mathrm{mol}^{-1}$	
Wheat					
Apolló	6.85	7.83	12.5	42.5	
Alba	5.25	6.53	20.0	36.2	
Bánkúti 1201	8.21	8.45	37.5	55.0	
Bezostaya 1	7.11	7.22	80.0	80.0	
Martonvásári 15	5.20	5.97	81.2	91.2	
Triticale					
Presto	6.01	7.60	38.7	63.7	
	·····		LSD <sub>5%</sub> =1.12	LSD <sub>5%</sub> =4.9	

adjusted to 750  $\mu$ mol·mol<sup>-1</sup>. Wheat and triticale varieties were grown under these conditions for 7 weeks with gradually decreasing temperature, and were then frozen at  $-15^{\circ}$ C as a substitution for the long winter cold. After freezing, the plants were grown for a further 3 weeks, after which those which had survived could be clearly distinguished from those which had died.

The plant number determined in this way is presented in Table 5 as a percentage of the number of plants before freezing. With the exception of a single variety, an increase in the atmospheric  $CO_2$  content had a favourable effect on the hardening process, so that more plants survived than for those raised under normal conditions. In the course of the preliminary growth period, measurements were also made on the leaf area of the plants, which is one index of biomass production. For all varieties a rise in atmospheric  $CO_2$  concentration led to an increase in leaf area; this difference was significant for

the varieties Alba and Presto.

# DISCUSSION

A close correlation (0.86) was found between the phytotronic frost resistance values and the field winter hardiness of winter wheat varieties, averaged over 15 years of field experimentation. Consequently, the frost resistance values obtained with the model used in the experiments led to the following conclusions with regard to the avoidance of severe frost damage:

# Optimisation of the hardening process can be promoted by a correct choice of sowing date

An optimum choice of sowing date has a favourable influence on wheat hardening (7, 19), since a specific length of time is required under given ecological conditions for the variety to achieve the genetically determined level of hardiness (8, 16, 25). From the point of view of reliable production, it is an advantage to grow winter wheat varieties which acquire a satisfactory level of hardiness after a relatively short hardening period and which retain this hardiness for a long time. In this case frosts in early autumn or in late winter, early spring do not destroy the crop (1, 3, 13).

#### Optimum sowing depth has a favourable effect on overwintering

Field overwintering values are substantially influenced by low temperature and its duration. The freezing temperature applied for 24 hours in the phytotron is the value measured at the depth at which the tillering node is found. In nature, when the air temperature is -15 or  $-18^{\circ}$ C and there is no snow cover, the temperature 5 cm below the soil surface, where the tillering node is to be found in the case of optimum sowing depth, is not lower than -5 or  $-7^{\circ}$ C (26). If the tillering node is closer to the soil surface, the temperature at the critical depth may well be as low as -10 or  $-15^{\circ}$ C, depending on how long the cold lasts (3, 13, 18, 27).

# Likely effect of global climatic changes on overwintering

The environmental factors discussed here are those which have an influence on the overwintering of winter cereals and which are predicted to change in Hungary, as elsewhere. There is likely to be a drop in the quantity of winter precipitation, the temperature during the winter period is predicted to be higher and a rise in the atmospheric  $CO_2$  concentration is also to be expected.

The experimental results presented above confirm suggestions that all the changes will not necessarily be unfavourable for the living world. At the same time, it should not be forgotten that the combined effects of the changes will determine the final result, i. e. in the case of agriculture, the quantity and quality of crop production. The lack of precipitation may stunt plant development; in mild winters pathogens and pests also have a better chance

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to survive, thus causing damage leading to yield losses. This may mean that despite a rise in the number of plants overwintering, there may be a corresponding reduction in yield for the above reasons. A large number of further experiments will be required before this question can be adequately answered.

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