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BRIEF COMMUNICATION

Determination of frost tolerance in winter wheat and barley at the seedling stage

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Abstract

Detailed studies were made on changes in the quantity of 1.4 MDa rRNA precursor in barley and wheat cultivars with different degrees of frost tolerance. When analysing genotypes with different LT_{50} values a close negative correlation was found between the quantity of 1.4 MDa molecular mass rRNA precursor and the frost tolerance of the given barley or wheat cultivar. The results suggest that a technique based on low temperature phosphorus incubation at the seedling stage could be suitable for the selection of genotypes on the basis of this character in applied research.

Additional key words: DNA, *Hordeum vulgare*, RNA precursor, rRNA, *Triticum aestivum*.

Winter cereals, especially wheat and barley, are among the most important crops grown in Europe. One fundamental criterion for their reliable production is frost tolerance. The agronomic tests applied to date are reliable, but time- and cost-consuming (Andrews 1958, Veisz and Sutka 1989, Tischner *et al.* 1997). Based on previous results achieved concerning the role of nucleic acids in the development of frost resistance (Páldi and Dévay 1977a, 1983, Páldi *et al.* 1996), a method has been elaborated with which the frost tolerance of winter wheat and barley genotypes can be determined at the seedling stage.

Six winter barley (*Hordeum vulgare* L.) cultivars (Hohenturm, Martonvásári 34, Martonvásári 35, Béta 2 soros, Montpellier and Ager III), two spring barley cultivars (Mk 42 and Mk 47), ten winter wheat (*Triticum aestivum* L.) cultivars (Cheyenne, Norstar, Bezostaya 1, Mironovskaya 808, Rannyaya 12, Cappelle Desprez, Bánkuti 1201, Moisson, Libellula and Bersé), two chromosome substitution lines (CS/Ch5A and CS/Ch7A), two winter wheat lines (Mv 11-75 and Mv 13-74) and five spring wheat cultivars (Chinese Spring, Penjamo 62, Siete Cerros, Lutescens 62 and Super X) with different degrees of frost tolerance were used in the experiment. The two winter wheat lines were developed after nearly 10 years of selection from a population obtained by crossing the cv. Bezostaya 1, which has excellent frost tolerance, and cv. Moisson, which has poor frost tolerance. The two lines differ only as regards the degree of frost tolerance. The seeds were germinated under sterile conditions on simple medium with 1 % agar and 2 % sucrose at 20 °C for 72 h in darkness. The intact seedlings were incubated for 24 h at 1 °C with their root tips resting in 25 cm^3 1000-fold diluted Knop solution per 50 seedlings, containing 18.5 MBq $^{32}P_i$.

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Abbreviations: LT - lethal temperature; PAS - sodium 4-aminosalicylate; P_i - inorganic phosphate; rRNA - ribosomal ribonucleic acid; SDS - sodium lauryl sulphate.

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The chemicals used in the experiment were of analytical grade and were purchased from *Sigma-Aldrich* (Budapest, Hungary). Radioactive potassium orthophosphate was purchased from the *Radiochemical Centre* (Amersham, UK).

The wheat and barley seedlings were homogenised in buffered sucrose medium (Loening 1967) and the homogenate was centrifuged at 1 000 *g* for 5 min. This sedimented the cell debris and nuclear material and left the bulk of the cytoplasmic fractions in suspension. The debris fraction was resuspended in the same medium. SDS (0.5 %) and PAS (5 %, m/v) were added to both fractions (Kirby 1968) and the suspensions were shaken with phenol containing 0.1 % (m/v) 8-hydroxyquinoline at 5 °C. The phases were separated by centrifugation at 1 000 *g* for 10 min and the phenol extraction was repeated once or twice. The RNA was precipitated from the final supernatant by the addition of 2 % (m/v) sodium acetate and 2.5 vol. of ethanol at -20 °C. The final RNA precipitate was reprecipitated twice from 0.3 M sodium acetate, washed once with ethanol and partially dried for a few minutes *in vacuo*. The DNA-free rRNA, prepared according to Wells and Ingle (1970), was dissolved in electrophoresis buffer containing 5 % (m/v) sucrose to give a final concentration between 0.5 and 2.0 mg cm^{-3}. The purified RNA was fractionated by electrophoresis on 2.4 % polyacrylamide gel (50 V for 3.5 h). The distribution of radioactivity was determined by freezing the gels in dry ice prior to cutting 0.5 mm slices, which were dried on filter paper. The slices were counted in an *Intertechnique SL 30* (Saclay, France) liquid scintillation spectrometer (Loening 1967, 1969).

For the determination of LT_{50} values 2-week-old wheat and barley plants were frozen at 6 freezing temperatures in computer-controlled phytotron units and grown for a further 2 weeks after thawing at 15 °C with 16 h illumination. Survival percentages were then calculated. The temperature at which 50 % of the plants survived was taken as the critical temperature (Dévay and Páldi 1983, Tischner *et al.* 1997).

Previous results (Páldi and Dévay 1977b, 1983, Páldi *et al.* 1996) suggested a negative correlation between the quantity of 1.4 MDa rRNA precursor and the frost tolerance of wheat and barley genotypes, characterized by the LT_{50}. The 1.4 MDa rRNA precursor was chosen, as it is stable and easily demonstrated. The results of experiments carried out on 19 wheat genotypes and 8 barley cultivars with different degrees of frost tolerance confirmed this suggestion (Tables 1, 2). It was found that in winter wheat genotypes with good frost tolerance no 1.4 MDa rRNA precursor could be observed during incubation with $^{32}P_i$ at 1 °C. As the degree of frost tolerance, *i.e.* the LT_{50} value, decreased, the quantity of this precursor increased (correlation coefficient -0.91). This change was the most pronounced in frost-sensitive spring cultivars. A similar tendency was observed for winter and spring barley cultivars (correlation coefficient -0.89).

Table 1. Effect of low temperature on the quantity of 1.4 MDa rRNA precursor in wheat cultivars characterized by incorporation of $^{32}P_i$ into the 1.4 MDa rRNA precursor at 1 °C for 24 h in the dark. Data are the means of five experiments (n.d. - not determined, * - could not be determined).

	Cultivar	Incorporation of $^{32}P_i$ [counts s^{-1}]	LT_{50} [°C]
Winter wheats	Cheyenne	*	-18.4
	Mv 11-75	*	-16.8
	Norstar	*	-16.7
	Bezostaya 1	*	-16.2
	Mironovskaya 808	*	-16.0
	Rannyaya 12	*	-15.8
	Bánkuti 1201	14	-12.8
	CS(Ch5A)	17	n.d.
	Libellula	30	-10.7
	Cappelle Desprez	34	-8.6
	Moisson	44	-7.5
	Mv 13-74	53	-6.1
	CS(Ch7A)	55	n.d.
	Bersé	81	-4.8
Spring wheats	Penjamo 62	87	-4.1
	Siete Cerros	105	-3.0
	Chinese Spring	104	-2.9
	Lutescens 62	101	-2.8
	Super X	99	-2.8
$LSD_{0.01}$		9.3	1.19
$LSD_{0.05}$		6.8	0.86

Table 2. Effect of low temperature on the quantity of 1.4 MDa rRNA precursor in barley cultivars characterized by incorporation of $^{32}P_i$ into the 1.4 MDa rRNA precursor at 1 °C for 24 h in the dark. Data are the means of five experiments (* - could not be determined).

	Cultivar	Incorporation of $^{32}P_i$ [counts s^{-1}]	LT_{50} [°C]
Winter barleys	Hohenturm	*	-16.8
	Martonvásári 35	*	-15.6
	Martonvásári 34	*	-13.7
	Béta 2 soros	12	-12.6
	Montpellier	25	-10.3
	Ager III	46	-5.8
Spring barleys	Mk 42	68	-6.3
	Mk 47	69	-6.0
$LSD_{0.01}$		8.4	2.14
$LSD_{0.05}$		6.0	1.54

In frost-sensitive cultivars the increase in the quantity of 1.4 MDa rRNA precursor resulted primarily from the inhibition of maturation of the ribosomes, especially in the final nuclease step. The simplest interpretation of the results is to assume that frost-resistant wheat and barley cultivars are characterized by a lack of disturbance of rRNA synthesis and of the maturation processes of the ribosomes at low temperature. This is manifested by greater synthesis of the heavy (1.3 MDa) and light (0.7 MDa) cytoplasmic rRNAs (Páldi *et al.* 1996).

The results achieved using genotypes specially developed for the testing of frost tolerance indicate that the quantitative determination of 1.4 MDa rRNA could serve as a molecular biological method for the laboratory testing of frost tolerance. This technique could be of use not only in theoretical research on frost tolerance, but also in practice, since it can be carried out in a relatively short time.

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