IN VITRO SCREENING FOR FROST RESISTANCE OF SOME WINTER WHEAT GENOTYPES (*TRITICUM AESTIVUM* L.)

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ABSTRACT

In *vitro* response to low temperature of some winter wheat cultivars, respresentative for the germplasm used in wheat breeding in Romania, was investigated by measuring fresh weight of calli before and after freezing. Fresh weight of calli was significantly reduced by low temperature in all studied genotypes. Less winterhardy cultivars had higher callus weight at normal temperature and larger reduction after exposure to low temperature. However, correletion between calli weight after freezing or the ratio of weights with and without stress, and winterhardiness, as measured by necrosis index in a direct plant freezing test, was not significant. This suggests that *in vitro* response of calli weight to low temperature does not completely describe genetic differences in winterhardiness.

Key words: weight of callus, *Triticum aestivum*, frost tolerance

INTRODUCTION

The behaviour of the wheat varieties at the low temperatures during the winter is an essential element, with direct implications in obtaining high and stable yields. The improvement of the genetic background that controls winterhardiness remains the main way for obtaining plants which are able to produce high yields even under unfavourable conditions.

In vitro methods can be useful in assisting breeding programmes directed towards improving winterhardiness. Adding hydroxiproline as a constituent of in vitro-media, proline over-producing variants of several spring-wheat varieties were selected, which contained increased levels of free proline and proved to be more frost tolerant than the initial material (Tantau and Dorffling, 1991). Using *in vitro* techniques, it was possible to regenerate hydroxy-proline winter wheat plants with an increased mean proline content and a higher frost tolerance (Dorffling et. al., 1993).

Research on the direct effect of low temperatures on *in vitro* grown calli is less extensive. Kendall et al. (1990) obtained frost tolerant plants by cryoselection of callus in spring wheat. However it is not clear how much of the genetic differences in winterhardiness are due to mechanisms that also control the freezing tolerance of calli.

This investigation was carried out to evaluate the relationship between the *in vitro* reaction to low temperature of calli, obtained from winter wheat cultivars used in breeding programes in Romania, and winterhardiness as evaluated in direct freezing tests.

MATERIALS AND METHODS

Five winter wheat cultivars of different winterhardiness were chosen for this study. The winterhardiness of these cultivars was previously characterized by scoring the necrosis index following a direct freezing test, by Petcu and Ţerbea (1995) (Table 1).

Table 1. The necrosis index and winterhardiness classes of some winter wheat cultivars (naturally hardened and exposed to - 10^{0}C)

Genotype	Necrosis index	Class of resistance
Dropia	0.74	Resistant
Fundulea 29	0.75	Resistant
AF 93-2	0.80	Resistant
Lovrin 24	2.50	Sensitive
Libellula	3.20	Sensitive

Immature embryos (1.5 - 2 mm length) were placed on Murashige - Skoog medium, supplemented with 2 mg/l 2,4 D and 0,5 mg/l kinetine to obtain calli.

Calli cultures were grown at 28^{0}C and dark for four weeks, and after that transferred (one callus/tube) on the same medium and hardened for 14 days at low temperature (2^{0}C, for 4 days).

After hardening the calli cultures were exposed to freezing treatment, by reducing the temperature with 2^{0}C/hour to - 8^{0}C, where they were kept for 18 hours.

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The reaction to this low temperature was estimated by measuring the callus weight after the treatment. The results obtained in many crops (wheat, triticale - Mayer and Ottler, 1992; sunflower - Dozet and Vasic, 1995) confirm the hypothesis that callus weight might be an adequate parameter for describing the response to stress factors for *in vitro* experiments.

Calli growth response to freezing temperatures was expressed as percentage from control.

The analysis of variance and correlation analysis were used to evaluate the experimental results.

RESULTS AND DISCUSSIONS

ANOVA regarding the *in vitro* response to low temperature shows a strong and significant effect of temperature on callus weight. Cultivars were not significantly different in average over the two temperature treatments, as shown by the small and non-significant effect of cultivars, but the interaction between cultivar and temeprature is significant.

This result suggests that the response to low temeprature was dependent on cultivar, the effect of freezing being different on calli obtained from different cultivars (Table 2).

Table 2. ANOVA for the weight of calli of 5 winter wheat cultivars exposed to low temperature

Source of variance	SS	DF	MS	F-value
Temperature (A)	3.0920	1	3.0920	118.480**
Error A	1.5939	24	0.0664	
Cultivar (B)	0.1080	4	0.0270	0.4067
Interaction AxB	0.5935	4	0.1484	5.685**
Error B	0.7829	30	0.0261	

Cultivars had significantly different weight of calli at the control treatment (in the absence of freezing). The highest weight of calli was recorded in cultivars Libellula and Lovrin 24, both known as less winterhardy (Table 3). Significant differences were also recorded between cultivars for the weight of calli after the freezing treatment. The highest callus weight after freezing was recorded in cultivars Dropia and AF 93-2, both classified as winterhardy.

Table 3. Average weight of calli obtained from winter wheat immature embryos with and without exposure to low temperature

Cultivar	Control		Low temperature		Difference	
	(g)	%	(g)	%	(g)	%
Dropia	0.757 b	100	0.534 c	70.54	0.223*	29.46
Fundulea 29	0.901 a	100	0.482 cd	53.49	0.419***	46.51
AF 93-2	0.711 b	100	0.501 c	70.46	0.210*	29.54
Lovrin 24	1.001 a	100	0.338 f	33.76	0.663***	66.24
Libellula	1.014 a	100	0.427 de	42.11	0.587***	57.89
Average	0.876	100	0.456	54.07	0.426***	45.93

Values followed by the same letter are nost significantly different.
*,***) Significantly different from control for P<0.05 and P<0.001

On an average, freezing caused a reduction in the calli weight of more than 45%. In the winterhardy cultivars Dropia and AF 93-2 the callus weight after freezing still represented over 70% of the callus weight in the control treatment. In contrast, the callus weight after freezing in susceptible cultivars Lovrin 24 and Libellula represented less than 40% of the control weight.

Correlation analysis suggests that the ratio between the weight of treated and control calli was equally influenced by genotypic weight variation with and without the freezing stress (Table 4).

Table 4. Correlation between the parameters describing *in vitro* growth and winterhardiness described by the necrosis index after a plant freezing test

Specification	Necrosis index	Weight of control calli	Weight of treated calli
Weight of control calli	+0.84		
Weight of treated calli	-0.77	-0.82	
Ratio weight of control calli/ treated calli	-0.83	-0.97***	+0.94***

***) significant at P<0.001

As expected, the ratio is negatively correlated with the weight without stress (Figure 1) and positively with the weight after freezing (Figure 2). There is an obvious trend of calli which grow better under normal conditions to suffer a larger weight reduction after freezing. However, because of the small number of cultivars tested, the correlation is not significant.

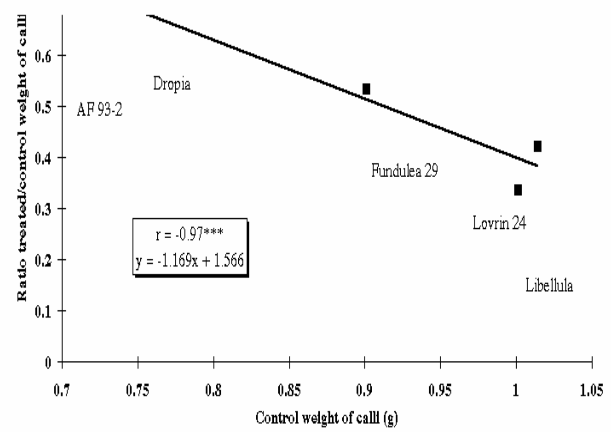

Figure 1. Relationship between weight of control calli and ratio treated/control weight of calli at some winter wheat genotypes

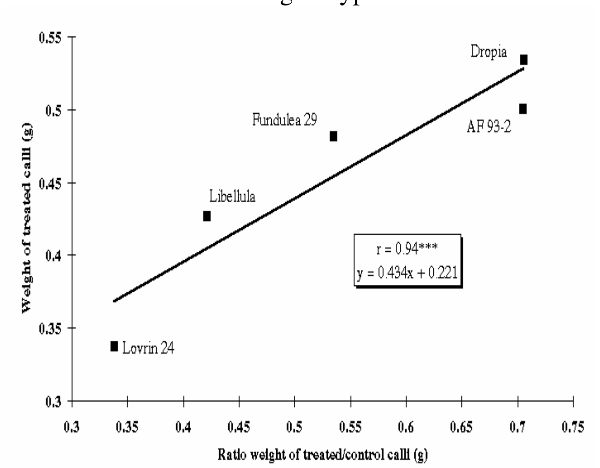

Figure 2. Relationship between ratio of treated/control calli and weight of treated calli at studied genotypes

Necrosis index determined by a direct frezing test in plants is not significantly correlated with any of the parameters obtained *in vitro*. The smallest correlation was obtained between necrosis index and the weight of calli after freezing. This could cast some doubt about the efficiency of cryoselection in the germplasm represented by the studied cultivars.

Our results suggest that genotypic differences in winterhardiness are only partially manifested *in vitro* as a differential response in callus weight after exposure to freezing temperatures. Only large differences in winterhardiness, such as those between susceptible Libellula and resistant Dropia might be detected at the level of calli weight after freezing, while other smaller differences might be due to mechanisms that are not working at the callus level.

The possible association between callus growth potential at normal temperatures and response to low temeprature deserves further attention, as it might interfere with detection of "true" response to freezing.

CONCLUSIONS

Wheat cultivars were different in their callus weight response to freezing temperatures. Less winterhardy cultivars had higher callus weight at normal temeprature and larger reduction after exposure to low temperature.

However, correlation between calli weight after freezing or the ratio of weights with and without stress, and winterhardiness, as measured by necrosis index in a direct plant freezing test, was not significant. This suggests that *in vitro* response of calli weight to low temperature does not completely describe genetic differences in winterhardiness.

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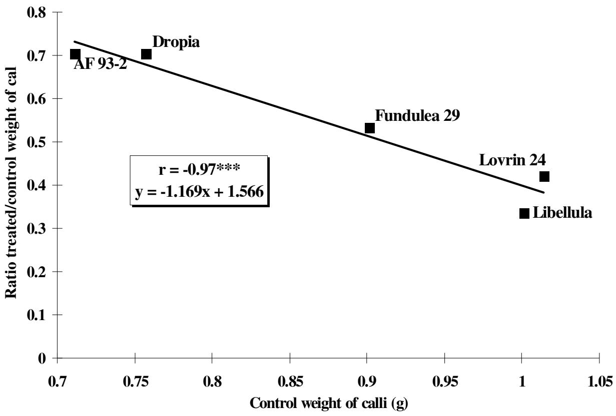

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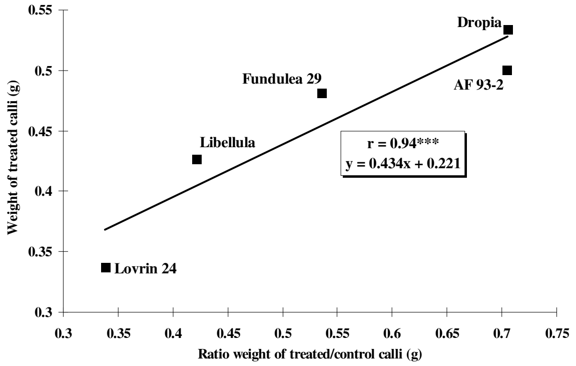

Figure 2. Relationship between ratio of treated/control calli and weight of treated calli at studied genotypes