Moving beyond the Winter Hardiness Plateau in U.S. Oat Germplasm

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ABSTRACT

Progress has been slow in the development of winter-hardy oat (*Avena sativa* L.) cultivars. No cultivar released in the last 40 yr has better freezing tolerance than the cultivar Norline, which was released in 1960. However, in an analysis of 65 yr of field testing, Norline was not more winter hardy than 'Wintok', which was released in 1940. An analysis of individual location–year combinations of Wintok and Norline suggested that progeny from a cross of these two cultivars might contain germplasm that was transgressive for freezing tolerance. The objective of this research was to use mass selection in controlled environment freeze tests on successive segregating generations of the cross between Wintok and Norline to identify inbred progenies with significantly greater winter hardiness than either parent. Following three generations of seed increase and three generations of selection for freezing tolerance in controlled freeze tests, several F_7 genotypes were identified with greater freezing tolerance than both parents. In the F_9 generation, two of the lines exhibited a higher level of freezing tolerance than either parent, and both were slightly more freezing tolerant than the moderately winter-hardy barley, *Hordeum vulgare* 'Trebi'.

Susceptibility to freezing temperatures is a major impediment to fall-sown oat production in the eastern USA. Very limited production occurs north of the state of North Carolina and the northernmost fringe of fall-sown production is approximately the 2.8°C isotherm (Marshall, 1992). Livingston and Elwinger (1995) found that overall winter hardiness of entries in the USDA-ARS coordinated Uniform Oat Winter Hardiness Nursery (UOWHN) improved at a rate of 0.26% per year during the period from 1935 to 1992. Furthermore, they found that the rate of progress had slowed considerably since the 1970s.

Improvement of winter hardiness in oat during the twentieth century was based primarily on selection in field environments. Although progress was impressive, the experimental error in field trials due to inter- and intraplot variability of the stresses that cause winter injury made mean separation of genotypes difficult (Marshall, 1965). In addition, progress was slowed by the unpredictable occurrence of winters severe enough to kill tender genotypes and damage those of intermediate hardiness (Marshall, 1992). Early evaluations of oat in controlled environment tests designed to supplement field evaluations used juvenile whole-plant assays (Murphy et al., 1937; Amirshahi and Patterson, 1956a, 1956b). Subsequent improvements in controlled environment techniques involved evaluation of individual lateral crown meristem tissue from which root and shoot regrowth is regenerated (Marshall, 1965). Marshall and Kolb (1982) increased the winter hardiness of two heterogeneous populations of homozygous genotypes through successive cycles of controlled crown freezing. This technique was employed as a component in the development of the winter-hardy germplasm line Pennline 40 (Livingston et al., 1992).

The earliest improvements in oat winter hardiness in the USA resulted from selection of variants within the heterogeneous landrace cultivar Red Rustproof (Marshall, 1992). The next major improvement was the release of the Oklahoma cultivar Wintok in 1940. The pedigrees of both parents of Wintok traced to selections from Red Rustproof and underscored the diversity of winter survival alleles in that landrace cultivar. Another historically important winter-hardy cultivar was Norline, released by Purdue University in 1960 (Patterson and Schafer, 1978). The pedigree of one parent of Norline traced directly to Red Rustproof, and the second parent contained 25% Red Rustproof germplasm. In addition, the pedigree of Norline included 'Winter Turf', a winter oat landrace from England, and 'Victoria', a winter oat from Argentina (Souza and Sorrells, 1988). Wintok and Norline together represent the most winter-hardy cultivars developed to date in the USA.

Wintok and Norline have been evaluated in the UOWHN since 1954. In 495 location–years of side-by-side comparisons, the survival of the two cultivars was not different ($P < 0.05$) (Livingston and Elwinger, 1995). But, in a subsequent analysis of individual location–year combinations, Wintok outperformed Norline 41% of the time and significantly ($P < 0.05$) in about 12% of location–years, while Norline outperformed Wintok with a similar frequency (Fig. 1). These results suggested that both cultivars contained diversity in their winter hardiness alleles that were operative under different environmental stresses. Identification of transgressive segregates for improved winter hardiness among progenies from a cross between these cultivars could result in improvements in winter hardiness beyond the plateau represented by these cultivars. The objective of this research was to use mass selection in controlled environment freeze tests on successive segregating generations of the cross between Wintok and Norline to identify inbred progenies with greater winter hardiness than either parent.

MATERIALS AND METHODS

F_1 seeds were produced from the cross of Wintok and Norline in the greenhouse at North Carolina State University in fall 1992. F_2 seed was harvested from F_1 plants grown in the

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Abbreviations: UOWHN, Uniform Oat Winter Hardiness Nursery.

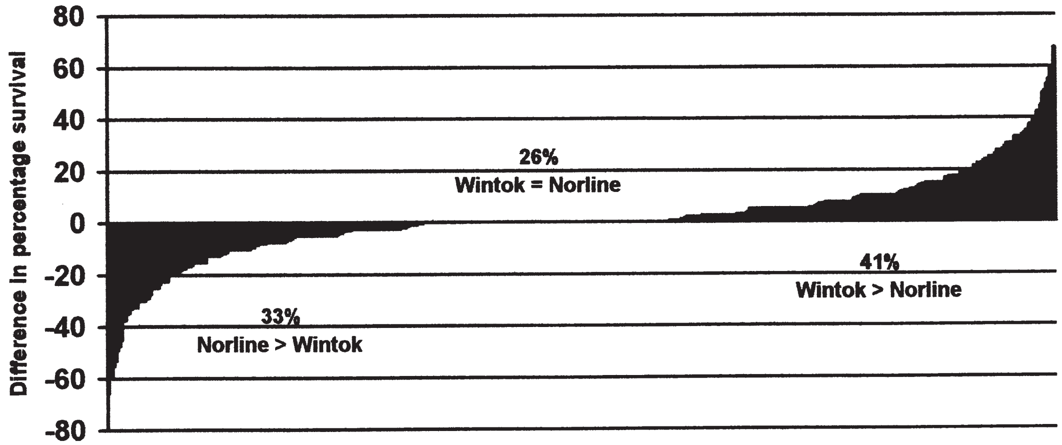

Fig. 1. Difference in winter survival between 'Wintok' and 'Norline' in 495 location years in which Wintok and Norline were in the same experiment. Data are from 1964 to 1992; each bar represents the difference in survival in a single year at a single location, sorted in the order shown.

greenhouse during fall 1993. Approximately 2500 F_2 seeds were planted in an 11-m^2 plot in October 1994 at the Central Crops Research Station, Clayton, NC. No selection was applied during the growing season and F_3 seeds were bulked following harvest in June 1995. F_4 seed were produced using a similar protocol during the 1995–1996 season.

Mass Selection on Individual Plants

Between October 1997 and May 1998, 10 440 random F_4 plants were subjected to selection for resistance to crown freezing in controlled environment tests. Seeds were planted in 12- by 16-cm disposable plastic trays filled to a depth of 3 cm with Metromix 200 (Scotts-Sierra Horticultural Products Company, Marysville, OH). A mean of 85 F_4 plants plus 10 plants of Norline and 10 plants of Wintok were evaluated per tray. Space constraints in the freezing chamber limited the number of trays to six or seven per freeze test. A total of 123 trays were used in 20 freeze tests during the 8-mo period. Plants were grown under controlled conditions as described by Livingston (1996). Briefly, plants were grown for 5 wk at 13°C with a 12-h photoperiod and cold hardened for 3 wk at 2°C (first phase hardening). They were watered with tap water three times per week and a nutrient solution was applied twice per week. Water was withheld from trays 3 d before freezing to promote uniform freezing in the tray. Plants were at the three-leaf growth stage when freeze tests were initiated.

At the start of each freeze test, approximately 10 mL of crushed ice was sprinkled over the soil surface to initiate freezing and prevent supercooling. The trays were enclosed in plastic bags to prevent desiccation, loosely sealed, and placed in a dark freezer at −3°C. Thermocouples were placed in the soil to monitor temperature. The soil in the trays reached −3°C approximately 48 h after initiation of the test, and was maintained at that temperature for 3 d to initiate second phase hardening. The temperature in the freezer was then reduced to −14°C at a rate of $-1°C\ h^{-1}$. The target temperature was maintained for 4 h and then raised to 2°C at a rate of $2°C\ h^{-1}$. After 24 h at 2°C, the trays were removed, drenched with a 1% solution of Truban fungicide (5-ethoxy-3-trichloromethyl-1,2,4 thiadiazole; Scotts-Sierra Crop Protection Co., Maysville, OH) and placed in the original chamber at 13°C for 21 d. A total of 2589 F_4 plants from 18 separate tests survived. From the survivors, approximately 10% of the most vigorous plants from each test were transplanted to greenhouse benches. F_5 seed from surviving F_4 plants was bulked following harvest.

A total of 5117 F_5 plants were subjected to selection for crown freezing resistance between September 1998 and March 1999. The protocol was similar to that described for the F_4 generation, except the target temperature was reduced to −16°C and 20 seeds of each of the parents were planted per tray. In 11 separate tests, 542 plants survived; approximately 30% of the most vigorous survivors from each test were transplanted to a greenhouse bench. F_6 seed from surviving F_5 plants was bulked following harvest.

A total of 2252 F_6 plants underwent mass selection for crown freezing resistance in a single test in January 2000. The protocol was similar to that described for the F_4 and F_5 generations except the target temperature was −17°C. All 139 surviving plants were transplanted to a greenhouse bench. Seed supplies per plant were limited, but eight vigorous plants produced sufficient seed for replicated evaluation in the F_7 generation.

Replicated Line Evaluations Using Crown Meristem Tissue

A preliminary controlled freeze test was performed on the eight $F_{6:7}$ lines plus the parental cultivars Wintok and Norline. Three of the lines were less hardy than either parent (data not shown) and were eliminated from subsequent testing. The five most freezing-tolerant $F_{6:7}$ lines, along with the parents and the very winter-hardy barley 'Dictoo' were planted 2 cm deep in 15-cm-long plastic nursery tubes filled with Metromix 200 as described by Livingston (1996). One seed was planted per tube, and there were 10 tubes per entry in each replicate. Plants were grown under the same temperature and light regime as described previously without the 3-d dry period before freeze testing. After hardening, crowns were separated and removed from each plant by trimming off roots and shoots. The crowns were inserted into slits made in circular moist sponges at 2°C, placed in a plastic bag to prevent desiccation, and immediately placed in a freezer at 2°C. To compensate for sponge-to-sponge variation in the freeze test, each sponge contained one plant of each entry. Ten sponges, each containing one plant of each of the five $F_{6:7}$ lines, the two parents, and Dictoo barley, were included in each replicate. The temperature in the freezer was reduced to −13°C. After freezing, the crowns were transplanted to trays similar to those used during the mass selection generations and allowed to grow for 21 d. Individual plants were then rated on a 0-to-5 scale, where 0 = dead; 1 = barely alive, may have roots or shoot: 3 = will survive, 2 to 3 roots, weak new shoot growth; 4 = slight damage, good new growth; and 5 = undamaged, similar to unfrozen

Table 1. Percentage survival following controlled freeze test among progenies of oat in the F_4, F_5, and F_6 generations, and the parental lines 'Wintok' and 'Norline' in comparison with the midparent value.

	F_4†	F_5‡	F_6§
	% survival (±SE)		
Progeny mean	24.8 (1.9)	10.3 (1.4)	6.4 (1.8)
Wintok	17.6 (3.2)	6.9 (2.1)	4.3¶
Norline	19.1 (3.7)	5.3 (1.3)	1.4¶
Midparent	18.4	6.1	2.8

† Frozen at −14°C.
‡ Frozen at −16°C.
§ Frozen at −17°C.
¶ SE not available because only one replication of parents were included.

plant. Ratings were based on the mean of 10 plants per entry. A three-replicate (across time) randomized complete block design was used.

On the basis of these results, four lines were selected and remnant $F_{6:7}$ seed was advanced to the next generation at the Cunningham Research and Education Center, Kinston, NC, during the 2001–2002 season. The four $F_{6:8}$ lines plus Wintok, Norline, Pennline 40, and the winter-hardy wheat cultivar Jackson were included in an eight replicate freeze test during winter 2002–2003 following the protocol described above. In October 2002, $F_{6:8}$ seed of the four lines plus Wintok and Norline were planted in 4.7-m^2 plots in Kinston in a two-replicate randomized complete block experiment. Heading was recorded when 50% of the panicles had emerged from the boot.

RESULTS AND DISCUSSION

Percentage survival of plants in the three generations that were subjected to selection decreased from 24.8 to 6.5% as the minimum temperature decreased from −14°C to −17°C (Table 1). However, the mean survival for the segregating population increased from 135% of the midparent mean in the F_4 generation to 169% of the midparent mean in the F_5 generation and to 229% of the midparent mean in the F_6 generation. These results suggest that mass selection was very effective in increasing the frequency of freeze-tolerant genotypes in the segregating population. Studies have shown that controlled environment freeze resistance is a quantitative trait controlled by genes exhibiting additive effects and the trait had moderate to high heritability (Amirshahi and Patterson, 1956a; Muehlbauer et al., 1970). Our results support these conclusions and provide evidence of the ability of this controlled environment technique to alter gene frequencies rapidly under mass selection. The selection intensity increased from 2.5% in the F_4 to 3.2% in the F_5 to 6.2% in the F_6.

In 20 oat crosses, Amirshahi and Patterson (1956b) found that the cross with the highest heritability (95%) for freezing tolerance and the one with the most lines exceeding the freezing tolerance of Wintok was a cross between Wintok and Norline (an experimental line at the time). Unfortunately, none of those lines were entered into the UOWHN and in storage, the seed quickly lost viability.

Crown meristem tissue freezing evaluations among the selected progenies in the F_7 and F_8 generations indicated that transgressive segregates exhibiting hardiness superior to both parents had been identified (Table 2). W/N-1, W/N-4, and W/N-10 all had significantly higher survival ratings than either parent. In the F_8 generation, the survival of the best genotype was better than the Wintok-Norline midparent value by 58%. This extrapolated to a difference in LT_{50} of about 2°C between Wintok-Norline and the best genotype.

None of the oat germplasm here was as hardy as Dictoo barley (Table 2), but this is not surprising since Dictoo has long been considered one of the most freezing-tolerant barley cultivars available. Nevertheless, the level of freezing tolerance of W/N-1 and W/N-10 suggested that these lines would compete favorably with barley in the hardiness range of Trebi. Trebi has been the moderately hardy barley check in the Uniform Barley Winter Hardiness Nursery for 60 yr.

Although most winter-hardy germplasm flowers later than nonhardy germplasm because of vernalization and/or daylength requirements, none of the four lines in this study matured as late as Norline in spite of their superior freezing tolerance (Table 3). This suggests that it may be possible to break the apparent linkage between late flowering and freezing tolerance. Oat cultivars with the freezing tolerance of germplasm described here but with a heading date similar to 'Brooks' (Table 3) would be ideal for North Carolina growers, but cultivars with heading dates similar to Wintok would be adapted to Virginia, Maryland, and Kentucky. Efforts are underway to develop early maturing germplasm with a high level of freezing tolerance.

These results do not necessarily indicate that crossing two hardy cultivars will always produce transgres-

Table 2. Mean survival ratings following controlled freeze testing at −13°C, of selected progenies of oat in the F_7 and F_8 generations, 'Wintok', 'Norline', 'Dictoo' barley, and the winter-hardy oat, 'Pennline 40'.

	Survival rating†	
	F_7‡	F_8§
Dictoo barley	3.0a¶	–
W/N-1	1.7b	1.5ab¶
W/N-10	1.5b	1.7a
W/N-4	1.3b	1.2bc
W/N-8	1.2bc	0.9cd
Norline	0.5cd	0.1e
Wintok	0.1d	0.5de
Pennline 40	–	0.5de
LSD (0.05)	0.74	0.45

† 0 = dead, 5 = undamaged, similar to unfrozen plant.
‡ Mean of three freeze tests with 10 plants in each test.
§ Mean of eight freeze tests with 10 plants in each test.
¶ Means in the same column followed by the same letter are not significantly different from each other at $P = 0.05$ according to the LSD test.

Table 3. Date of 50% panicle emergence in oat lines grown in 2003 at Kinston, NC.

	Day of year
'Brooks'	115a†
W/N-1	120b
W/N-4	120b
W/N-8	120b
W/N-10	120b
'Wintok'	121b
'Norline'	124c
LSD (0.05)	1.2

† Means in the same column followed by the same letter are not significantly different from each other at $P = 0.05$ according to the LSD test.

sive segregates. Wintok and Norline were selected because their average survival across 40 yr was the same, but under different environmental conditions, in certain years, their survival differed significantly from each other. This led us to suspect that the cultivars may possess different alleles for freezing tolerance and that it should be possible to combine them in a single genotype. While no data are presented here to confirm that specific freezing tolerance genes have been recombined, if they had been it is likely that we would observe germplasm that was hardier than both parents (Tables 1, 2). It should be possible to confirm the recombination of genes using genetic marker technology. Recombinant inbred lines of a Wintok–Norline cross have been developed and are currently being analyzed for polymorphisms.

REFERENCES

Amirshahi, M.C., and F.L. Patterson. 1956a. Development of a standard analytical freezing technique for evaluating cold resistance of oats. Agron. J. 48:181–184.

Amirshahi, M.C., and F.L. Patterson. 1956b. Cold resistance of parent varieties, F_2 populations, and F_3 lines of 20 oat crosses. Agron. J. 48:184–188.

Livingston, D.P., III. 1996. The second phase of cold hardening: Freezing tolerance and fructan isomer changes in winter cereal crowns. Crop Sci. 36:1568–1573.

Livingston, D.P., III, and G.F. Elwinger. 1995. Improvement of winter hardiness in oat from 1935 to 1992. Crop Sci. 35:749–755.

Livingston, D.P., III, H.G. Marshall, and F.L. Kolb. 1992. Registration of Pennline 40 winter oat germplasm. Crop Sci. 32:1512.

Marshall, H.G. 1965. A technique of freezing plant crowns to determine the cold resistance of winter oats. Crop Sci. 5:83–86.

Marshall, H.G. 1992. Breeding oat for resistance to environmental stress. p. 699–749. *In* H.G. Marshall and M.E. Sorrells (ed.) Oat science and technology. Agron. Monogr. 33. ASA and CSSA, Madison, WI.

Marshall, H.G., and F.L. Kolb. 1982. Individual crown selection for resistance to freezing stress in winter oats. Crop Sci. 22:506–510.

Muehlbauer, F.J., H.G. Marshall, and R.R. Hill, Jr. 1970. Winterhardiness in oat populations derived from reciprocal crosses. Crop Sci. 10:646–649.

Murphy, H.C., T.R. Stanton, and H. Stevens. 1937. Breeding winter oats resistant to crown rust smut and cold. J. Am. Soc. Agron. 29:622–637.

Patterson, F.L., and J.F. Schafer. 1978. Registration of Norline oats. Crop Sci. 18:356.

Souza, E., and M.E. Sorrells. 1988. Coefficients of parentage for North American oat cultivars released from 1951 to 1986. Bull. No. 33. Cornell University Agric. Exp. Stn., Geneva, NY.