

Evaluation of freezing tolerance in advanced progeny from a cross of *Avena sativa* X *A. macrostachya*

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ABSTRACT

Avena macrostachya is a perennial, autotetraploid oat. In its natural habitat it has a high degree of winter hardiness. The objective of this experiment was to screen 53 backcross progenies of an interspecific cross between the cultivar Brooks (*A. sativa*) and *A. macrostachya* using a controlled freezing test. No entry tested was more freezing tolerant than the recurrent parent Brooks. These results indicated that freeze tolerance from *A. macrostachya* had not been introgressed into the progeny. It was suggested that the mechanism for winter hardiness in *A. macrostachya* is different from that in *A. sativa* and may involve an escape mechanism rather than a genetically controlled freezing resistance mechanism.

Key Words: *Avena macrostachya*, *A. sativa*, winter hardiness, freezing tolerance, perennial oat.

INTRODUCTION

In 1853, Benjamin Balansa (1825-1891) discovered *A. macrostachya* on Mt. Djurdjura, Algeria (Baum and Rajhathy, 1976). It was originally classified within the genus *Helictotrichon* as *H. macrostachyum* (Bal. ex Coss. et Dur) Henr. due to its perennial growth habit. Baum (1968) moved it to the genus *Avena* based on gross morphological habit, shape and structure of glumes. *A. macrostachya* is confined to Mt. Djurdjura and other mountain areas in Algeria at altitudes between 1500 and 2200m.

Due to its unique traits, such as perenniality, cross-pollination, winterhardiness, and some disease resistance, *A. macrostachya* may be important in breeding programs. The production of hybrids between *A. macrostachya* and cultivated oat is possible, although difficult, and requires embryo rescue (Leggett, 1985). A number of techniques have been used to produce hybrids, such as backcross using *A. sativa* as the recurrent parent, doubling the number of chromosomes via colchicine treatment, and radiation to promote homologous chromosome pairing (Leggett, 1985). Leggett (1985) suggested that the chromosome homology between domesticated and wild species is quite low, so it is not surprising to observe infrequent pairing and recombination between these two species.

In an attempt to enhance oat germplasm for cold tolerance, the North Carolina State University Small Grains Program made interspecific crosses between *A. sativa* and *A. macrostachya*. The winter oat cultivar 'Brooks' (CI 9260, Murphy, 1979) served as the female parent in these crosses. Brooks is productive, tolerant to barley yellow dwarf virus and moderately resistant to soil-borne mosaic virus. The objective of this research was to evaluate the winter hardiness of these backcross-derived lines using controlled environment crown-freeze tests.

MATERIALS AND METHODS

Avena macrostachya ($2n=4x=28$) plants were grown from two seeds obtained from J.M. Leggett of the Institute of Grassland and Environmental research, Aberystwyth, U.K. A female hexaploid oat parent Brooks ($2n=6x=42$, AACDD) was crossed with *A. macrostachya* by Dr. J. P. Murphy in late 1980's and embryo rescue was performed on the developing F_1 seeds. F_1 s with 35 chromosomes were treated with colchicine to obtain plants with 70 chromosomes. Pollen of the amphidiploid F_1 was treated with X-rays and applied to the female plants of Brooks. Progenies were self-pollinated for six generations. During the selfing generations, selection was made for heading date, plant height and fertility using the Brooks phenotype as the benchmark. Selfing generations were grown at the Cunningham Research and Extension Center, Kinston, NC where winter weather is moderate. Little, if any, selection was imposed for winter hardiness.

Freeze test

A total of fifty-three $F_{6:7}$ derived lines and the recurrent parent Brooks were evaluated for crown freeze tolerance. Since *A. macrostachya* would not set seed, it could not be included. The experiment was a randomized complete block design with three replicates. Due to size limitations in the freeze chamber, two freeze tests were conducted with replications over time. Freeze test 1 included entries 1 to 18 and Brooks, freeze test 2 included entries 19 to 53 and Brooks.

Seeds were planted 2 cm deep in plastic tubes filled with Metromix 220 (Scotts-Sierra Horticulture Products Co., 14111 Scotslawn Rd., Marysville, OH 43041) with drainage holes at the bottom. One seed/tube, and five tubes/line were planted.

The plants were grown in a growth chamber for five weeks with a 12h photoperiod and a light intensity of $225 \mu\text{mol m}^{-2} \text{s}^{-1}$. The plants were then transferred to a different chamber at 2°C for three weeks of cold acclimation with a 12h photoperiod and a light intensity of $320 \mu\text{mol m}^{-2} \text{s}^{-1}$.

After cold acclimation, five plants of each line were washed with ice water, roots were trimmed to 1 cm and shoots to 4-5 cm. These plantlets were inserted into slots cut into damp, circular sponges (one line per sponge), placed in a plastic bag, to prevent desiccation and set on an iron flange. The sponges containing plantlets were inoculated with crushed ice, to prevent supercooling and loosely sealed in the plastic bags.

Sponges were placed in a custom designed freezer and subjected to the following temperature sequence.

1. -3°C for 48 h for subzero acclimation.
2. Reduce temperature at 1°C h⁻¹ to -8°C (replication one) or -9°C (replication two and three), and maintain for 3 h.
3. Raise temperature at 2°C h⁻¹ to 2°C and maintain for 12-h to thaw.

After the freeze cycle, roots were trimmed and plantlets were transplanted in trays, and allowed to regrow for three weeks in the 13°C growth chamber.

Efforts were made to choose a freeze temperature at which Brooks would be killed but genotypes hardier than Brooks would survive. A temperature of -8°C was selected in the first replication and -9°C in the second and third replications.

Crown freeze data

The following rating scale was used to evaluate the transplants after three weeks regrowth:

- 0 = dead,
- 1 = barely alive, may have roots or shoot,
- 2 = will survive, 2-3 roots, weak new growth, and dead old growth,
- 3 = some damage, good root system, 10-20% leaf growth, dead old growth and good new growth,
- 4 = slight damage, good new growth, old growth only has slight burning of the tips,
- 5 = undamaged, similar to unfrozen plant.

Because all the survival rating data were smaller than 30, the square root transformation was utilized. And 0.5 was added to each data point before the statistical analysis performed (Gomez and Gomez, 1984). SAS V8.2 (SAS, Inc., 1999) software was used to perform the ANOVA analysis.

The entry means were compared by the protected LSD test.

RESULTS

All freezing tolerant genotypes were more similar to Brooks morphologically than to *A. macrostachya* (not shown). NC 00-C3-17 had 2n=56 chromosomes. Jellen identified 19 chromosomes in this line as coming from *A. macrostachya* (Santos, 1997). The backcross progenies were morphologically similar to *A. macrostachya* to some extent, i.e. similar blue gray plant color (Santos 1997). This suggested that *A. macrostachya* genes were indeed present in the progeny.

Controlled freeze test results showed that no entry was significantly more freezing tolerant than Brooks in freeze test 1 (Table 1). Only three lines had a higher survival rate than the recurrent parent Brooks, but the differences were not significant. In freeze test 2 (Table 2), three lines had a higher survival than Brooks, and only one was significantly higher than Brooks at 0.05 level (Table 2) which is within the number that would be expected to occur by chance.

Table 1. Mean survival rating and transformed rating for set one of Brooks/*A. macrostachya* progeny

Entry	Genotype	Rating Mean	Transformed Rating Mean
15	NC 00-4277	2.36	1.68
12	NC 00-4257	2.15	1.63
16	NC 00-4313	2.04	1.59
19	Brooks	1.45	1.39
8	NC 00-3969	1.33	1.35
9	NC 00-4004	1.28	1.26
4	NC 00-3899	1.20	1.30
1	NC 00-3671	1.03	1.12
13	NC 00-4264	1.00	1.17
6	NC 00-3916	0.93	1.14
18	NC 00-C3-17	0.87	1.16
2	NC 00-3817	0.83	1.11
14	NC 00-4266	0.70	1.05
5	NC 00-3910	0.67	1.05
3	NC 00-3886	0.53	0.99
7	NC 00-3931	0.47	0.96
11	NC 00-4193	0.33	0.88
17	NC 00-4332	0.33	0.88
10	NC 00-4180	0.20	0.82
LSD _{0.05}			0.34

DISCUSSION

The primary factor delineating the northern boundary of winter oat production in the USA is the level of winter hardiness available in cultivars (Marshall, 1992). Breeding for increased winter hardiness in cultivated oat has been studied and implemented during the last century, but improvement has slowed since the mid 1970s. This decline was mostly due to elimination of oat improvement programs and exhaustion of genetic variation in the gene pool (Livingston and Elwinger, 1995). Further increases in the winter hardiness of oat depend on the creation and selection of genotypes that transgress known level of hardiness in either domestic or wild germplasm (Marshall and Shaner, 1992 Livingston *et al.*, 2004).

Table 2. Mean survival rating and transformed rating for set two of Brooks/*A. macrostachya* progeny

Entry	Genotype	Score Mean	Transformed Score Mean
29	NC 00-3813	2.10	1.54*
47	NC 00-4316	1.93	1.47
48	NC 00-4317	1.60	1.38
41	NC 00-4189	1.20	1.21
54	Brooks	1.15	1.25
44	NC 00-4276	1.03	1.18
35	NC 00-3925	1.01	1.17
28	NC 00-3809	0.90	1.17
31	NC 00-3845	0.73	1.06
38	NC 00-3997	0.54	1.02
37	NC 00-3989	0.54	0.99
24	NC 00-3717	0.47	0.95
21	NC 00-3686	0.40	0.92
49	NC 00-4322	0.40	0.91
43	NC 00-4261	0.39	0.93
50	NC 00-4333	0.33	0.88
32	NC 00-3849	0.30	0.89
45	NC 00-4292	0.30	0.88
39	NC 00-4008	0.27	0.87
36	NC 00-3963	0.17	0.81
46	NC 00-4302	0.17	0.81
20	NC 00-3682	0.13	0.79
27	NC 00-3797	0.13	0.79
52	NC 00-4338	0.11	0.78
22	NC 00-3705	0.10	0.77
51	NC 00-4337	0.10	0.77
19	NC 00-3620	0.07	0.75
23	NC 00-3710	0.07	0.75
34	NC 00-3912	0.07	0.75
42	NC 00-4217	0.07	0.75
53	NC 00-P6-6	0.07	0.75
33	NC 00-3890	0.03	0.73
25	NC 00-3735	0.00	0.71
26	NC 00-3750	0.00	0.71
30	NC 00-3842	0.00	0.71
40	NC 00-4165	0.00	0.71
LSD _{0.05}			0.29

The controlled freeze test conducted by Santos (1997) showed that freezing tolerance of single *A. macrostachya* crowns test is poor. The results above agree with his observation in that lines with obvious evidence of introgression from *A. macrostachya* are vulnerable to single crown-freezing test. Leggett found that *A. macrostachya* has similar freezing resistance to susceptible cultivars using an ethylene glycol freezing test (Santos, 1997). It was suggested that *A. macrostachya* might have a different winter hardiness mechanism than Brooks.

One explanation for the winter hardiness of *A. macrostachya* could be its profuse tillering. It has been suggested that the outer tillers may provide insulation for the inner tillers. The inside of the a cereal crown is a complex region of the plant and whole plant survival after freezing is dependant on the survival of only a portion of the tissue within the crown (Livingston *et al.*, 2005). It is possible that the whole plant would survive if a critical number of inner tillers survive, even though most of the outer ones are killed in cold weather (Santos, 1997) Individual crown freezing would not be appropriate to test this type of mechanism. Breeders will need more information on the mechanism of winter hardiness in *A. macrostachya* before it can be effectively used as a source of cold tolerance.

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