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Somaclonal variation for freezing tolerance in a population derived from norstar winter wheat*

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Summary. Progeny of 66 plants regenerated from callus cultures derived from immature embryos of Norstar winter wheat were evaluated as seedlings for tolerance to controlled freezing. Greater freezing tolerance than the parent cultivar was observed in both R_2 and R_3 regenerate families. LT_{50} values (predicted temperatures at which mean survival frequencies are 50%) for four families in the R_2 generation and three families in the R_3 were significantly lower than that of Norstar. In both R_2 and R_3 generations, most families did not differ significantly from the cultivar Norstar, by three separate measures of tolerance. Significant variation among families was observed in both R_2 and R_3 generations for survival, but not for plant height. Variation within family in the R_3 generation was also significant, though smaller than that among families. In the R_3 generation, eighteen families were significantly less freezing tolerant than Norstar according to LT_{50}, while thirteen were significantly less tolerant according to survival at a minimum temperature of −17 °C.

Key words: *Triticum aestivum* – Winter wheat – Tissue culture – Somaclonal variation – Freezing tolerance

Introduction

Winter wheat (*Triticum aestivum* L.) in comparison to spring wheat, makes more efficient use of early spring moisture, matures earlier, yields more, reduces weed competition and decreases soil erosion (Gusta and Fowler 1979). However, it has not been adopted widely in the northern plains due to the risk of winter kill, primarily as a result of low temperature damage to the crown (Chen et al. 1983). Further, lack of sufficient genetic variability for winter survival, difficulty in early identification of winter hardiness, and limited understanding of interactions between genotype and environment have made breeding progress difficult (Fowler and Gusta 1979; Grafuis 1981; Limin and Fowler 1983).

In a recent review, Limin and Fowler (1983) concluded that "the most hardy wheat cultivars available today contain most of the major cold hardiness genes available in the winter wheat gene pool. Therefore, only limited improvement may be possible through the use of traditional breeding methods." Related species, including members of *Secale, Agropyron* and *Triticum*, are a potential source of genetic variability for winter hardiness (Grafius 1981; Limin and Fowler 1981, 1982). Also, it has been suggested that epistatic effects generated in some winter × spring hybrids may result in improvement (Limin and Fowler 1983). However, even allowing that relatively small incremental improvements in low temperature tolerance (e.g. 2° to 3 °C) would be economically important, no encouraging results have yet been obtained from wide hybridization.

It has been recently observed that heritable variation is frequently obtained upon regeneration of plants from many forms of tissue, cell and organ culture (Larkin and Scowcroft 1981). Wheat has been one of

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the principal subjects for investigation of this phenomenon, and several laboratories have reported the occurrence of positive agronomic characteristics among regenerated progeny (Larkin et al. 1984; Ahloowalia and Sherington 1985; Maddock et al. 1983; Chen et al. 1987).

In this study, a population of selfed progeny was generated from immature embryo-derived callus culture of Norstar winter wheat (Chen et al. 1987) as Norstar is one of the most winter hardy, elite quality North American wheat cultivars and the progeny was screened for low temperature tolerance employing controlled freezing tests.

Materials and methods

Donor plants

Winter wheat (*Triticum aestivum* L.), cv. Norstar, was grown from seed in 6-inch pots, one plant per pot, in a greenhouse, maintained between 22° and 27 °C, with a 16-h photoperiod for two weeks. All plants were vernalized at 4 °C for six to seven weeks with 8-h photoperiod and light intensity of 12 Wm^{-2} and grown to maturity under the indicated greenhouse conditions. Plants were fertilized weekly with 20:20:20 (N:P:K). Tillers were tagged at anthesis, and embryos were excised for culture 13 days later.

Tissue culture and regeneration

Harvested kernels were surface sterilized by immersion in 70% (v/v) ethanol for 1 min., followed by 20% (v/v) commercial bleach (approx. 1% sodium hypochlorite) for 15 min., and five rinses with sterile, distilled water. Embryos were aseptically excised and placed with the scutellar tissue against a medium after Sears and Deckard (1982). Embryos which germinated were discarded. Calli which developed were transferred after three to four weeks to the same medium containing half the concentration of 2,4-D (0.5 mgl^{-1}, instead of 1.0 mgl^{-1}). Throughout the culture period calli were maintained in a controlled environment chamber at 26 °C with a 16-h photoperiod and light intensity of 40 Wm^{-2}. For plant regeneration, calli were transferred after three to four weeks to the same medium lacking 2,4-D. Regenerated plantlets were removed from the culture at the 2–3 leaf stage and planted in soil, but maintained at high humidity (> 90% rh) for about two weeks. Plants were vernalized at 4 °C for six to seven weeks with 8-h photoperiod and light intensity of 12 Wm^{-2} and grown to maturity under the greenhouse conditions noted above. Seed was harvested from each individual tiller. Regenerate plants are referred to here as the R_1 generation after the notation of Yurkova et al. (1982). Seed produced from the R_1 population is referred to as R_2 seed.

Generation of the R_3 population

Six R_2 seeds from each R_1 tiller were germinated in test tubes (16 mm × 150 mm) containing vermiculite and water. Seed from tillers which did not produce more than six seeds were not included in the experiment. After one week of growth, the seedlings were placed in the vernalizer on April 26, 1985. Vernalized seedlings were transplanted to the field in space-planted head rows, unreplicated, along with 12 rows of identically treated Norstar seedlings, at the University of Alberta experimental farm, Edmonton, Alberta, on June 3, 1985. R_2 families (derivatives of single regenerate plants) were placed at random throughout the field. R_3 seed were collected from these plants.

Hardening and freezing

One hundred forty seeds of each line were germinated on filter paper, and the seedlings were transplanted after three days into soil:peat:vermiculite (3:2:2). Plants were grown for two weeks in a controlled environment chamber maintained at 20/15 °C with a 12-h photoperiod at 160 Wm^{-2} (day/night). These plants were cold hardened for two weeks at 2 °C with an 8 h photoperiod of 12 Wm^{-2}. Crowns were prepared for freezing by excising the roots and all the leaf tissue 5 cm above the base of the crown. The procedures for evaluating freezing resistance were as described by Chen et al. (1983), employing a cooling rate of 3 °Chr^{-1} to a final temperature of −29 °C. Following thawing at 4 °C, the crowns were planted in flats in the soil mix noted above. Survival was determined after three weeks growth at 20–25 °C with a 16 h photoperiod of 100 Wm^{-2}.

Experimental design, data collection and analyses

R_2 families were sampled by inclusion of equal numbers of seeds from each R_1 tiller producing at least 30 viable seeds. R_3 lines within families were the progeny of the six randomly selected R_2 seedlings grown in the field, as noted above. Sampling within lines was done by including equal numbers of seeds from each plant producing sufficient seed so that the total was greater than 200 viable seeds. This was the case for only eight families, including the Norstar control.

R_2 families were compared in a randomized block design, with freezing temperature as blocking factor, and variable numbers of observations (R_1 tillers) per family. For the R_3 generation, the experimental design was the same, except that lines were nested within families, and the maximum number of observations per line was 6. For both generations, family was considered a fixed effect. The nested effect, line within family, in the R_3 generation, was considered random. In both generations, individual plants were considered random observations. Missing values, or missing cells, in the case of the R_3, occurred as a result of R_2 plants failing to reach maturity.

Data collected after three weeks of regrowth following freezing included: (1) number of crowns surviving of the total planted from each temperature treatment, and (2) plant height from surviving crowns. Plant height was measured as distance from soil level to the tip of the longest leaf. Percent survival data were transformed by arcsin ($x^{1/2}$) prior to statistical treatment, while plant height data were logarithmically transformed. Pearson correlation coefficients for measures of freezing tolerance were computed for families in the R_2 and R_3 generations. For each test line, survival vs. temperature data were fit to regression equation by the method of least squares. For each family, a linear model explains at least 80% of the variation ($R^2 \geqq 0.8$), and quadratic or square root model in no case explain significantly more variation than linear model. The LT_{50} value was predicted from the regression equation as the temperature at which 50% survival should occur. As LT_{50}'s are negative values, lines with < Norstar are considered superior in performance.

Results

Freezing tolerance in these experiments was measured as (1) LT_{50}; (2) percent survival at −17 °C; and (3)

Table 1. Freezing tolerance of R_2 and R_3 families derived from Norstar winter wheat compared to Norstar

Generation	No. of families	LT (50)	Height[d]	Survival at −17 °C
R_2	> Norstar[a]	17	0	2
	= Norstar[b]	45	66	55
	< Norstar[c]	4	0	9
R_3	> Norstar[a]	18	0	0
	= Norstar[b]	44	65	52
	< Norstar[c]	3	0	13

[a] Values significantly greater ($P=0.05$) than those of the Norstar control
[b] Values are not significantly different from those of the Norstar control
[c] Values significantly smaller ($P=0.05$) than those of the Norstar control
[d] Height of plants 21 days after freezing treatment

Table 2. Analyses of variance for freezing tolerance in R_2 and R_3 generations derived from Norstar wheat

Generation	Source	df	Mean Square	
			Height[a]	Survival[b]
R_2	Family (F)	65	0.52	0.11**
	Temperature (T)	9	115.51**	33.01**
	F×T	585	0.45	0.04
R_3	F	64	0.79	0.29**
	T	9	97.22**	30.28**
	F×T	576	0.71	0.08

** F-test significance at $P=0.01$
[a] Plant height 21 days after freezing treatment. Data were logarithmically transformed
[b] Percentage data were transformed by Arcsin ($x^{1/2}$)

Table 3. Analysis of variance for freezing tolerance among and within R_3 families derived from Norstar wheat

Source	df	Mean square[a]
Family (F)		
among	7	0.37**
within	32	0.11**
Temperature (T)	6	18.44**
F×T		
among	42	0.07**
within	192	0.04

** F-test significant at $P=0.01$
[a] Percentage data for survival were transformed by Arcsin ($x^{1/2}$)

height of surviving plants after three weeks of regrowth. In both R_2 and R_3 generations following freezing, no families differed significantly from Norstar for plant height (Table 1). For survival, significant differences were observed in both generations. For LT_{50}, three and four families were found to have significantly greater

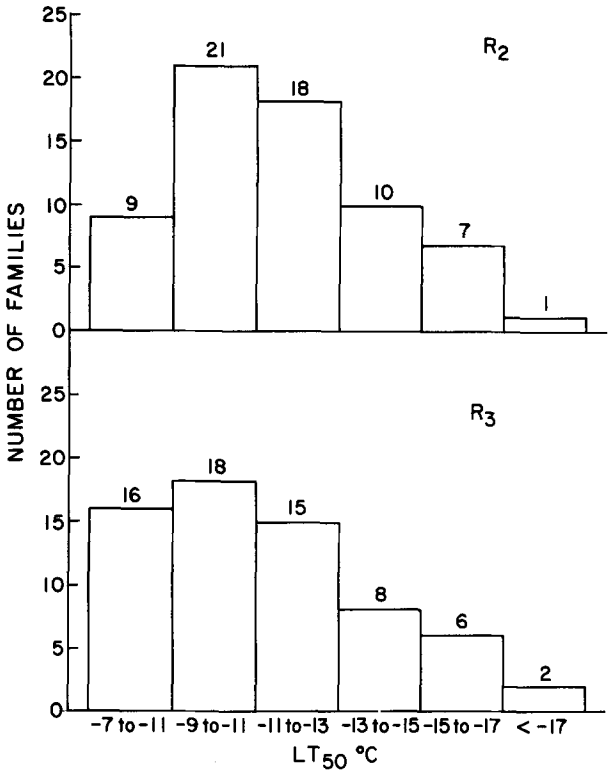

Fig. 1. Freezing tolerance distributions of R_2 and R_3 populations derived from Norstar wheat

freezing tolerance than Norstar in the R_2 and R_3 generations, respectively (Table 1). Correspondingly, high significant F-tets for variation among families were obtained for survival frequency in both generations, but not for height of surviving plants (Table 2). The blocking treatment, temperature, was the major source of variation for both dependent variables. In the analysis of the R_3 generation, the variance estimate among families was greater than that within families, though both were significant. A significant temperature × family interaction effect was also observed (Table 3). A significant, positive correlation coefficient ($r=+0.859$) was found for survival frequency of families between R_2 and R_3 generations.

In the R_2 generation, only family 32 had a greater freezing tolerance than −17 °C (Fig. 1). The LT_{50} for family 32 in the R_2 was −17.7 °C, while the control Norstar seedlings had an LT_{50} of −15.3 °C. In the R_3 generation, families 20 and 32 had LT_{50}'s of −17.1 and −17.5, respectively, while the Norstar control population had an LT_{50} of −14.5 (Fig. 2). The LT_{50} for the R_3 population as a whole, not including the Norstar control plants, was −12.6 (Fig. 2). In general, in both generations, far more families had less freezing tolerance than Norstar (Table 1, Fig. 2).

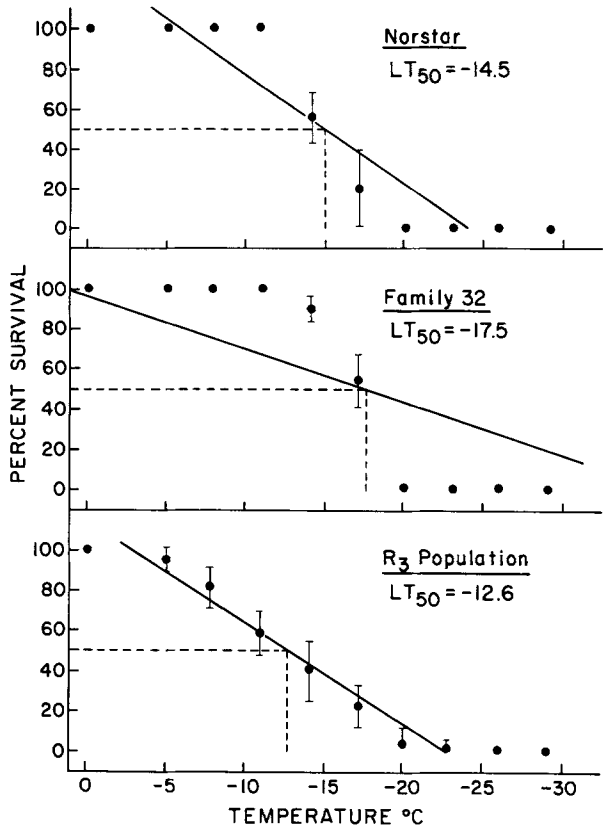

Fig. 2. Least squares regressions of survival vs. temperature for Norstar wheat and R_3 lines derived from Norstar

Discussion

This is the first report which describes heritable variation for tolerance to low temperature stress, which was observed in populations of somaclones without benefit of prior selection. Such variation is now well established for a variety of agronomically important traits in wheat as well as in other crops (Larkin 1985). We recently reported on such agronomic traits in a population of somaclones derived from the winter wheat cultivar, Norstar (Chen et al. 1987). As winter survival is a very important trait to wheat growers in many northern climates, and one which had recently proved intractable to conventional breeding methods (Limin and Fowler 1983), it was considered valuable to screen this population, derived from one of the most winter hardy cultivars available.

It has been suggested by Larkin (1985) that selection for tolerance to environmental stresses, including temperature stress, would be powerfully performed utilizing in vitro selection devices. Indeed, several stress tolerance selections have been performed in this way (Ojima and Ohira 1983; Nabors et al. 1980), including low temperature tolerance selections (Templeton-Somers et al. 1981; Dix and Street 1976). Several authors have suggested induced alterations, even if heritable, are not necessarily expressed constitutively. Further, it is necessary to first establish the effectiveness of a proposed selection scheme. To date, for wheat cultures available to us, it has not been possible to establish effective selection for tolerance to temperatures below −15 °C in cultures capable of subsequent plant regeneration.

Selection for freezing tolerance in wheat crowns is, by contrast, a well established technique (Chen et al. 1983), and the finding of significant variation, including some lines more tolerant than the parent, in a population derived from only 66 R_1 plants, suggests that freezing tolerance is not more difficult to approach using this breeding tool than other traits which have been similarly examined (Larkin et al. 1984; Chen et al. 1987). In fact, the distribution observed for freezing tolerance in this population was not markedly different from those of agronomic traits (Chen et al. 1987). As was found in our previous study, freezing tolerance in this population was not correlated with abberant mitotic or meiotic chromosomal behavior.

Segregation for freezing tolerance appeared to have occurred in the R_3 generation, as evidenced by the significant within family variance estimate. Among family variation was greater as would be expected if mutations were the exception rather than the rule. The finding that most families did not differ significantly from the Norstar control indicated that mutation for this trait was not ubiquitous in the population.

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