EVALUATION OF FROST TOLERANCE IN WINTER WHEAT

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ABSTRACT

Changes in membrane stability, free proline content and peroxidase isoenzyme expression, induced by low temperatures and exogenous ABA in some wheat genotypes, were investigated. Both low temperature and exogenous ABA induced larger increases in free proline content and stability of cell membranes in frost resistant wheat genotypes than in frost sensitive ones. Proline levels in hardened plants and in plants treated with ABA were correlated with frost tolerance, expressed as TL_{50}. Low temperatures, more than ABA treatment, determined the increasing of peroxidase isoenzymes activity with medium and high molecular weight. Genotype ranking for frost tolerance in hardened plants at low temperatures in controlled condition was consisted with that recorded in ABA treated plants. Our results suggested that ABA pays an important role in the acclimatization process of wheat and indicated the possibility to use the treatment with ABA for frost tolerance screening.

Key words: ABA, free proline, frost tolerance, membrane stability, peroxidase, winter wheat.

INTRODUCTION

Cold acclimation (hardening) is a complex adaptive process by which plants increase their tolerance to extracellular freezing. This process is induced by exposure of the plants to low but nonfreezing temperatures (Levitt, 1980) and is accompanied by biochemical and structural changes in plant cell (Guy, 1990; Thomashow 1990).

Abscisic acid (ABA) seems to have a specific function in the induction of cold hardening. Exogenous ABA can replace low temperature, and during cold hardening the endogenous free ABA content increases in some genotypes (Chen and Gusta, 1983; Guy, 1990, Dorffling et al., 1990; Ryu and Li 1993).

The purpose of this paper was to compare if changes induced by exogenous ABA on wheat plants (stability of membranes, free proline content and peroxidase isoenzymes expression) are similar to changes induced by low temperature in wheat plants in relation with the frost tolerance of some Romanian wheat genotypes.

MATERIALS AND METHODS

Two sets of near isogenic lines of wheat for *Vrn* genes were used. Both cultivars originate from crosses involving Mexican spring wheat Nadadores 63, which is *Vrn* carrier.

Three contrasting genotypes regarding frost tolerance (Odesskaia 51 as frost resistant, Iulia - medium resistant and Libellula as frost sensitive genotype) were used as control.

The experimental variants were the followings:

- control plants: wheat seeds were germinated in plastic boxes on filter paper at 24-18^{0}C (day/night) with 16 hours photoperiod for 10 days;

- cold hardened plants: ten days old seedlings were hardened at 2°C temperature and 10 hours photoperiod for one, two and three weeks respectively;

- abscisic acid treatment: for the treatment with $2x10^{-4}$M ABA solutions, seedlings (ten days old), obtained under the same conditions as described above for control plants but in pots kept closed, were used.

Cell membrane stability was conductometrically determined, as electrolyte leakage, after freezing treatment and was expressed as index of injury (%). The freezing treatment consisted in gradually exposure of the plants to negative temperature (-8°C, -10°C, -12°C, -14°C, -16°C).

Free proline content was determined after the methods proposed by Bates (1973).

Peroxidase isoenzymes were qualitatively determined by polyacrylamide gel electrophoresis after the method described by Hagima and Moisa (1992).

Frost tolerance of wheat cultivars was expressed as lethal temperature (temperature at which 50% of the population is killed) and was calculated by analysis from conductivity data.

RESULTS AND DISCUSSIONS

Frost resistance potential of wheat genotypes, expressed by lethal temperatures, is shown in table 1. Frost resistance of the wheat genotypes, hardened under controlled condi-

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tions (2°C), was almost consistent with that of the plants treated with ABA. Fundulea 4 (winter form) was more resistant to frost than Lovrin 34. The presence of *Vrn* 3 allele increased the lethal temperature by 4°C in Fundulea 4 for the plants treated with ABA or artificially hardened at low temperatures and by 2°C in Lovrin 34. The effect of the *Vrn* 3 allele is dependent on genetic background and treatment.

Table 1. Frost tolerance of wheat genotypes expressed by lethal temperature (TL_{50})

Genotypes	Plants treated with ABA (TL_{50})	Plant hardened at 2°C (TL_{50})
Odesskaia 51	-7	-18
Fundulea 4	-14.08	-15.89
Lovrin 34	-13.29	-13.82
Iulia	-12	-13.75
Lovrin 34 *Vrn*	-11.78	-11.68
Fundulea 4 *Vrn*	-10.69	-11.41
Libellula	-10.50	-12

Cell membrane stability at low temperatures increased progressively during the period of hardening and, also, after ABA treatment for all wheat genotypes. Differences among genotypes were recorded: frost resistant genotypes (Odesskaia 51, Fundulea 4, Lovrin 34) had a higher stability of membranes after freezing treatment than the frost sensitive ones (Libellula, Fundulea 4 Vrn, Lovrin 34 Vrn) (Figures 1-3).

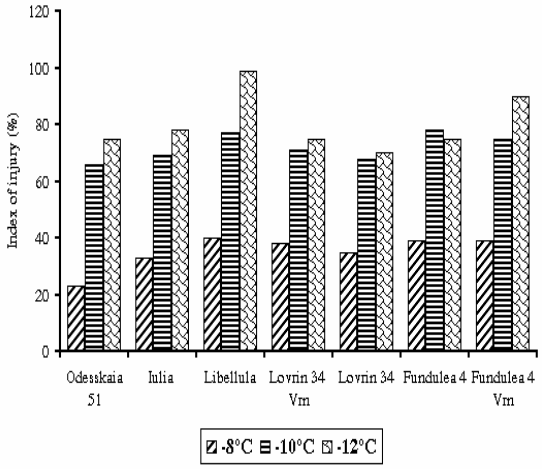

Figure 1. Influence of freezing treatment on cell membrane stability of non-hardened wheat plants

A similar effect after ABA treatment of winter calli was obtained by Papenbrock and Dorffling (1994). It is known that cell membranes lose their selective semipermeability during freezing treatment, which destroys the cells (Levitt, 1980).

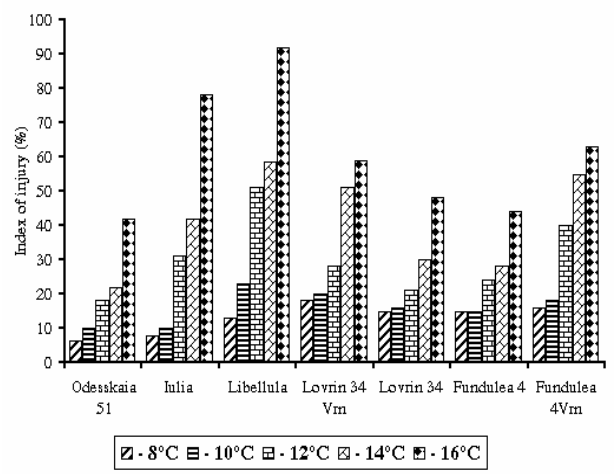

Figure 2. Effect of cold acclimation (2°C) on cell membrane stability of wheat genotypes

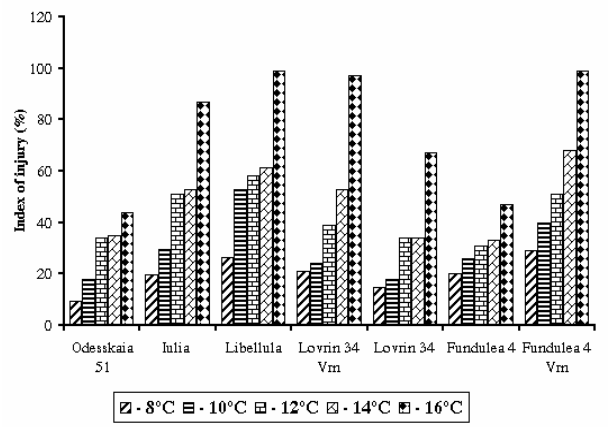

Figure 3. Effect of exogenous ABA on cell membrane stability of wheat genotypes

In control plants, free proline content was lower and the differences between genotypes were not significant. The ABA treatment and hardening of plants at low temperature lead to an increase in proline content in all genotypes. Our results show that the changes were faster and more pronounced in frost resistant genotypes (winter form) than in sensitive ones (spring form) (Figure 4).

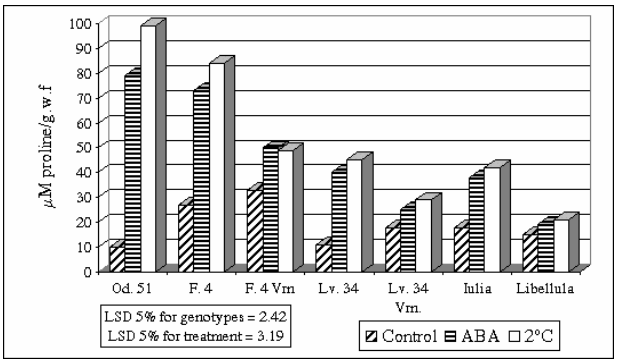

Figure 4. Free proline content of wheat plants after different treatments: non hardened, ABA and hardened at low temperatures

Data obtained for stability of membranes, free proline content and frost resistance of cultivars mentioned above, in all treatments studied, were highly and significantly correlated (Table 2).

Table 2. The correlation coefficients between frost resistance of 2°C hardened wheat and frost resistance of wheat plants treated with ABA, free proline content of cold hardened plants or ABA treated

Specification	Frost resistance of wheat plants hardened at 2°C
Frost resistance of wheat plants treated with ABA	r= 0.95***
Proline content of wheat plants hardened at 2°C	r= -0.90**
Proline content of wheat plants after ABA treatment	r= -0.82*

Differences in frost tolerance between Fundulea 4 and Lovrin 34 could be also associated with peroxidase isoenzyme patterns. Hardening induced two peroxidase isoenzymes with high molecular weight (which migrated slowly trough the gel) in Fundulea 4 and only one in Lovrin 34. These isoenzymes persisted after freezing treatment but their activity was reduced for Fundulea 4.

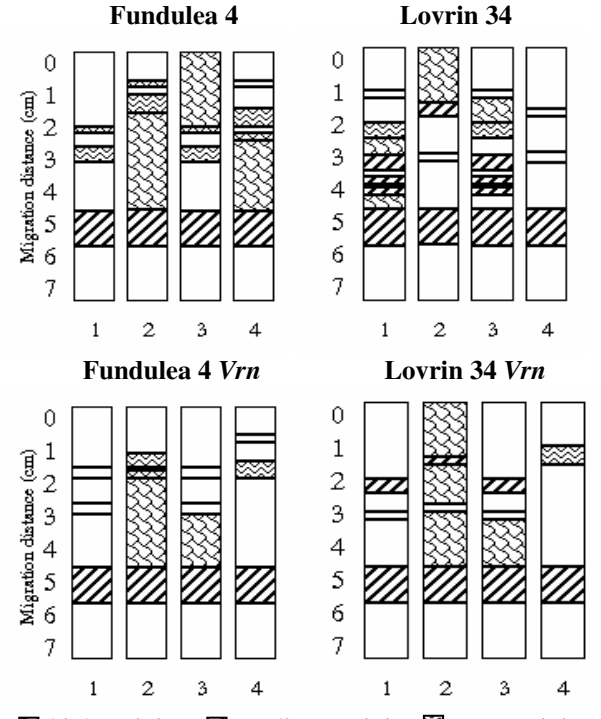

Figure 5. Peroxidase isoenzyme patterns from non-hardened plants (1), hardened plants (2), plants treated with ABA (3) and freezing exposed plants (4) in wheat cultivars

The peroxidase isoenzyme pattern of plants treated with ABA was compared with control plant, differences in intensity of peroxidase activity - higher in plants treated with ABA - being observed (Figure 5).

CONCLUSIONS

The data obtained suggested that both low temperatures and exogenous abscisic acid (ABA) induced some similar changes in wheat plants concerning proline accumulation and cell membrane stability.

The high significant coefficients of correlation between free proline content of plants and 50% of lethal temperature (TL_{50}) in the ABA treatment and the same parameters in the plants hardened at low temperatures, showed that ABA plays an important role in cold hardening process of wheat and suggested the possibility to use ABA treatment as a rapid method for frost resistance screening of winter wheat breeding material.

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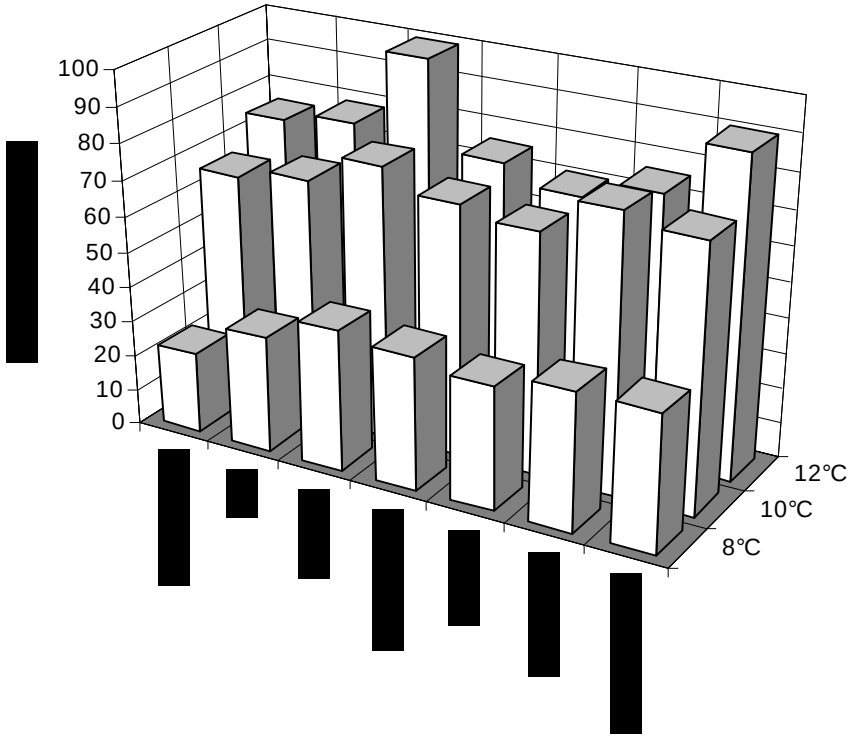

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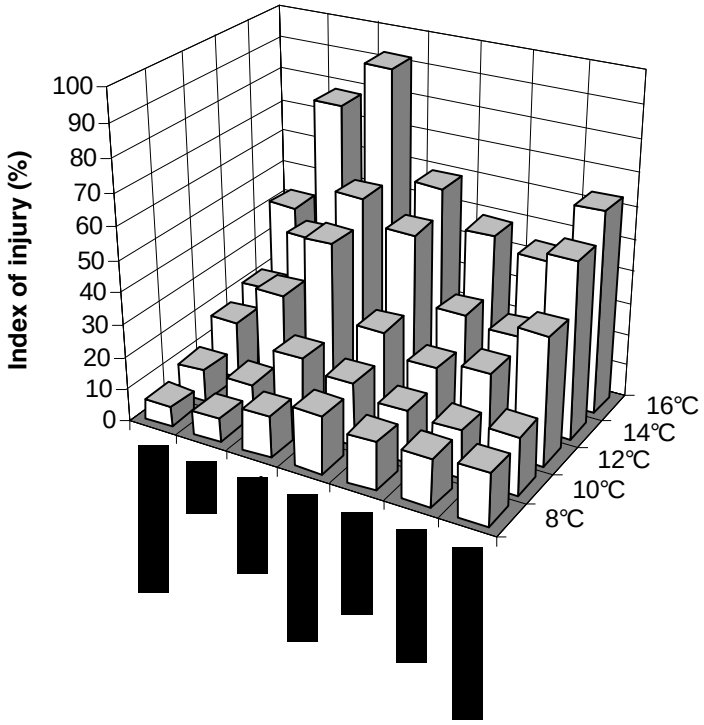

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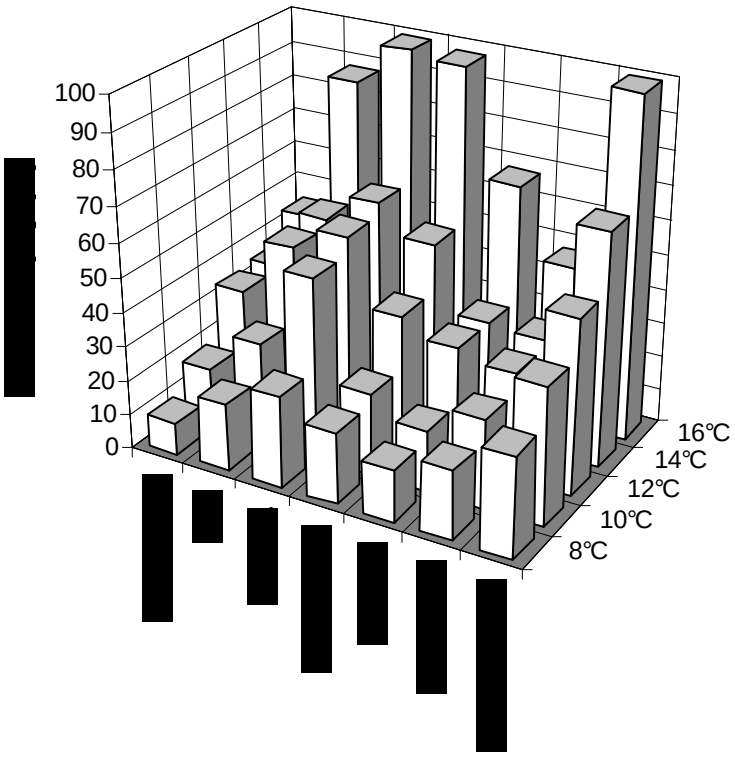

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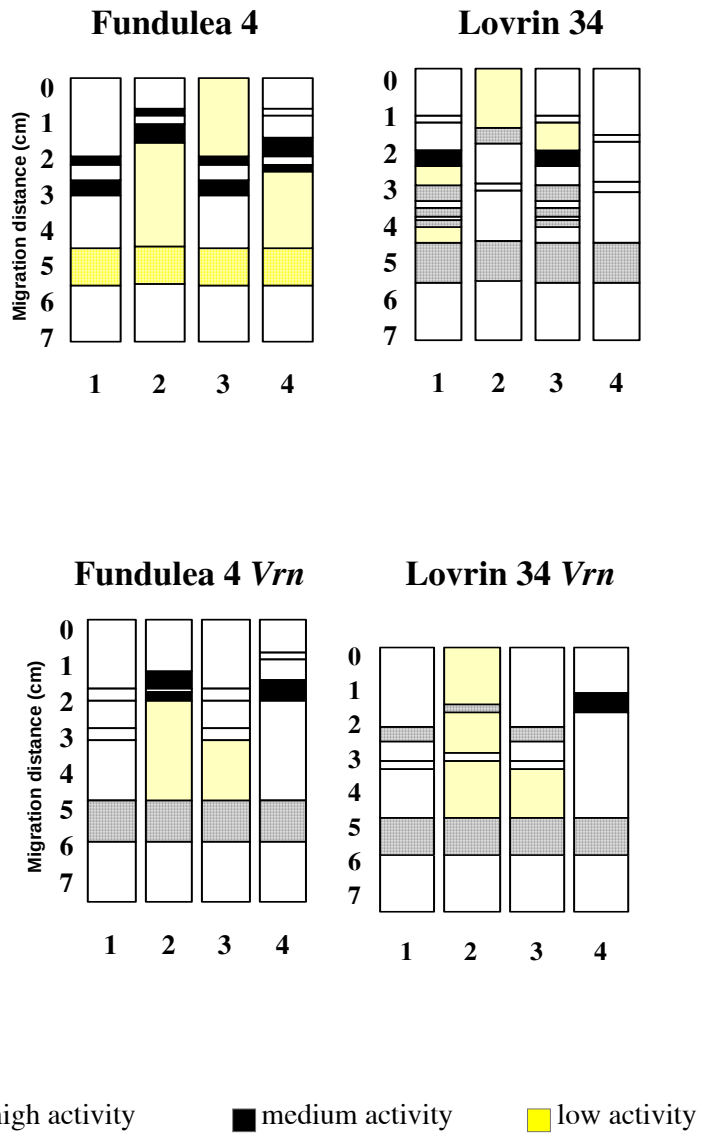

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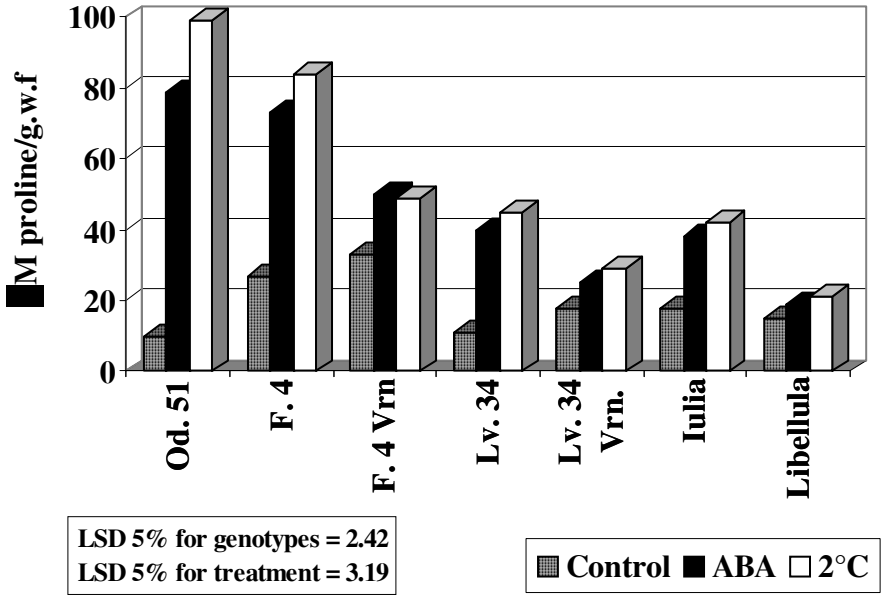

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