STUDY ON THE RELATIONSHIP BETWEEN FROST RESISTANCE AND FREE PROLINE CONTENT IN SOME WINTER WHEAT AND BARLEY GENOTYPES

Elena Petcu, Maria Þerbea, Zoe Duþã and Dumitru Ionescu[1)]

ABSTRACT

The evolution of free proline content in leaves and roots of some winter wheat and barley genotypes, during cold hardening process, under natural and controlled conditions (2°C and 10 hours photoperiod) and the relationships with frost resistance were established. The results showed that low temperatures induce the increase of free proline content in winter barley in smaller quantities as compared to proline content in winter wheat and that the frost resistant genotypes (wheat or barley) accumulated a higher proline quantity than the susceptible ones. A significant correlation between frost resistance and free proline content from leaves of winter wheat plants hardened under controlled conditions was found. For winter barley genotypes hardened under control conditions (2°C, 10 hours photoperiod), after seven days free proline content was positively correlated with frost resistance (r= 0.97*) but for winter barley genotypes naturally hardened, the proline content did not correlated with frost resistance (r=0.12).**

Key words: winter wheat, winter barley, frost resistance, free proline content.

INTRODUCTION

The physiological and biochemical processes which lead to frost tolerance, or the adaptation of plants to low temperature, are extremely complex. The mechanisms which regulate these processes in Romanian wheat and barley genotypes are not sufficiently well known. In recent years, however, some information has been reported, for example on the interaction between low temperatures effect and cell membrane stability and the relationship between frost resistance of some Romanian wheat genotypes and proline content (Petcu and Þerbea, 1993; Petcu and Atanasiu, 1994). Such information is very useful because frost resistance in winter cereals may be evaluated by direct methods, based on a visual appreciation of damage produced in plants after having been exposed to frost (Palta et al., 1978; Calkinns and Swanson, 1990), or by indirect methods, which involve the utilization of correlation between morphophysiological and biochemical or genetic markers and plants frost resistance (Cerny et al., 1990).

Regarding free proline content, this is one of biochemical markers frequently used in breeding programmes due to its involvement in plant adaptation both to frost and to drought and salinity. Thus, in wheat, Dorffling et al. (1990), Petcu and Atanasiu (1994) found positive correlation between frost resistance and proline content of plants during hardiness. In barley nevertheless it is not clear whether exist genetic variability for proline content under field or controlled conditions (Kolar, 1991; Hayes et al., 1993).

The aim of this study was, on one hand, to compare the evolution of proline content during hardiness under controlled conditions in some winter wheat and barley genotypes, and on the other hand to reveal the existence of differences in proline accumulation under natural and controlled conditions in some Romanian barley genotypes in relation with their frost resistance.

MATERIALS AND METHODS

Four winter wheat and eight winter barley genotypes, provided by the wheat and barley Breeding Department of Research Institute for Cereals and Industrial Crops of Fundulea (RICIC), were grown and hardened under natural and controlled conditions.

The seeds were sown in soil-sand mixture (3:1) in wooden boxes and the plants were hardened under natural conditions during October - December 1994, in green house.

For hardening under controlled conditions, the seeds were germinated on filter paper rolls in a diluted Hoagland solution (1:2) for 10 days at 20-23°C. The plants were hardened at 2°C and 10 hours photoperiod for three weeks. The nutritive solution was changed every second day.

In every week free proline content was analysed after Bates methods (1973).

Frost resistance was estimated according to the direct method: the winter wheat plants naturally hardened were exposed at -17°C for 18 hours while winter barley plants were exposed to -14°C, for 18 hours. These temperatures were achieved in a growing

[1)] Research Institute for Cereals and Industrial Crops, 8264 Fundulea, , Cãlãraºi County, Romania

chamber, where temperature was gradually reduced with 2°C/hour.

After freezing treatment, the plants were exposed at 18-20°C for recovery and frost resistance was established after 10-12 days on the basis of plant damage degree. A score scale was used ranging from 1 to 9 (1- for plants having green leaves, not destroyed by frost, therefore considered very resistant, and 9 - for plants with dead leaves, completely destroyed by freezing treatment, therefore considered very susceptible).

ANOVA analyses and linear correlation coefficients were calculated for statistical data interpretation.

RESULTS AND DISCUSSIONS

For hardening of winter cereals it is necessary to expose the plants to low temperatures (generally ranging between 0-10°C) (Hincha and Schmitt, 1994).

Temperatures over 10°C were registered in October 1994 and in this case the hardening process of winter wheat and barley began relatively late, when daily minimum temperatures were below 10°C. The conditions for the first phase of hardening was registered in November (9-30 November) when daily minimum temperatures were between 0°C and 5°C and for the second phase of hardening process between 1 to 10 December when temperatures around -3°C were registered (Figure 1).

Among the studied barley genotypes, in winter of 1994 -1995, the most resistant one was Odessa 86 genotype. Within the same group of frost resistance we also include Precoce, Fundulea 208 and Fundulea 204 genotypes, but with a difference of 1 respectively, 2 scores on the assessment scale as compared to Odessa 86. Frost resistance of Mãdãlin genotype is reduced and so is that of Fundulea 471 and Fundulea 442. Among the studied wheat genotypes, Odesskaia 51 was very resistant, Iulia and Rodur middle resistant and Libellula was the most frost susceptible genotype (Table 1).

Table 1. Frost resistance of winter wheat and barley studied genotypes

Genotypes	Score	Resistance category
Odesskaia 51 (winter wheat)	2	Very resistant
Iulia (winter wheat)	6	Middle resistant
Rodur (winter wheat)	6	Middle resistant
Libellula (winter wheat)	8	Very susceptible
Odessa 86 (winter barley)	3	Resistant
Precoce (winter barley)	4	Resistant
Mãdãlin (winter barley)	7	Susceptible
Fundulea 471 (winter barley)	6	Middle resistant
Fundulea 442 (winter barley)	7	Susceptible
Fundulea 208 (winter barley)	5	Resistant
Fundulea 204 (winter barley)	5	Resistant
Griviţa (winter barley)	6	Middle resistant

ANOVA analyses showed that duration of

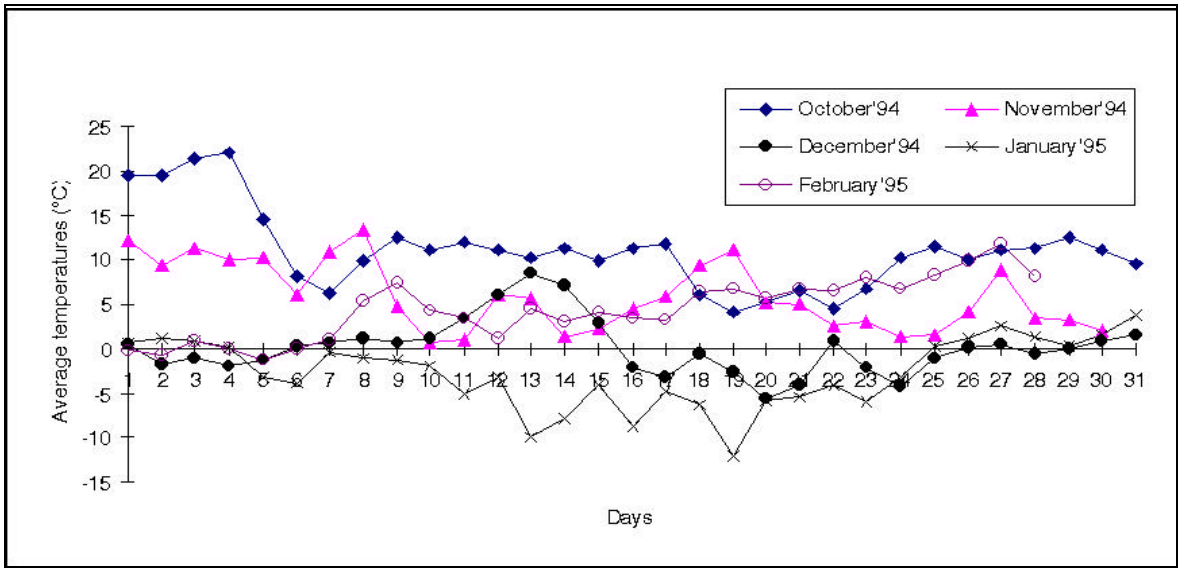

Figure 1. The evolution of average daily temperatures during natural hardened process of winter wheat and barl ey at Fundulea (October 1994 – February 1995

hardening process (factor A), genotype (factor B) and interaction of these factors influenced very significantly the proline accumulation from leaves and roots (Tables 2 and 3).

Table 2. ANOVA analyses for proline content in leaves and collums of winter wheat and barley genotypes

Source of variation	SP	FD	S^2	F value
Duration of hardening (Factor A)	72733.242	4	18183	449.544**
Error A	323.586	8	40.448	
Genotype (Factor B)	105364.322	6	17.560	967.871***
Interacţion A*B	105864.634	24	4411.026	243.117***
Error B	1088.618	60	18.143	

Table 3. ANOVA analyses for proline content in roots of winter wheat and barley genotypes

Source of variation	SP	FD	S^2	F value
Duration of hardening (Factor A)	9999.530	4	2499.882	227.331***
Error A	87.973	8	10.996	533.524***
Genotype (Factor B)	22256	6	3709.365	209.234***
Interacţion A*B	34913.237	24	1454.718	
Error B	417.154	60	6.95	

Ostampliuk (1967, quoted by Drăghici et al., 1978) showed that during natural hardening the rate of osmotic substance accumulation, among which also free proline is slower in barley than in wheat.

Recent studies have shown that accumulation of proline in barley plants starts in the first day of hardiness and the maximum accumulation is after seven days of hardiness (Murelli et al., 1995).

Our results have shown that in the first week of cold hardening at 2°C, one pick for winter barley genotypes was registered and for winter wheat genotypes the peak was registered in a second week of cold hardening at 2°C.

After three weeks of hardening, proline content decreased both in barley genotypes and in wheat genotypes but was still higher in the resistant wheat genotype Odesskaia 51 (Figures 2 and 3). It is obvious that under controlled conditions (2°C and 10 hours photoperiod), proline content increased more in leaves than roots both for winter wheat and barley genotypes (Figures 2 and 3). The highest proline content in roots was registered by wheat genotype Odesskaia 51 (55 µM) followed by Mădălin (barley) in the first week of hardening at 2°C.

Proline content in the roots does not correlate with frost resistance of genotypes but a correlation is evident between frost resistance of the studied genotypes and proline content of the upper part of plants hardened for one, respectively two weeks (r = -0.86***, r = 0.75*, Table 4).

Table 4. Relationship between frost resistance of cereal studied genotypes and free proline content

Proline content		Frost resistance
Proline content in leaves and collums	Plants hardened 1 weeks	r = -0.86***
	Plants hardened 2 weeks	r = -0.75*
	Plants hardened 3 weeks	r = -0.34
Proline content in roots	Plants hardened 1 weeks	r = -0.60
	Plants hardened 2 weeks	r = -0.22
	Plants hardened 3 weeks	r = 0.10

Murelli et al. (1995) did not find significant differences as regards proline content during hardening (4/2°C; 8 h daylight/16 h dark) in two different genotypes in terms of frost resistance potential.

The genetic variability for proline content was studied also in barley genotypes. So, proline content in the seven barley Romanian genotypes (except Odessa 86 which is an Ucrainian genotype) hardened under natural conditions is relatively lower as compared with proline content of some genotypes hardened under controlled conditions (Figure 4).

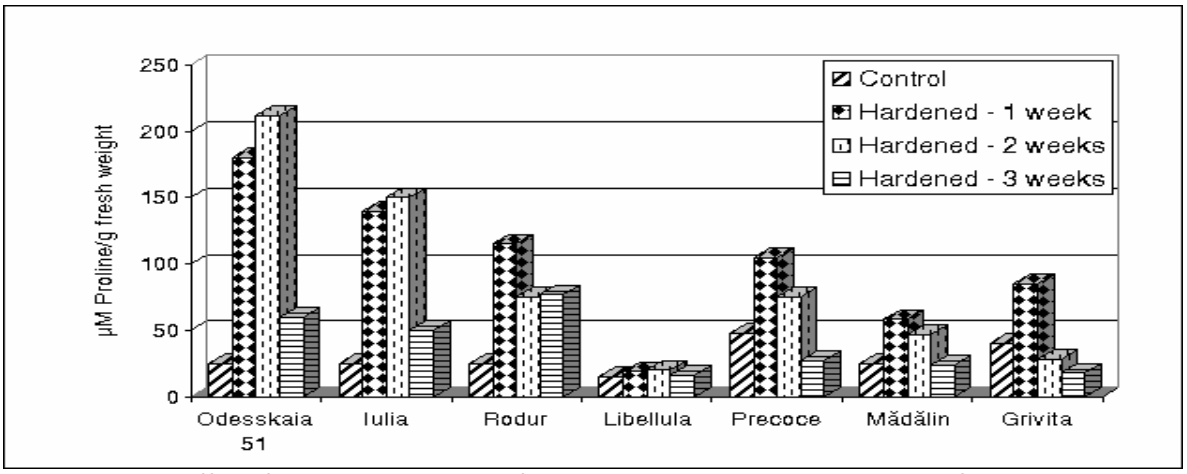

Figure 2. The effect of hardening treatment on free proline content in leaves and collums of winter wheat and barley genotypes

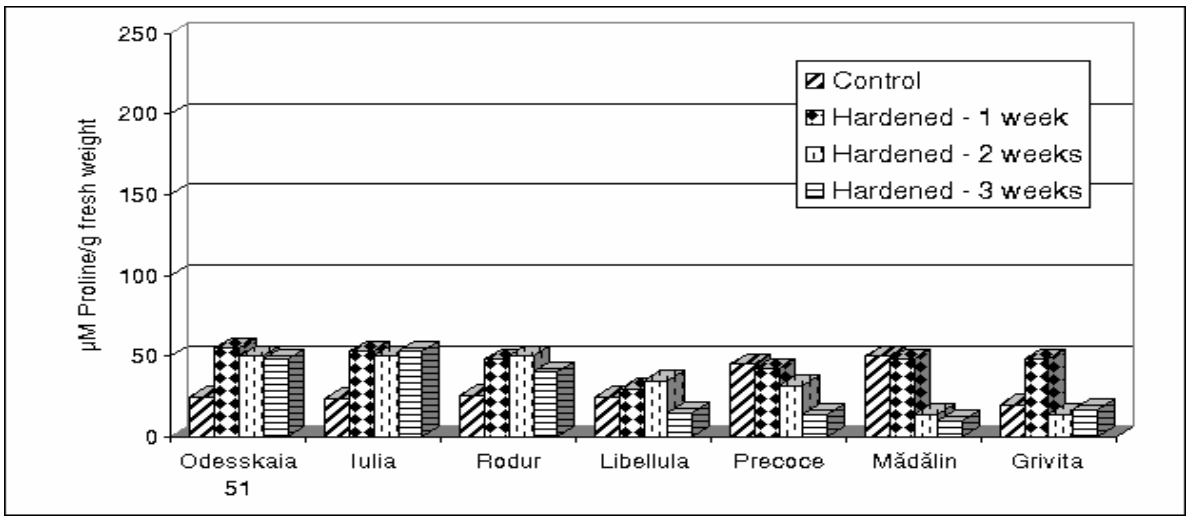

Figure 3 . The effect of hardening treatment on free proline content in roots of winter wheat and barley genotypes

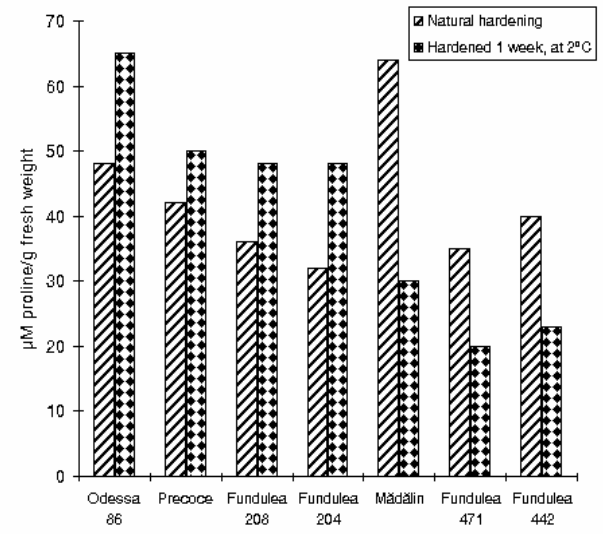

Figure 4. The effect of natural (three months) and controlled hardening (1 week at 2°C) on free proline content in leaves of seven winter barley genotypes

The correlations study has shown that in the first case (natural hardening) there is not any correlation between proline content and frost resistance (r = -10, table 4), whereas for the second case (hardened under controlled conditions) proline content was correlated with frost resistance (r = -0,97***, Figure 5 A and B).

The accumulation of free proline content in wheat plants during hardening is correlated with frost resistance (Machackova et al., 1989; Dorffling et al., 1990) and *in vitro* selection and regeneration of wheat lines with a high proline content determined an increase of frost resistance (Dorffling and Lesselich., 1993). In the same direction, in barley, lines have been obtained with resistance to hydroxiproline whose frost resistance was higher than in check (Tantau et al., 1994).

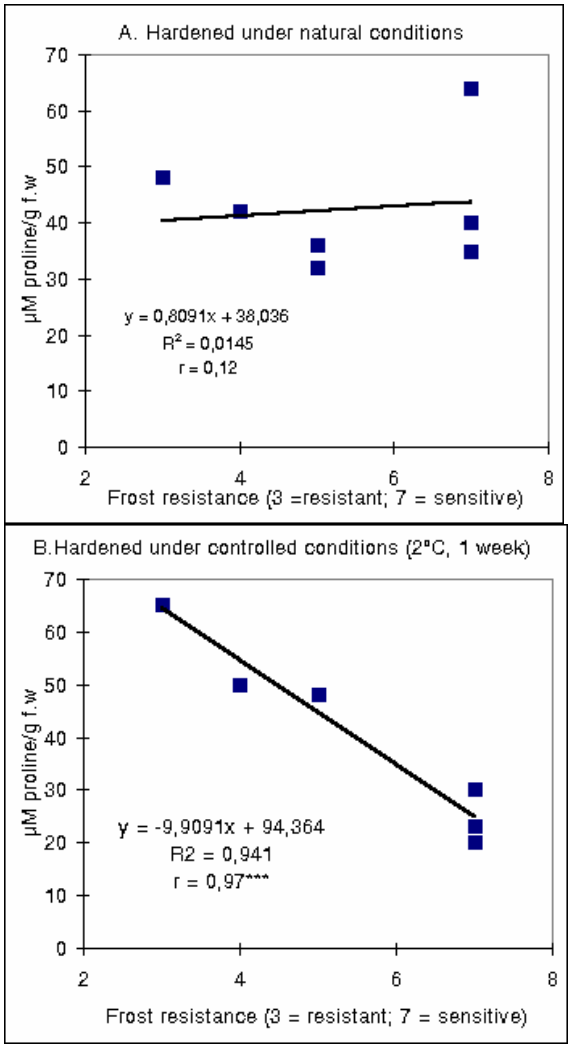

Figure 5. Relationship between frost resistance and free proline content in plants hardened under natural (A) and controlled conditions (B)

In oats these correlation are not clear (Alberdi et al., 1993) while in wheat and barley a decrease of proline content was observed in the case of hardening without light (Petcu and Þerbea, 1993).

CONCLUSIONS

The proline accumulation in winter wheat and barley plants was influenced by genotype and duration of cold hardening. Frost resistant genotypes accumulated more proline in leaves than the susceptible ones.

A significant correlation between frost resistance of some winter barley and proline content in leaves of plants hardened seven days at 2°C and 10 hours photoperiod was found.

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