RESEARCH

An Intergenomic Reciprocal Translocation Associated with Oat Winter Hardiness Component Traits

David R. Wooten, David P. Livingston III, Eric N. Jellen, Kathryn J. Boren, David S. Marshall, and J. Paul Murphy*

ABSTRACT

The reciprocal intergenomic translocation between hexaploid oat (*Avena* sp.) chromosomes 7C and 17 (T7C-17) has been associated with the division of cultivated oat into *A. sativa* L. and *A. byzantina* K. Koch species and differences in crown freezing tolerance and winter field survival. The objectives of this experiment were: (i) to validate the association of T7C-17 with crown freezing tolerance and winter field survival in a population derived from a cross of the non-winter-hardy 'Fulghum' (non-T7C-17) with winter-hardy 'Norline' (T7C-17); (ii) to determine if preferential selection for T7C-17 occurred during inbreeding; and (iii) to examine the association of T7C-17 with the winter hardiness component traits heading date, plant height, and vernalization and photoperiod responses. Crown freezing tolerance and vernalization and photoperiod responses were evaluated in controlled environment studies. Heading date, plant height, and winter field survival were evaluated in field experiments during two seasons. The presence of the translocation was associated with greater crown freezing tolerance, winter field survival, and days to flowering. Translocation status was not associated with vernalization and photoperiod responses or plant height. The T7C-17/non-T7C-17 segregation ratio was 2:1. These results confirmed the importance of T7C-17 in conferring winter hardiness traits in winter oat and preferential selection for the translocation during inbreeding.

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Abbreviations: RIL, recombinant inbred line; QTL, quantitative trait locus; T7C-17, intergenomic reciprocal translocation involving chromosomes 7C and 17.

Low levels of winter hardiness limit the area of commercial winter oat (*Avena sativa* L.) production in much of North America. Winter hardiness in cereals is related to several quantitative traits including crown freezing tolerance, vernalization and photoperiod responses, heading date, and plant height (Fowler et al., 1999). Crown freezing tolerance is the most important winter-hardiness trait (Olien, 1967). Photoperiod and vernalization responses combined with heading date act as freezing stress avoidance mechanisms that delay growth of freezing-sensitive reproductive tissues until warmer temperatures arrive (Snape et al., 2001). Consequently, winter field survival and crown freezing tolerance are often correlated with other winter-hardiness component traits such as heading date and vernalization and photoperiod responses through pleiotropy or linkage (Brulebabel and Fowler, 1988; Francia et al., 2004; Kobayashi et al., 2005; Pan et al., 1994; Toth et al., 2003).

A reciprocal intergenomic translocation involving chromosomes 7C and 17 (T7C-17) is found in most hexaploid oat species (Jellen et

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al., 2004; Zhou et al., 1999). The absence of the translocation has been associated with early U.S. winter-type germplasm, but important exceptions have been noted. For example, the fall-sown oat cultivar 'Winter Turf', introduced from England in the colonial era, contains T7C-17 and is found in the pedigrees of many winter oat cultivars. Spring oat germplasm and some of the most winter-hardy modern cultivars contain T7C-17 (Jellen and Beard, 2000).

Santos et al. (2006) found that the translocation was significantly correlated with both crown freezing tolerance and field winter survival in a recombinant inbred line (RIL) population derived from a cross of the cultivars Fulghum and Wintok. In addition, they observed almost threefold as many homozygotes with the translocation as homozygotes without the translocation, which indicated preferential selection for T7C-17 during inbreeding. Fulghum is a traditional winter oat cultivar derived from a single plant selection from the land race 'Red Rustproof' in the late 19th century (Coffman, 1977). It has a low level of winter hardiness (Livingston and Elwinger, 1995) and does not have T7C-17 (Jellen and Beard, 2000). Norline is a winter-hardy cultivar developed by the Indiana USDA-ARS oat breeding program and released in 1960 (Patterson and Schafer, 1978). It is winter hardy (Livingston and Elwinger, 1995; Livingston et al., 2004) and contains T7C-17 (Jellen and Beard, 2000). Norline and Wintok are the long-term winter-hardy checks in the Uniform Oat Winter Hardiness Nursery and have similar mean winter hardiness, but often differ for winter field survival in some environments (Livingston and Elwinger, 1995). Both lines have T7C-17.

The objectives of this experiment were: (i) to validate the association of T7C-17 with crown freezing tolerance and winter field survival in a population derived from a cross of the non-winter-hardy Fulghum (non-T7C-17) with winter-hardy Norline (T7C-17); (ii) to determine if preferential selection for T7C-17 occurred during inbreeding in this population; and (iii) to examine the association of T7C-17 with the winter hardiness component traits of heading date, plant height, and vernalization and photoperiod responses.

MATERIALS AND METHODS

Plant Material

The experiment was conducted using a population of 128 F_6–derived RILs developed from the cross of non-winter-hardy Fulghum (non-T7C-17) × winter-hardy Norline (7C-17 translocation). Plants in the Fulghum × Norline population were selfed to the F_6 generation by single seed descent. Each RIL was derived from a single F_6 plant, and each F_6 plant descended from a different F_2 plant; however, seed set and production of nonviable seed was not recorded. Translocation status was determined for seedlings grown from $F_{6:7}$ seed using the C-banding technique as described in Santos et al. (2006), with presence or absence based on observation of multiple cells from root-tip meristems of three plants per RIL.

Controlled Crown Freezing Tolerance Test

The experimental design was incomplete blocks within complete replications, with 14 incomplete blocks of 10 entries within each of five complete replications with time. Each complete replication consisted of 120 RILs (eight lines were not included because of limited $F_{6:7}$ seed) plus 20 check entries consisting of seven entries of each parent, Fulghum and Norline, and six entries of the winter-hardy check cultivar Wintok. Ten $F_{6:7}$ seeds of each entry were germinated on moist paper in petri dishes, one dish per entry, for 4 d. Five seedlings of each entry were then planted 1.5 cm deep in five adjacent 20-cm-long nursery tubes held in racks of 100 tubes. Plants were grown in Metromix 200 (Scotts-Sierra Horticultural Products Co., Marysville, OH) and lightly watered daily with a complete nutrient solution (Livingston, 1991). The plants were grown for 5 wk in a 9-m^2 growth chamber in the Southeastern Plant Environment Laboratory at North Carolina State University. The chamber was illuminated for a 10-h photoperiod with a photosynthetic photon flux density of 300 μmol m^{-2} s^{-1}, with a day temperature of 13°C and night temperature of 10°C. At the five-leaf stage, plants were transferred to a hardening growth chamber for a 3-wk cold acclimation treatment. The hardening chamber held a constant 3°C, with a 10-h photoperiod of photosynthetic photon flux density of 300 μmol m^{-2} s^{-1}. While cold acclimating, plants were watered with a complete nutrient solution three times per week, and watered with deionized water on alternate days.

Plants were removed from the nursery tubes after cold acclimation, and soil was washed off the roots with ice water. Roots were trimmed to 0.5 cm and crowns were trimmed to 5 cm in length. The crowns were placed in slits in cold, slightly moist sponges. The crowns and sponges were sprinkled with crushed ice to prevent supercooling, and sealed in plastic bags. The sealed unit was placed on a steel plumbing flange to provide thermal and structural stabilization. The prepared units were then placed in a freezer at −3°C for 48 h to induce subzero acclimation (Livingston, 1996). Subsequently, the freezer temperature was decreased by 1°C h^{-1} to −10°C. The temperature was held at −10°C for 3 h and then raised by 2°C h^{-1} to 2°C. Within each replication, the entries were assigned to an incomplete block of 10 entries using an alpha (0,1) lattice structure. The same 10 entries were frozen in each of five sponges. One of the five congruent sponges from each entry group was placed on each shelf in the freezer.

After the crowns and sponges thawed, the roots were trimmed from the crowns to prevent the growth of microbes as roots deteriorate, and the crowns were planted in an entry × sponge grid pattern within 50- by 30-cm plastic flats filled 5 cm deep with moist Metromix 200. The flats were returned to the growth chamber in the Southeastern Plant Environment Laboratory, where environmental conditions were the same as those which provided prehardening. After 3 wk of regrowth, recovery for each crown was visually measured on a scale of 0 to 10 (0 = complete plant death, 10 = no freeze damage), double the scale used in Santos et al. (2006).

Field Trials

Heading date and plant height were evaluated using a randomized complete block design with two replications. The $F_{6:7}$ seed of each RIL plus the parents and Wintok as checks were planted on 23

Oct. 2002 at the Cunningham Research and Education Center, Kinston, NC. Plots were single rows 1.3 m long and mean row spacing was 0.3 m. Heading date was recorded as the day of the year when 50% of panicles had emerged. Severe lodging prevented measurement of plant height in 2002–2003. Seed of each plot was harvested and threshed to collect $F_{6:8}$ seed for winter field survival testing. The experiment was repeated in 2003–2004 using remnant $F_{6:7}$ seed and a similar protocol, except plot size was increased to two adjacent 1.3-m-long rows with mean row spacing of 0.6 m. Plant height was estimated as the distance between the soil surface and the tip of the panicle of an average plant.

Winter field survival was evaluated using a randomized complete block design with five replications in each of five environments. The $F_{6:8}$ seed of the RILs plus five entries of the parental checks and Wintok was used. The experiment was planted on 16 Sept. 2003 at the Upper Mountain Research Station (elevation = 895 m) near Laurel Springs, NC, and 9 Oct. 2003 at the Mountain Research Station (elevation = 727 m) near Waynesville, NC. Soil type at Laurel Springs was Toxaway (fine-loamy, mixed, nonacid, mesic Cumulic Humaquept) and soil type at Waynesville was French (fine-loamy over sandy or sandy skeletal, mixed, mesic Fluvaquentic Dystrochrepts). Single-row plots were hand planted with 6 g of seed per plot in single rows 2.3 m long with a mean row spacing of 0.3 m. Fall plant emergence and growth were recorded for each plot in early November. Laurel Spring reached a minimum temperature of −14.5°C on 31 Jan. 2004 and Waynesville reached a minimum temperature of −14.2°C on 7 Jan. 2004. Field survival was estimated for each plot in March 2004 as the survival percentage for the plots corrected for plot variation in germination or fall growth. The experiment was repeated in the 2004–2005 season at both North Carolina locations with the addition of the Virginia College of Agriculture and Life Sciences' Kentland Research Farm, near Blacksburg, VA. Soil type at Blacksburg was Hayter (fine loamy, mixed, mesic Ultic Hapludalfs). Plots in 2004–2005 were two collinear row segments each 1.3 m long and with a row spacing of 0.3 m. Laurel Springs was planted on 24 September, Waynesville on 8 October, and Blacksburg in the second week of October. In the winter of 2004–2005, Laurel Springs reached a minimum temperature of −19.2°C, Waynesville reached a minimum temperature of −15.7°C, and the city of Blacksburg reached a minimum temperature of −17.9°C. All three locations reached their minimum temperatures on 20 Dec. 2004 (National Climatic Data Center, www.ncdc.noaa.gov, verified 6 July 2007). No winter damage was observed in Waynesville in either season, so the data were not included in the analysis of winter field survival.

Photoperiod and Vernalization Responses

Photoperiod and vernalization responses were evaluated in a growth chamber experiment conducted at the Southeastern Plant Environment Laboratory at North Carolina State University. A split-plot factorial design with three replications over time was used. Photoperiod was the whole-plot factor and vernalization and genotype were factorial subplot factors. Seedlings for the nonvernalized treatment were germinated in moist paper towels for 4 d at 20°C. Seedlings for the vernalized treatment were germinated in moist paper towels for 4 wk in the dark at 2°C. Two plants of each treatment were planted in 10-cm^2 square pots, with all plants in a replication planted on the same day. Long- and short-day effects were simulated in two separate growth chambers. Both chambers were illuminated for a 10-h photoperiod with photosynthetic photon flux density of 550 μmol m^{-2} s^{-1}, and the long-day treatment was simulated using a 2-h midnight interruption with low-intensity incandescent lights. This provided long-day stimulus to the plants, while minimizing the difference in photosynthetically active radiation. After 42 d with differing photoperiod treatments, both chambers were increased to a 16-h photoperiod to facilitate flowering. Each plot consisted of two plants grown in the same pot, and days to panicle exertion were recorded for each plant. To normalize error variance and simplify analysis, the natural log of the mean of the two plants for each pot was used for ANOVA.

Data Analysis

Translocation segregation ratios were examined using the FREQ procedure in SAS. The phenotypic data were analyzed using the MIXED procedure of SAS (Littell et al., 1996) with the Satterthwaite option for calculating degrees of freedom. Narrow-sense heritabilities were estimated for the population excluding checks, using an all-random-effects model following the method described by Holland et al. (2003, Table 2.1, Section 11) but adjusted for the differences in experimental design. The translocation status was then added as a fixed effect to each model, and estimate statements were used to estimate the additive and dominant effect of the translocation; the LSMEANS statement generated least-squares means (means) for the three translocation classes. Entries (including parents and checks) were then considered a fixed effect and the LSMEANS statement generated entry least-squares means (means). The DIFF option ($\alpha = 0.05$) was used to test for transgressive segregation. Correlations were estimated among crown freezing tolerance, winter field survival, heading date, and translocation status using the CORR procedure. Procedure GLM was used to regress winter field survival on crown freezing tolerance. The univariate procedure was used to test normal distributions for heading date, winter field survival, and crown freezing tolerance for both the whole population and the two translocation classes.

RESULTS

Eighty-one Fulghum × Norline lines had T7C-17, and 39 lines had a non-T7C-17 karyotype. These frequencies did not follow the expected 1:1 segregation ratio ($P < 0.0001$) for a two-parent RIL population. The data did not significantly deviate from a 3:1 ratio ($P = 0.058$), but a 2:1 ratio ($P = 0.85$) was the best fit for the observed segregation pattern. Six lines were heterogeneous for T7C-17, which was not significantly different from the expected value of four lines in a population of 126 F_6–derived lines. One line was nullisomic for chromosome 7C[17] (Fig. 1) and was excluded from the analyses of the translocation classes, but included in analyses treating genotype as a fixed effect.

Crown Freezing Tolerance

Norline was significantly more crown freezing tolerant than Fulghum (Table 1). Five lines were significantly more freezing tolerant than Norline (4%), but no lines were

significantly less freezing tolerant than Fulghum, as the measured freezing tolerance of Fulghum was not significantly different from zero. Distribution of crown freezing tolerance means was bimodal (Fig. 2). Dividing the population by translocation status, however, produced two populations with normal distribution for crown freezing tolerance (Fig. 2). Translocation status was highly significant, with an additive effect of 1.4 (Table 1). The heritability of crown freezing tolerance was 83 ± 2%. Mean crown freezing tolerance was significantly correlated with the presence of the translocation (r = 0.72) (Table 2).

Winter Field Survival

Winter field survival ranged from 44% at Laurel Springs in 2004–2005 to 100% at Waynesville in 2003–2004 and 2004–2005. The results from Waynesville were not included in further analyses. Three environments had differential winter field survival (Table 1). The effect of T7C-17 on winter field survival was highly significant in all three environments, with the magnitude of the effect closely tracking the observed winter field survival heritability. Thus the translocation had a greater influence on phenotype in environments where genotype had a greater effect on phenotype. No transgressive segregation for increased field survival was observed in any environment or in the combined analysis. Although significant genotype × environment interaction was observed, we used genotype means from the combined analysis as the best indicator of winter field survival. Mean winter field survival from the full population had a bimodal distribution (Fig. 3). The distribution of winter field survival, however, was normal for the non-T7C-17 class (Fig. 3), but the population of RILs with T7C-17 was not normally distributed, being skewed toward greater field survival. Winter field survival was significantly correlated with the presence of the translocation (r = 0.61) (Table 2). Mean

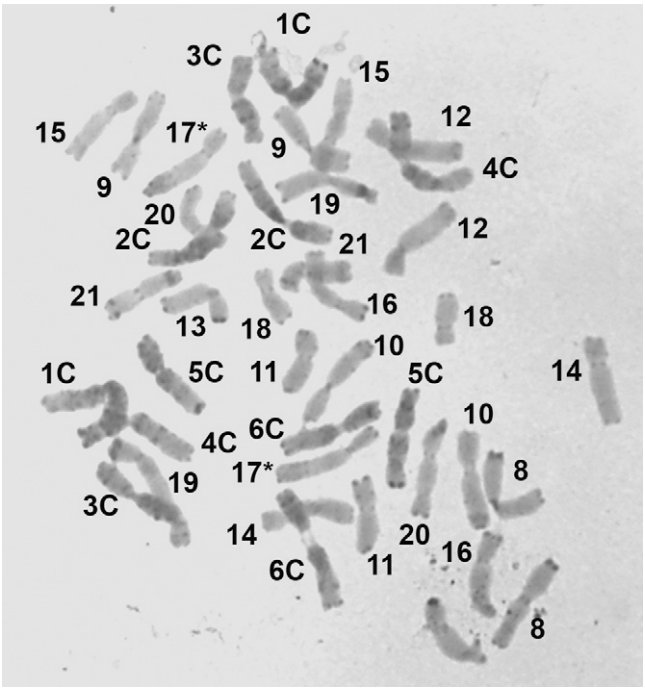

Figure 1. C-banded somatic chromosomes from chromosome $7C^{17}$ nullisomic oat line. Chromosomes are labeled with their number; 17* identifies chromosome 17^{7C}.

Table 1. Population mean and extremes, parental phenotypes, translocation class means, additive translocation effect, and heritabilities for the oat winter hardiness component trait from a recombinant inbred line population derived from a cross of 'Fulghum' × 'Norline'.

Parameter	Crown freezing tolerance	Heading date	Winter field survival			
			Laurel Springs 2004	Laurel Springs 2005	Blacksburg 2005	Combined
	0–10†	DOY‡	%			
Population mean	3.9	118.8	64.8	44.0	73.2	60.6
Population min.	0.2 NS	109.5 NS	4.6 NS	6.0 NS	36.0***	15.5 NS
Population max.	7.5***	128.0***	95.0 NS	81.0 NS	86.0 NS	87.0 NS
Fulghum	0.5 ± 0.6	110.9 ± 1.5	14.0 ± 3.5	7.6 ± 3.8	53.2 ± 2.3	24.9 ± 10.5
Norline	5.7 ± 0.6	122.5 ± 1.5	88.2 ± 3.5	66.2 ± 3.8	81.2 ± 2.3	78.5 ± 10.5
Translocation class mean	4.9 ± 0.6	119.4 ± 1.7	74.1 ± 2.5	51.1 ± 1.8	75.3 ± 1.3	66.9 ± 1.5
Nontranslocation class mean	2.1 ± 0.6	117.9 ± 1.7	45.7 ± 3.3	29.5 ± 2.6	69.1 ± 1.5	48.2 ± 2.0
Heterogeneous class mean	4.4 ± 0.8	117.3 ± 2.5	73.5 ± 9.6	46.1 ± 7.9	71.5 ± 3.6	64.2 ± 5.8
Chromosome $7C^{17}$ nullisomic	2.6 ± 0.7	122.5 ± 1.8	70.0 ± 7.6	16.0 ± 8.4	74.0 ± 4.8	53.3 ± 11.3
Additive translocation effect§	1.4***	0.74*	14.2***	10.8***	3.1***	9.3***
Heritability	83 ± 2	81 ± 17	89 ± 2	78 ± 3	63 ± 5	76 ± 4

*Significance level of $P < 0.05$ between population extreme and most similar parent; NS = not significant.

**Significance level of $P < 0.01$ between population extreme and most similar parent.

***Significance level of $P < 0.001$ between population extreme and most similar parent.

†0 = complete plant death, 10 = no freezing damage.

‡DOY = Day of the Year.

§Additive translocation effect calculated as (mean of translocation class − mean of nontranslocation class)/2.

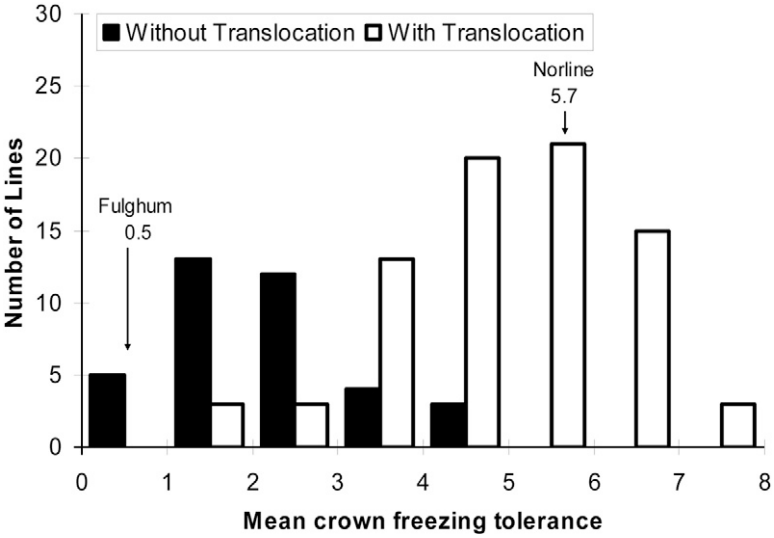

Figure 2. Frequency distribution of crown freezing tolerance line means for homogeneous translocation classes. A population of 116 oat recombinant inbred lines and their parents, non-winter-hardy 'Fulghum' and winter-hardy 'Norline', were evaluated for crown freezing tolerance on a scale from 0 to 10, where 0 = complete plant death and 10 = no freezing damage.

crown freezing tolerance was very highly significantly correlated with winter field survival (Table 2, Fig. 4).

Heading date for the full population was normally distributed, with a heritability of 81 ± 17% (Table 1). The additive effect of the translocation was to increase heading date by 0.74 d. This effect was only significant ($P < 0.05$) for the lines that were homogeneous for the translocation, because there were not enough heterogeneous lines to precisely estimate the heterogeneous class mean. Therefore, it may be more accurate to state that lines uniform for T7C-17 had a heading date 1.5 d greater than lines without T7C-17. The small relative effect of the translocation indicated that the heading date gene associated with the translocation was of minor importance, or not as closely linked to the translocation as the crown freezing tolerance and winter field survival genes.

Very significant genotype, vernalization, photoperiod, genotype × vernalization, and genotype × photoperiod effects were detected in the controlled environment vernalization and photoperiod responses study (data not shown). Translocation status, however, did not have a significant effect on either vernalization or photoperiod response. Translocation status did have a significant effect on days to flowering for vernalized plants and plants under long photoperiod, that is, the conditions most similar to field environments. Because we directly measured field heading date, we present field heading date results instead of days to flowering in the growth chamber. Genotype effect was very significant in ANOVA of the field plant height data, but translocation status was not.

Analysis of lines heterogeneous for the translocation produced inconclusive results. In almost all cases, the heterogeneous class mean was more similar to one of the translocation class means (Table 1), indicating dominant gene effects. The winter field survival and crown freezing tolerance genes from Norline appeared to show dominant gene action, while heading date genes from Fulghum appeared dominant. Orthogonal linear contrasts modeling additive and dominant gene action, however, showed that the dominant gene action was not significant for any trait. This results from the small number of heterogeneous lines in comparison to the lines uniform for the translocation, and the loss of heterozygosity from inbreeding to generate the $F_{6:7}$ and $F_{6:8}$ lines that were evaluated. A RIL population has little power to detect dominant gene action, so the lack of significant dominant gene action effects should not be interpreted as evidence that there were no such effects.

One RIL was nullisomic for chromosome 7C[17] (Fig. 1). This line had normal fecundity and could not be identified as abnormal based on phenotype. It had intermediate crown freezing tolerance (Table 1) and was significantly different from both parents. The comparative winter field survival of the nullisomic line seemed to vary with environment. At Laurel Springs in 2004–2005, the nullisomic line did not have significantly greater field survival than Fulghum, but in the other two environments, it did have significantly greater field survival than Fulghum (Table 1). The nullisomic RIL had a relatively late heading date, equal to Norline. It was significantly shorter than either parent, with a height of 75 cm, compared with 112 cm for Fulghum and 108 cm for Norline. Identification of genetic factors using nullisomic lines can be complicated by the deficiency of nontarget genes, but the data indicated that chromosome 7C[17] carries genes for increased crown freezing tolerance, increased winter field survival, and increased height.

Table 2. Correlation of winter hardiness component traits and translocation status from a population of 120 oat recombinant inbred lines derived from a cross of 'Fulghum' and 'Norline'.

Trait	Winter field survival	Crown freezing tolerance	Heading date	Translocation status
Winter field survival	1	0.73***	0.46***	0.61***
Crown freezing tolerance		1	0.23**	0.72***
Heading date			1	0.20*
Translocation status				1

*Significant at $P < 0.05$.

**Significant at $P < 0.01$.

***Significant at $P < 0.001$.

DISCUSSION

Phenotypic Data

The distribution of RIL freezing tolerance means showed a classic distribution pattern for a population segregating

for a major gene (or linked genes) affecting a quantitative trait. Examination of the distributions of the two translocation classes revealed that the translocation was the controlling factor resulting in the bimodal distribution. The crown freezing tolerance heritability of 83% was greater than the 67% reported by Santos et al. (2006). The greater heritability in this population probably enabled the identification of a bimodal distribution with two normal subpopulations, in contrast to the Fulghum × Wintok population, wherein the larger error variance resulted in a more normal crown freezing tolerance distribution (Santos et al., 2006).

We identified transgressive segregants for increased crown freezing tolerance, indicating that Fulghum donated some alleles for greater crown freezing tolerance, but these genes were not on or near the translocation. No RILs were significantly less crown freezing tolerant than Fulghum, but the crown freezing tolerance of Fulghum was not significantly different from zero. There were only two RILs, however, with crown freezing tolerance means numerically less than Fulghum, so even if the experiment had the power to detect RILs that were less crown freezing tolerant than Fulghum, there may not have been any in this population. These results closely paralleled those in the Fulghum × Wintok population evaluated by Santos et al. (2006), wherein 20% of the RILs were identified as transgressive segregants for increased crown freezing tolerance, and no lines were identified with significantly reduced crown freezing tolerance compared with Fulghum.

The crown freezing tolerance of Wintok, a check cultivar in this experiment, was equivalent to the score reported by Santos et al. (2006). Norline and 51 RILs (43%) were significantly more crown freezing tolerant than Wintok in this experiment. The pedigree of Norline is Lee/Victoria//Forkedeer*2. Forkedeer was a selection from Fulghum (Marshall, 1992), so it is possible that some of the alleles that Fulghum contributed to increased freezing tolerance in the Fulghum × Wintok population were already present in Norline. This would account for the greater percentage of lines that were more freezing tolerant than Wintok, and a lesser percentage of transgressive segregants for freezing tolerance. Only 4% of lines were transgressive segregates for increased freezing tolerance in this experiment vs. 20% for Santos et al. (2006).

The asymmetric distribution of transgressive segregants in both populations suggested that there may have been epistatic interaction leading to greater crown freezing tolerance. Wooten et al. (2008) found an asymmetric distribution of transgressive RILs for crown freezing tolerance in a cross of 'Kanota' × 'Ogle'. They identified complementary gene action among quantitative trait loci (QTLs) for crown freezing tolerance, leading to a greater number of RILs with decreased freezing tolerance. In the Fulghum × Wintok and Fulghum × Norline populations, the number of transgressive segregants indicated that there could be complementary gene interaction for increased crown freezing tolerance. Because all of the RILs that were more crown freezing tolerant than Norline had the translocation, it was possible that alleles from Fulghum on other chromosomes may have interacted through complementary epistasis with the alleles on the translocation, resulting in increased freezing tolerance. It is also possible

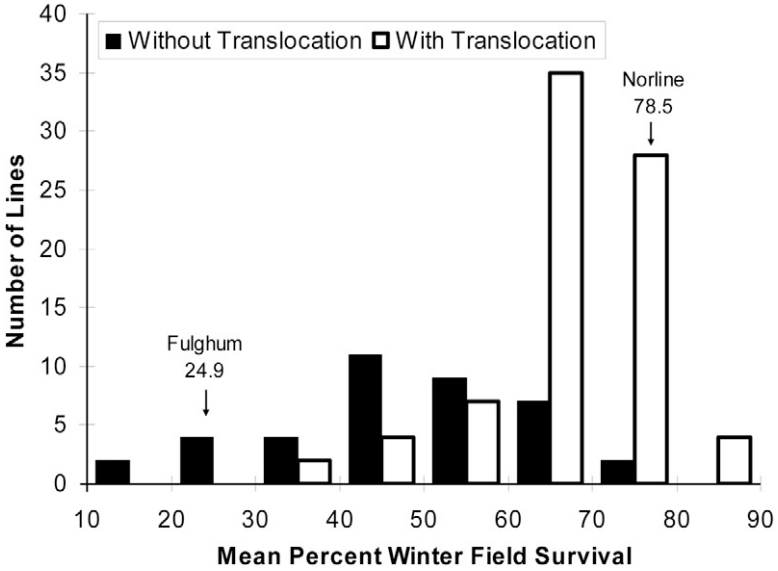

Figure 3. Frequency distribution of winter field survival line means for homogeneous translocation classes. A population of 120 oat recombinant inbred lines and their parents, non-winter-hardy 'Fulghum' and winter-hardy 'Norline', were evaluated for winter field survival in three environments.

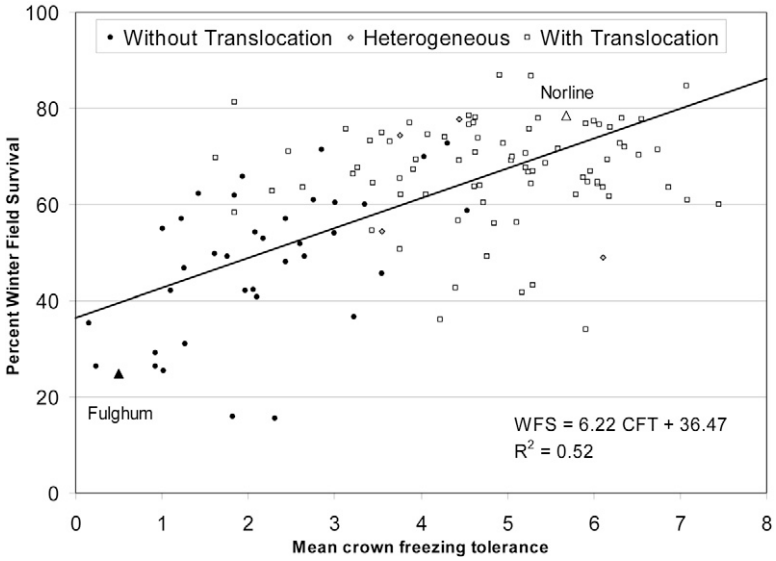

Figure 4. Scatter plot and regression of winter field survival vs. crown freezing tolerance for a population of 120 oat recombinant inbred lines and their parents, non-winter-hardy 'Fulghum' and winter-hardy 'Norline'; WFS = winter field survival, CFT = crown freezing tolerance.

that the segregation distortion of the translocation caused the asymmetric distribution of transgressive segregants. Quantitative trait locus mapping in these populations could determine if these transgressive segregants were the results of epistasis or additive gene action, and such QTLs could be excellent targets for marker-assisted selection to improve crown freezing tolerance.

The great variation between the selection environments for winter field survival illustrates the difficulties in conducting field evaluations for winter hardiness and the need for indirect selection methods such as cytogenetic and molecular markers, or crown freezing tolerance testing. Santos et al. (2006) also observed no winter damage at the Waynesville location in the winter of 1998–1999. Both the field survival mean and heritability for the Fulghum × Wintok population during the 1998–1999 season at Laurel Springs were intermediate between the results from the 2 yr of this study (Santos et al., 2006).

In this study, Fulghum had significantly less winter field survival than Norline or Wintok, which were not significantly different from each other. These results agreed with those reported by Livingston and Elwinger (1995) based on evaluations in 495 environments from the Uniform Oat Winter Hardiness Nursery. It appears that while Norline had a greater crown freezing tolerance than Wintok as measured with this protocol, there was little difference between the winter field survival of the two lines. In the Fulghum × Norline population, it appeared that genes for increased crown freezing tolerance conferred greater field survival. In the Fulghum × Wintok population, there was an additional genetic factor conferring greater winter field survival on Wintok and its progeny that did not increase crown freezing tolerance to the same extent. When considering the number of lines that were transgressive segregants for increased crown freezing tolerance in the two populations, we speculate that the genes for increased crown freezing tolerance that Norline may have inherited from Fulghum may not increase winter field survival to the same degree that they increase crown freezing tolerance. Otherwise, Norline should have had greater winter field survival than Wintok, and there should have been transgressive segregants for increased winter field survival in the Fulghum × Wintok population.

Translocation Effects

Santos et al. (2006) reported a similar segregation distortion for T7C-17; however, they found a ratio closer to 3:1 rather than the 2:1 ratio identified in this study. Duplicate-deficient lines and other cytogenetic abnormalities are relatively common in oat (Wilson and McMullen, 1997), and could contribute to segregation distortion resulting from crosses between oat cultivars. Portyanko et al. (2001) identified several regions of segregation distortion in their map from a cross of 'Ogle' and 'TAM O-301', both of which have the T7C-17. Four major QTLs affecting photoperiod or vernalization responses were associated with regions of segregation distortion (Holland et al., 2002). The molecular markers from linkage groups in the region of T7C-17, however, did not have segregation distortion in the Ogle × TAM O-301 population (Portyanko et al., 2001) or in the Kanota × Ogle population (Wight et al., 2003).

Jellen and Beard (2000) suggested that the translocated portion of chromosome 7CL carries a gene or linked genes critical for survival, noting a lack of homozygous-deficient lines for this chromosomal region. The spontaneous generation of the $7C^{17}$ nullisomic line in this population supports this theory because the translocated portion of chromosome 7CL was on chromosome 17^{7C} in the nullisomic line. The 2:1 segregation ratio found in this population suggests there might have been a nonviable gene combination in this cross. We speculate that Fulghum may carry a homeolog of one or more linked critical genes that are typically on the translocated region of 7CL at some other unlinked location in the genome. The critical genes typically located on chromosome 7CL of non-T7C-17 lines may be missing or nonfunctional in Fulghum 7CL. When crossing Fulghum with a T7C-17 line, those progeny that received the translocated chromosomes had all of the critical genes. Alternately, those lines that received the nontranslocated chromosomes had only a 50% chance of survival, depending on whether they received the unlinked (functional) critical genes from Fulghum. Examination of intergenerational survival during inbreeding could possibly have assisted in identifying the cause of segregation distortion for T7C-17.

This conjecture could explain several observations regarding this translocation. It explains the segregation ratio found in this population, and the viability of the nullisomic line. Also, there has been a shift in winter oat cultivars from non-T7C-17 types to cultivars with T7C-17, possibly resulting from natural selection for the translocation during inbreeding after crossing lines differing for the translocation. Jellen et al. (2004) found that T7C-17 predominated in hexaploid *Avena* species, while non-T7C-17 were only common in *A. byzantina* K. Koch and some *A. sterilis* L. lines that were likely progenitors of *A. byzantina* (Zhou et al., 1999). The placement of two critical genes in close proximity by the T7C-17 may cause selection pressure favoring this arrangement in hexaploid oat species.

The actual untransformed additive effect of the translocation on crown freezing tolerance was almost exactly the same in the Fulghum × Wintok (Santos et al., 2006) and Fulghum × Norline populations. In this experiment, however, the effect of the translocation on winter field survival was less than that reported by Santos et al. (2006). Even in the Laurel Springs 2003–2004 season, which had

the greatest heritability and translocation effect, the additive effect of the translocation was only 14.4% in Fulghum × Norline, compared with 20.5% in the Fulghum × Wintok population (Santos et al., 2006). These similar T7C-17 effects on crown freezing tolerance and different T7C-17 effects on winter field survival seemed to indicate that while Norline and Wintok had the same genes for crown freezing tolerance on or near the translocation, Wintok had an additional gene or genes associated with the translocation that increased winter field survival. Because Norline had greater crown freezing tolerance, but the same translocation effect, Norline probably had genes for increased freezing tolerance at another genomic region or regions. Livingston and Elwinger (1995) found that Wintok and Norline had similar mean winter field survival but often performed differently in some environments. Germplasm lines with increased crown freezing tolerance were developed from a cross between Norline and Wintok (Livingston et al., 2004) and some of these were significantly more winter hardy than either Norline or Wintok (Livingston, Uniform Oat Winter Hardiness Nursery, unpublished data, 2004–2006). These results from crosses of Norline and Wintok support the hypothesis that Wintok has a gene for winter field survival associated with T7C-17 not present in Norline, while Norline has genes not present in Wintok for crown freezing tolerance that are not associated with T7C-17. Quantitative trait locus mapping of winter field survival and crown freezing tolerance in a population from a cross of Norline × Wintok could identify these genes and provide a means to select extremely winter-hardy lines.

Crown freezing tolerance and winter field survival for the RILs heterogeneous for the translocation were more similar to the performance of lines homogenous for T7C-17. Similar results were reported in the Fulghum × Wintok population (Santos et al., 2006). This indicates that the freezing tolerance and winter field survival genes on T7C-17 are at least partially dominant. Dominant or partially dominant alleles for increased crown freezing tolerance or winter survival may allow effective early-generation selection within segregating populations resulting from crosses between parents with differing T7C-17 status. The heading date of heterogeneous RILs was earlier than the mean heading date of the RILs lacking T7C-17. This suggests that the earliness genes from Fulghum near the translocation are probably dominant, and also that the apparent dominant gene action was not caused by segregation distortion within heterogeneous lines. Segregation distortion could have caused lines to be more similar to Norline, as was the case with crown freezing tolerance and winter field survival, but segregation distortion within the heterogeneous RILs could not have caused them to be more similar to Fulghum, as was the case for heading date.

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