ABSTRACT

WOOTEN, JR., DAVID RUDOLPH. New genetic tools for improving oat winter hardiness. (Under the direction of David P. Livingston, III and J. Paul Murphy).

Winter hardiness is a complex trait, and poor winter hardiness limits commercial production of winter oat. The identification of new genetic tools to improve oat winter hardiness will allow for continued increases in oat winter hardiness. The objectives of this research were: identify quantitative trait loci (QTL) for crown freeze tolerance in the 'Kanota' x 'Ogle' mapping population, evaluate the winter hardiness component traits winter field survival, crown freeze tolerance, heading date, plant height, and vernalization responses in a population derived from a cross of winter tender 'Fulghum' with winter hardy 'Norline', examine the relationships between winter hardiness component traits and a reciprocal intergenomic translocation between chromosomes 7C and 17 in the Fulghum x Norline population, develop a genetic linkage map in the Fulghum x Norline population using SSR markers and selected RFLP markers, and identify QTL for winter hardiness component traits in the Fulghum x Norline population.

In the Kanota x Ogle population seven QTL and four complimentary epistatic interactions were identified that accounted for 56% of the phenotypic variation. Ogle contributed alleles for increase crown freeze tolerance at three loci, while Kanota contributed alleles for increase crown freeze tolerance at four loci. All loci where Kanota alleles increased crown freeze tolerance showed complementary epistasis for decreased crown freeze tolerance with the QTL near UMN13. Two of the major QTL identified were in the linkage groups associated with the 7C-17 translocation.

In the Fulghum x Norline population, 7C-17 translocation did not segregate in the expected 1:1 ratio, and twice as many translocation types as non-translocation types were observed. The translocation was significantly correlated with crown freeze tolerance (r=0.72) and winter field survival (r=0.62). The heritability of crown freeze tolerance was 83% and the heritability of winter field survival was 76%. Field heading date was significantly correlated with translocation status (r=0.20). Plant height, vernalization response, and photoperiod response were not associated with the translocation. QTL were identified for winter field survival, crown freeze tolerance, vernalization response, plant height and heading date, and epistatic interactions were identified for all of these traits except plant height. Major QTL for winter field survival (R^2=35%) and crown freeze tolerance (R^2 =52%) were identified on linkage group FN3 which was associated with the 7C-7 translocation.

In this research, QTL for freeze tolerance in known linkage groups were identified for the first time in oat and specific QTL for winter hardiness component traits were identified in winter oat for first time. The importance of the 7C-17 translocation to oat winter hardiness traits was confirmed. Comparison with previous results explained differential performance between the hardy winter oat cultivars Norline and Wintok. These results suggested how the 7C-17 translocation conferred increased biological fitness in most *Avena* germplasm.

NEW GENETIC TOOLS FOR IMPROVING OAT WINTER HARDINESS

by

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DEDICATION

I dedicate this work to my grandparents Harry and Fran Rouse. One of my earliest memories is of planting watermelons on their farm in western Illinois. They started me on this path many years ago. I am very happy where it has led me.

BIOGRAPHY

David Wooten was born in West Columbia, South Carolina. He was raised in Colorado Springs, Colorado, until moving to Houston, Texas just before high school. He graduated from Klein High School and attended both the University of Texas at Austin, and Texas A&M, College Station before graduation from Texas A&M with B.S. degrees in Genetics and Plant and Environmental Soil Science in 1999. He continued his studies at Texas A&M working in the sorghum breeding program under the direction of Dr. William Rooney. In 2001 he received a M.S. degree in Plant Breeding and left Texas to begin studies at North Carolina State University. Beginning in 2002, he worked in the small grains breeding program at NC State. His dissertation research examined genetic tools for improving oat winter hardiness, under the direction of David Livingston and Paul Murphy. He finished his PhD in Crop Science in 2006 and accepted a position as a soybean breeder for Monsanto in Stonington, Illinois.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

Literature Review

Introduction

Winter hardiness is an important limitation to winter oat (*Avena sativa* L and *A. byzantina* K. Koch) production in North America. Oat is more sensitive to low temperature than other winter cereals which confines winter oat production to warmer areas of the US. Although breeding for winter hardiness can be difficult, substantial progress was made early in the 20th century (Marshall, 1992; Livingston and Elwinger, 1995). This resulted in the expansion of winter oat adaptation from Southeast coastal areas in 1920 to most areas south of the Ohio River by 1960 (Marshall, 1992). However, as US oat acreage declined, breeding efforts were curtailed and progress improving winter hardiness slowed. Little progress was made after 1970 (Marshall, 1992; Livingston and Elwinger, 1995).

Breeding for winter hardiness is difficult due to trait genetic complexity and confounding environment factors. Winter hardiness genetics are influenced by several quantitative traits including crown freeze tolerance, vernalization response, photoperiod response, and heading date. Additional localized environmental factors make selection for winter hardiness difficult (Marshall, 1992). Yearly variation in winter severity alters the suitability of particular locations for winter hardiness from year to year (Livingston and Elwinger, 1995).

To address the difficulty in screening oat lines for winter hardiness, the Uniform Oat Winter hardiness Nursery was initiated in 1926 (Coffman, 1965). Researchers also developed methods to screen lines for specific components of winter hardiness (such as freeze tolerance) in controlled environments (Marshall, 1965). Molecular biology introduced possible alternatives to traditional winter hardiness screening procedures. Several investigators reported QTL for some winter hardiness component traits in oat

(Holland et al., 1997; Holland et al., 2002), diploid wheat (*Triticum monococcum*) (Vagujfalvi et al., 2003; Yan et al., 2003), bread wheat (*T. aestivum* L.) (Fowler and Limin, 2004; Kobayashi et al., 2005; Limin and Fowler, 2002; Toth et al., 2003), and barley (*Hordeum vulgare* L.)(Francia et al., 2004; Hayes et al., 1993; Pan et al., 1994). This approach is particularly suitable for a trait like winter hardiness that is sporadically expressed. If winter hardiness QTL are identified in winter oat parental material, then more experimental progeny could be screened without dependence on variable environmental conditions.

The objectives of this research were:

1. Identify quantitative trait loci for crown freeze tolerance in the Kanota x Ogle mapping population.
2. Evaluate the winter hardiness component traits winter field survival, crown freeze tolerance, heading date, plant height, and photoperiod and vernalization responses in a population derived from a cross of winter tender 'Fulghum' with winter hardy 'Norline'.
3. Examine the relationships between winter hardiness component traits and a reciprocal intergenomic translocation between chromosomes 7C and 17 in the Fulghum x Norline population.
4. Develop a genetic linkage map in the Fulghum x Norline population using SSR markers and selected RFLP markers.
5. Identify QTL for winter hardiness component traits in the Fulghum x Norline population.

Oat Usage and Production

Oat (*Avena sativa* L and *A. byzantina* K. Koch) is primarily utilized as a food, a feed and for forage. Dehulled oat grain is consumed by humans, oat grain is used as quality animal feed, and oat is used as a forage source for grazing animals. Additionally, oat groats and oat extracts are used in a variety of personal care products, such as soaps and skin creams.

The majority of oats produced worldwide is used as animal feed (Murphy and Hoffman, 1992). The majority of North American oat consumption comes from on-farm feeding, and oat production closely tracks oat consumption (Murphy and Hoffman, 1992). The quantity of oat used for animal feed in North America decreased dramatically since 1950. However, publicity relating the health benefits of consuming whole grain oats has resulted in increased human consumption, but net oat consumption is substantially lower. In 1999 approximately 2.5 million oat acres were harvested. This contrasts with historic maximums of approximately 42 million acres in 1926 and 1946 (USDA, 2000). World production also has decreased, but increased acreage in former USSR has offset some oat decreases in North America.

Oat cultivars generally are classified as spring or winter types. Spring oat accounts for the majority of world production (Marshall, 1992), while winter oat accounts for almost all production in North Carolina and areas to the south. Spring oat is planted in early spring and harvested in summer. Winter oat is planted in the fall and harvested in late spring or early summer. Winter oat grows vegetatively in the fall, then is dormant in winter. In the spring, if it recovers from winter damage, winter oat grows vegetative before flowering in early spring and maturing in the late spring or early summer. In the deep-south, farmers

graze oat during the winter providing a key source of winter forage. Cattle are removed in late winter to allow the oat to recover, flower, and produce grain.

Oat is the most sensitive winter cereal to temperature extremes. Hot or dry weather can cause severe damage, especially during or after flowering, while freezing cold temperatures can cause freeze damage (Marshall, 1992). However, oat tolerates cool summer growing conditions better than other cereals (Marshall, 1992). Temperature sensitivities dictate where oats are cultivated in North American. Winter oat is planted in warmer areas where mild winters lower winter damage, and maturation in late spring avoids the heat stress of hot southern summers. In northern areas, cold winters make winter oat cultivation impossible, but cooler summers are less likely to cause heat stress damage (Marshall, 1992).

Origin

Most *Avena* species are native to the Mediterranean Basin or the Fertile Crescent region of southwest Asia. Oat most likely was domesticated in conjunction with cultivation of domesticated wheat and barley (Murphy and Hoffman, 1992). The spread and development of agriculture based on the early domestication of wheat, barley and several animals in southwest Asia caused a simultaneous dissemination of the progenitor of cultivated oat, *A. sterilis* L. (Murphy and Phillips, 1993; Zhou et al., 1999) in weedy mixes of cereals consumed by humans and animals (Murphy and Hoffman, 1992). Human-imposed selection pressure in these weedy cereal mixes, and possibly their induced reproductive separation from related weeds, drove the domestication of oat and rye (*Secale cereale* L.) as secondary crops (Murphy and Hoffman, 1992). Additionally, as agriculture

developed, humans were more likely to recognize and attempt to exploit variation within cultivated plants. These attempts continue today.

Cytogenetics

Oat has a large and complex genome. Both species of cultivated oat (*A. sativa* and *A. byzantina*) are hexaploid with chromosome number 2n=6x=42 (Thomas, 1992). The three genomes in cultivated oat species are denoted AACCDD. All species of hexaploid *Avena* (*sativa*, *byzantina*, *fatua* s*terilis*, *hybrida*) are inter-fertile when hybridized, share the same ploidy level, and can be considered a single biological species (Ladizinsky 1988). However, there are several translocations and inversions that can disrupt some chromosome pairing in interspecific hybrids (Thomas, 1992; Jellen et. al., 1994). Researchers have found diploid and tetraploid species of *Avena* with similar chromosome structure to the A and C genomes of hexaploid *Avena*, but the specific donors of the A and C genomes found in hexaploid species have not been identified (Thomas, 1992). No diploid or tetraploid species have been found that contain the D genome. Unlike wheat, the specific development of the oat genome remains unknown (Thomas, 1992).

Cytogenetic changes have accompanied the adaptive radiation of oat. The genomes of cultivated hexaploid oat species (*A. sativa* and *A. byzantina*) show multiple genomic rearrangements between species (Jellen et al., 1994) and even cultivars (Jellen et al., 1997; Fox et al., 2001). These translocations, combined with the genetic flexibility conferred by the hexaploid nature of oat, allow for the survival of duplicate deficient lines (Wilson and McMullen, 1997). These lines do not have a complete hexaploid genome. Almost all cultivars of *A. sativa* have a reciprocal translocation between chromosome 7C and 17L

(T7C-17), while most cultivars of *A. byzantina* do not (Jellen et al., 1994; Zhou et al., 1999). Jellen and Beard (2000) suggested this translocation may be an example of the "adaptive gene cluster hypothesis" as proposed by Stebbins (1971). Further, the authors noted the difficulty in deriving aneuploid oat lines that are missing the translocated segment of 7C. A near complete series of nullisomic have been derived from the cultivars 'Kanota' (non T7C-17) and 'Sun II' (T7C-17) but no lines have been derived that are missing the translocated portion of 7CL. This suggested that essential genes may be located on this translocated chromosome segment.

Most spring oat lines are derived from *A. sativa* and most winter oat lines are derived from *A. byzantina* (Zhou et. al., 1999), although breeding efforts have resulted in extensive hybridization between the two species (Brown and Patterson, 1992). As a result, almost all spring oats have the 7C-17 translocation, while most traditional winter oats do not (Zhou et al., 1999; Jellen and Beard, 2000). These results suggested that the genes near the T7C-17 influence growth habit and are worthy of investigation as possible QTL for winter hardiness.

Winter Hardiness

Winter hardiness in small grains is the ability to survive and recover from the variety of stresses associated with growth in the winter season. Generally, rye (*Secale cereale* L.) is the most winter hardy followed by triticale (x *Triticosecale* Wittmack), wheat, and barley, while oat is the least winter hardy of the winter cereals. Winter hardiness is a complex trait controlled by several interacting quantitative traits and heavily influenced by the environment. Freeze (frost) tolerance, specifically the ability of the crown to survive freezing temperatures, is the most important genetic component of winter hardiness,

(Marshall, 1965; Gullord et. al., 1975; Fowler et. al., 1999). Other traits affecting winter hardiness include vernalization response (Brule-Babel and Fowler, 1988; Roberts, 1989; Fowler et. al., 1999,), photoperiod response (Holland et al., 2002; Mahfoozi et al., 2001; Snape et al., 2001)), and *per se* maturity (Prasil et al., 2004).

Freeze Damage

In most freeze-adapted plants species, freeze damage results from ice formation within the plant, also called internal ice stress. Three primary types of freeze induced stresses damage plants: intracellular ice formation, ice adhesion, and ice induced dehydration. Thawing produces additional specific stresses during the freeze-thaw cycle.

Intracellular Ice Intracellular ice formation is the most sudden and destructive form of freeze damage, usually resulting in cell death (Olien, 1967). Super-cooling is the primary cause of intracellular ice formation although super-cooling *per se* does not result in intracellular ice formation (Olien and Smith, 1977; Steponkus, 1984). When protoplasts become super-cooled in the presence of an ice nucleation agent, the cell contents can suddenly freeze (Steponkus, 1984). During the freezing process there are two possible causes of super-cooling. When the temperature of the plant drops below freezing the entire plant becomes super-cooled and intracellular ice formation is a possibility. However, ice nucleation, and subsequent freezing, typically occurs in the extra-cellular space, especially in cold acclimated plants (Olien, 1967). The cell membrane effectively excludes ice from the cell protoplast, so intracellular ice formation generally does not occur in freeze hardy plants (Olien, 1967; Olien and Smith, 1977; Levitt, 1980; Steponkus, 1984). As the water in the extra-cellular space freezes, the water potential is lowered and water within cells diffuses

out of the cell to maintain equilibrium. The equilibrium freeze process effectively prevents intracellular ice formation, however intracellular ice formation is possible under non-equilibrium freezing conditions (Levitt, 1980; Steponkus, 1984). When temperatures drop quickly, water may not diffuse from the cell quickly enough and super-cooling (with the possibility of intracellular ice formation) results (Levitt, 1980; Steponkus, 1984).

Adhesion Olien and Smith (1977) proposed the theory of adhesive stress during freezing. As temperature decreases, the quantity of liquid water in the plant decreases which leads to competition for the remaining liquid water between the growing ice lattice and the hydrophilic portions of the cell membranes. The competition for water causes the cell membrane to adhere to growing ice crystals. As ice crystals are generally angular and protoplasts are not, this adhesion can cause severe distortion in the cell walls and membranes as observed by Olien and Smith (1977). The angular distortion of the cell membrane causes damage and may result in cell death (Olien and Smith, 1977). Adhesive freezing stress is predicted thermodynamically in the temperature range near –10 °C (Olien and Smith, 1977), corresponding to the LT50 temperature range of most oat cultivars (Livingston, 1996). Thus, freeze stress in the form of adhesion may be an important aspect of freeze damage in oat.

Dehydration Freeze dehydration, also called frost desiccation, is the primary cause of freeze injury at temperatures lower than –10 °C (Olien, 1967; Levitt, 1980; Steponkus, 1984). Freeze dehydration may cause cell damage through several mechanisms. Some authors hypothesize that "solution effects"—disruptive changes in pH and cellular solute concentrations—cause cellular damage. However, cell membrane damage is the most important (reviewed by Steponkus, 1984). Freeze dehydration may cause transition of

membrane phospholipids from laminar arrangement to a hexagonal II arrangement (Steponkus, 1984). Steponkus (1984) suggested that this may cause the plasma membrane to lose its osmotic responsiveness, resulting in frost plasmolysis and cell death.

Dehydration causes the cell to contract, necessitating a decrease in membrane surface area. When temperatures rise, cell membranes must expand to accommodate infusing water. However membranes may not be capable of expansion resulting in expansion-induced lysis (Dowgert and Steponkus 1984; Steponkus 1984; Dowgert et al., 1987).

Freeze Tolerance

Because plants are unable to self regulate temperature, the only method of freeze resistance is freeze tolerance (Levitt, 1980). Plant freeze tolerance is not constitutive, but must be induced (Olien, 1976; Levitt, 1980). Exposure to low non-freezing temperatures increases freeze tolerance through a process termed cold acclimation or cold hardening. Cold acclimation dramatically improves the cold tolerance of winter cereals (Levitt, 1980; Livingston, 1996; Fowler et. al. 1999). Cold acclimating effects accumulate during the time plants are exposed to cold temperatures, but these effects can be quickly lost when exposed to warm temperatures.

In addition to cold acclimation at temperatures just above freezing, some species of acclimated plants will accumulate additional low temperature tolerance if kept frozen at subzero temperatures near freezing (Trunova, 1965; Livingston, 1996). This additional hardening is called subzero acclimation. Livingston (1996) showed that winter hardy oat cultivars showed a greater response subzero acclimation (measured by change in LT50) than winter tender oats.

Cold acclimation results in a large variety of changes in cells that generally fit three non-exclusive classes: alterations in cell membranes, production or excretion of carbohydrates and specific protein production.

A freeze-thaw cycle causes great stress on the cell membrane. Freeze dehydration causes the cell to shrink, while subsequent thawing can cause a rapid increase in cell volume resulting from diffusion of thawed water into the cell. Several changes in cell membranes during cold acclimation help prepare the cell for the stress of the freeze thaw cycle. Dowgert and Steponkus (1984) found cold acclimated protoplasts tolerated a much greater increase in cell surface area following freeze dehydration. Later authors observed that the plasma membrane of freeze-dehydrated cold-acclimatized cells were more uniformly convoluted and that the cells had more exocytotic vesicles available for later membrane expansion during thawing (Singh and Johnson-Flanagan, 1987). Steponkus et al. (1988) showed that increasing the proportion of unsaturated lipids in the cell membrane caused non cold-hardened rye protoplast membranes to behave and survive freeze tests similar to cold hardened rye protoplasts. The authors also showed that the proportion of free phospholipids and free sterols increased in the plasma membrane fraction of cold acclimated protoplasts, while the proportion of sterol glycosides and other glycosides decreased (Steponkus et al., 1988). Apparently plant cells increase plasma membrane low temperature fluidity resulting in increased freeze tolerance through tolerance of contraction and expansion during a freeze thaw cycle. However, Steponkus (1984) cautioned that some of these changes may result from adaptation to cold temperatures, and may not increase low temperature tolerance.

Winter cereals accumulate carbohydrates in crowns during cold acclimation (Olien, 1984; Livingston, 1991; Livingston and Henson 1998). Winter cereals store carbohydrates

as fructans (fructose polymers) and as the simple sugars fructose, glucose and sucrose. This contrasts with warm season cereals such as corn (*Zea mays*), which store carbohydrate as starch, a glucose polymer. Winter cereals convert simple sugars to fructans with a higher degree of polymerization during cold acclimation. During subzero cold acclimation this trend reverses. Total carbohydrate and fructan concentrations decrease while simple sugar concentration increases (Trunova, 1965; Livingston, 1996; Livingston and Henson, 1998). Livingston and Henson (1998) found that the concentration of simple sugars outside of the cell increased during subzero cold acclimation. This occurs after a portion of the plant water has already frozen.

Several mechanisms have been proposed for the role of carbohydrates in cryoprotection. Carbohydrates have been shown to stabilize membranes during freezing (Santerius, 1973), and fructans have been shown to be particularly adept at membrane stabilization during freeze or dehydration stress (Amiard et al., 2003; Hincha et al., 2000; Hincha et al., 2002). Additionally, de-polymerization of fructan during freeze desiccation increases the osmotic concentration of carbohydrates in the cellular contents, thus lowering the water potential of the cell. This allows the cell to resist freeze desiccation and maintain a greater cell volume. The increase in extra-cellular simple sugars detected by Olien and Lester (1985) and Livingston and Henson (1998) indicated that extra-cellular carbohydrates may confer resistance to adhesive stress during freezing. As the volume of liquid water decreases, the hydrophilic portions of the cell membrane and cell walls compete with the growing ice crystals for the remaining liquid water resulting in adhesion stress. Increasing the extra-cellular sugar concentration near the plasma membrane theoretically would decrease cell membrane stress from adhesion (Olien, 1984; Livingston and Henson, 1998),

and slightly inhibit the freezing of the remaining liquid water. Both of these processes would reduce the adhesive stress in the cell

Cold acclimation induces the synthesis of numerous proteins (Perras and Sahran, 1989). Alterations in metabolic enzymes have been reported by numerous authors, however the role of these proteins in freeze tolerance is unknown as they may only be a response to metabolism at low temperature (Reviewed by Guy, 1990). Other specific proteins, or protein families, have specific effects on the freeze process and freeze tolerance. Later researchers discovered that intracellular proteins prevent the growth of ice crystals in acclimated plant cells (Antikaine and Griffith, 1997). Other proteins are thought to stabilize membranes during the freeze thaw cycle. Danyluk et al. (1998) used antibodies to illustrate how a specific group of acid dehydrin proteins accumulated near the plasma membrane during cold hardening, and this accumulation correlated with low temperature tolerance among cultivars. Numerous other proteins in the hydrophilic non-enzymatic family of late-embryogenesis abundant proteins have been identified with cold acclimatization (Thomashow, 1998). Investigators believe that these proteins serve as cryoprotectants by stabilizing cell membranes during freeze dehydration (Thomashow, 1998).

Freeze Testing

The roots and leaves of oats may be damaged during winter, however it is the ability of the crown of the plant to regrow roots and shoots that insures winter survival. If the crown is killed, recovery is not possible and the plant will die (Olien, 1967). More precisely, it is irreversible damage to either the apical region or the crown support structures in acclimated plants that prevents recovery from freeze stress (Livingston et al., 2005). Marshall (1965)

utilized the importance of crown survival to develop the crown freeze test for determining cold resistance. This procedure freezes only the crown of the plant, which is then re-planted and later scored for survival or recovery (Marshall, 1965). Modified versions of the technique have been developed (Gullord et al. 1975; Marshall and Kolb, 1982; Brule-Babel and Fowler, 1988; Livingston, 1996; Santos et al., 2006), demonstrating good correlation between crown freeze tests and field winter survival (Marshall, 1965; Santos et al., 2006). Crown freeze tolerance testing has proven an effective indirect selection technique for improving field winter survival in oat (Livingston et al., 1992; Livingston et al., 2004; Marshall and Kolb, 1982).

Although low temperature tolerance may be the most important plant trait determining winter hardiness, other traits including vernalization and photoperiod response (Doll et al., 1989; Dubcovsky et al., 2006; Flood and Halloran, 1986; Fowler et. al., 1999; Holland et al., 2002; Roberts, 1990; Snape et al., 2001) also play important roles. While winter cereals are relatively freeze hardy in the vegetative portion of the life cycle, the inflorescence and floral primordia (*i.e*. reproductive organs) are easily damaged by freeing temperatures. Vernalization and photoperiod requirements combine with *per se* maturity genes to insure that plants will flower in the spring and avoid winter freezes (Prasil et al., 2004; Snape et al., 2001; Worland and Snape, 2001).

Vernalization response interacts with cold acclimatization which affects freeze hardiness during exposure to cold temperatures. Initially, cold temperatures induce cold acclimation, which increases freeze tolerance. However low temperature exposure eventually induces vernalization response which decreases freeze tolerance, and renders crowns unable to re-harden after periods of warmer temperatures (Fowler and Limin, 2004).

After several weeks of cold temperatures the freeze tolerance of some cultivars begins to decrease (Sutka, 1994; Prasil et al., 2004). While photoperiod and vernalization response interact to determine maturity, *per se* maturity tends to have a simple effect on winter hardiness (Prasil et al., 2004). Later maturity increases winter hardiness by avoiding the exposure of sensitive organs to low temperature.

Genetics of Winter Hardiness

The genetics of winter hardiness are complex because winter hardiness is controlled by several interacting quantitative traits. Genes for freeze tolerance, vernalization response, photoperiod response, and other traits interact to determine winter hardiness.

The winter hardiness of wheat and barley has been extensively researched and provided a valuable understanding of the genetic basis of oat winter hardiness. Inheritance studies in wheat and barley have shown that winter hardiness is controlled by multiple loci, but contradictory results with regard to specific genetic effects have been reported. Brul-Babel and Fowler (1988) found additive-dominant resistant effects. Two diallel crosses of six and ten winter wheat parents by Sutka (1994) showed general combining ability to be most important in predicting progeny cold tolerance, indicating additive and dominant effects. Gullord (1974) found primarily additive effects with some partial dominance in wheat. Later examination showed significant interaction of cultivars and freeze intensity (Gullord et al., 1975) with genes switching from dominant at low freeze intensity to recessive at high freeze intensity, possibly explaining the contradiction. In barley, Enus et al. (1962) found most of the 18 parents in a diallel contributed dominant winter hardiness genes, but the hardiest parent contributed primarily recessive freeze tolerance genes.

Oat genetic studies have not been as intensive but have had similar mixed conclusions. Amirshahi and Patterson (1956) found that cold tolerance of progeny from 20 crosses were normally distributed about the mean of the parental values, which indicated simple additive genetic effects. However, Muehlbauer et al. (1970) found that specific combining ability was the most important factor in determining the cold tolerance of progeny from 18 winter by spring crosses which indicated the importance of epistatic interaction and dominant effects rather than additive gene effects. Interestingly, both authors concluded that winter hardiness had a high heritability and that breeding for winter hardiness should be successful. However, no new cultivars have been released with greater winter hardiness than the checks used in these early experiments.

Due to the complex interactions among winter hardiness component traits, some molecular and cytogenetic techniques have been more successful in discovering the genetic basis of winter hardiness. Wheat researchers initially identified chromosomes controlling winter hardiness traits, subsequently identified QTL, and recently have cloned specific winter hardiness genes. Initial studies using chromosome substitution lines provided the first means of identifying and localizing winter hardiness loci on chromosome 5A. Snape et al. (1985) examined the role of the *Vrn1* gene on chromosome 5A in determining winter or spring type. Later, chromosome 5A was associated with cold tolerance (Sutka and Veisz, 1988; Roberts 1990). Sutka and Veisz (1988) used chromosome substitution lines and found that a gene on chromosome 5A controlled freeze tolerance and that it was recessive at mild freeze temperature and dominant at low temperature. Roberts (1990) made crosses with chromosome 5A substitution lines and was able to associate freeze resistance with the vernalization gene *Vrn1*. He suggested the possibility of a freeze resistance gene linked to

the *Vrn1* gene but could not find evidence of recombination between them. Eventually Galiba et al. (1995) observed and tracked recombination between the *Vrn1* and *Fr1* loci (a freeze tolerance gene) using RFLP markers; this confirmed the presence of two distinct genes.

Other chromosomes were found to contribute to winter hardiness when scientists examined the complete set of chromosome substitution lines. Veisz and Sutka (1989) found that chromosomes 5A, 5B, 5C, 4B, and 7A increased frost tolerance when they were introduced from winter hardy cultivar Cheyenne to winter tender cultivar Chinese Spring. Later Sutka (1994) showed that chromosomes 2B, 4B, 5A, 5B, 5D, 6A, and 6D increased field winter hardiness using the same genetic stocks. Snape et al. (1997) used QTL mapping techniques to locate a *Vrn2–Fr2* interval on chromosome 5D. Eventually the position of these QTL were more definitely identified allowing the cloning of the *Vrn1* and *Vrn2* genes in diploid wheat (Yan et al., 2003; Yan et al., 2004), and later the cloning of vernalization and freeze tolerance genes in common wheat (Kobayashi et al., 2005). The sequences of these cloned genes allowed for the identification of orthologous genes in other grass species (Andersen et al., 2006; Jensen et al., 2005). Similar studies are currently underway examining orthologs of these genes in oat (J. Preston, University of Missouri, St. Louis , personal communication). This methodology may provide conclusive evidence of the role of specific winter hardiness component trait QTL that have been or will be identified in oat.

QTL mapping of winter hardiness traits in barley found a chromosome interval controlling winter hardiness, freeze tolerance, maturity, and crown fructan content on chromosome 7 (Hayes, et al., 1993; Pan et al. 1994), homoeologous to group 5 wheat chromosomes. Later candidate genes were identified for the vernalization genes

(Dubcovsky et al., 2005; Von Zitzewitz et al., 2005). QTL or candidate genes for winter hardiness component traits have been identified on other chromosomes with the likely location of *Vrn-H1* locus on chromosome 7 and photoperiod QTL on chromosome 2 (Snape et al., 2001)

Oat winter hardiness quantitative trait loci analysis was first conducted by Santos (2000) using a population of 220 recombinant inbred lines from the cross of winter hardy Wintok by winter tender Fulghum. He found several loci for winter hardiness but the interval with the greatest winter hardiness effect also had the greatest effect for crown freeze resistance, maturity, vernalization, and growth habit (Santos, 2000). It is possible that this interval represents a winter growth region homoeologous to the regions on wheat group 5 chromosomes and barley chromosome 7. However due to the system of molecular markers used, it was not possible to associate the markers with specific chromosomes or other oat linkage maps.

The introduction of microarray technology has allowed for the potential to detect differential mRNA expression between winter hardy and winter tender cultivars, or even between plants under different environmental conditions, such as cold acclimating plants vs. plants in warm temperatures. To begin this process, Bräutigam et al. (2005) identified a large collection of EST from oat plants exposed to low temperatures. It seems likely that many of these will be genes for freeze tolerance or other winter hardiness traits. In the mean time, the use of reverse transcriptase PCR has been successful in examining the differential expression of mRNA for winter hardiness genes in wheat (Yan et al., 2004) and oat (J. Preston, personal communication).

Genetic Mapping

Gregor Mendel proposed the law of independent assortment after his famous investigation into the genetics of peas (*Pisum sativum*). To paraphrase his findings, alleles for different traits assort independently. For most alleles this law is true. However in the early 1900s, T. H. Morgan showed that two recessive alleles in *Drosophila* (white eye and miniature wing) assorted together more often than not, that is inheritance of one was linked to the other. He knew both traits were sex-linked so he knew both were on the X chromosome and correctly concluded that this was the reason they violated the law of independent assortment. With the chromosome linkage theory, independent assortment provides the null hypothesis for a chi-square linkage test, while significant violations are evidence of linkage disequilibrium.

Once loci are shown to be linked, it is informative to determine how tightly they are linked and how often genetic recombination allows for non-parental assortment. The tighter two loci are linked the less frequent is genetic recombination (r). Mather (1951) demonstrated a maximum likelihood method for determining recombination frequency; computer genetic linkage mapping programs (like Mapmaker/EXP) use modifications of such methods (Lander et al., 1987; Lander and Botstein, 1989; Lincoln et al., 1993a).

When recombinations between multiple loci are known, it is possible to order the loci in a linkage group which theoretically represents a chromosome or a portion of a chromosome. This process is simple for a small number of linked loci, but becomes extremely complex as loci numbers increase. Computer linkage mapping programs use multipoint maximum likelihood linkage mapping to order large numbers of loci (Lander et

al., 1987; Lincoln et al., 1993a). This ordering of loci is a key step for creating a genetic map.

When examining recombination frequencies between an ordered loci set, it becomes apparent that recombination frequencies between loci are not additive with the intervening loci due to the maximum recombination frequency between two loci of 0.5. In an attempt to correct this problem, several authors have proposed mapping functions. Mapping functions are used to convert recombination frequencies to map distances. In theory, the map distances should be additive. Haldane (1919) proposed the mapping function

$$m= -[\ln(1-2r)]/2$$

where m is (additive) map distance and r is recombination frequency. This function is derived simply from mathematical principles. Kosambi (1944) proposed the function

$$m=(1/2)*\ln[(1+2r)/(1-2r)].$$

This function assumes that when one recombination event occurs, it prevents other nearby recombination events, a phenomenon known as interference (Kosombi, 1944). While these (and other) mapping functions attempt to convert recombination frequencies to map distances, they are only approximations.

O'Donoughue et al. (1992) developed a Restriction Fragment Length Polymorphism (RFLP) based genetic linkage map using a cross of two diploid species, *A. atlantica* and *A. hirtula*. Later O'Donoughue et al. (1995) developed a linkage map of cultivated hexaploid oat using 71 recombinant inbred lines from a cross of *A. byzantina* cv. Kanota and *A. sativa* cv. Ogle. This linkage map originally consisted of 38 linkage groups, but further investigation has reduced the number to 29 linkage groups (Wight et al., 2003), closer but still greater than the 21 linkage groups suggested by the 21 pairs of chromosomes in

cultivated oat. A number of other investigators have developed linkage maps using different oat populations or markers (Jin et al, 2000; Kremer et al., 2001; Portyanko et al., 2001).

Using crosses between monosomic lines and the parental lines of the Kanota x Ogle mapping population, Fox et al. (2001) were able to associate some linkage groups from the KO map (O'Donoughue et al., 1995; Wight et al., 2003) with physical chromosomes. The association is quite informative in oat where there are multiple translocations between cultivars (Jellen et al., 1994).

Quantitative Trait Loci

Quantitative Trait Loci (QTL) are regions of the genome containing alleles affecting a quantitative trait. A QTL may be as small as a single gene, but in QTL detection and discussion, the region usually is near or between specific genetic markers. Detection of QTL is dependent on three factors: The presence of QTL, the presence of a suitable QTL-linked marker(s), and quality phenotypic data. The presence of QTL is not limiting due to the large number of quantitative traits in most organisms. After the development of quantitative genetic theory, the availability of suitable genetic markers was the primary limitation in QTL detection. Sax (1923) illustrated early QTL detection methods. He showed that the seed coat loci for white beans (*Phaseolus vulgaris* L.), a qualitative trait, was linked to a quantitative trait locus for small seed size, a quantitative trait. Seed color served as the genetic marker for a seed size QTL. Unfortunately, there are few instances where nature provided such readily available genetic markers, hence much of the early work in QTL detection was conducted using the model organism *Drosophila* (Falconer and Mackay, 1996). This limitation was removed by the development of DNA based genetic

markers such as RFLP, RAPDS (random amplified polymorphic DNA), and SSRs (simple sequence repeats). Numerous authors have reported QTL studies of small grains based on DNA markers (Hayes et al., 1993; Galiba et al., 1995; Siripoonwiwat et al, 1996; Holland et al., 1997; Bai et al., 1998).

After the development of DNA markers, collection of informative phenotypic data became the primary limitation to QTL detection. Fortunately, populations for efficient QTL location (Falconer and Mackay, 1996) can be generated in plant species such as oat. A population of recombinant inbred lines is one example (Falconer and Mackay, 1996), and is the most commonly used population in small grains. Precise mapping of QTL depends on phenotype data for each genotypic class with good accuracy and utilization of a great number of genotypes (Kearsey and Farquhar, 1998). The greater the genotype number examined, the greater the chance of informative recombination events (recombination between linked markers and QTL), and better QTL detection.

Statistical t-test or linear regression procedures can be utilized for QTL detection through single factor analysis methodology, as conducted by Siripoonwiwat et al. (1996). This is a simple QTL detection method and does not require a genetic linkage map, but has less power and usually is less informative depending on recombination rates between the actual QTL location and the genetic markers (Falconer and Mackay, 1996). Interval mapping and composite interval mapping, are more complex and powerful procedures for detecting QTL (Falconer and Mackay, 1996). Unlike single factor analysis, both interval mapping procedures utilize information from genetic linkage maps to refine the QTL search. Interval mapping computer programs use maximum likelihood methods (Basten et al., 1999; Lincoln et al., 1993b; Wang et al., 2006) or multiple regression procedures (Utz and

Melchinger, 1996) to estimate likely QTL locations. More recently, multiple interval mapping (MIM) was developed to simultaneously estimate the position and effects of all identified QTL for a given trait (Kao et al., 1999). This method improves the accuracy of estimating the effects of QTL and can even identify and estimate epistatic interactions between QTL, further improving the accuracy of genetic effect estimate (Wang et al., 2006; Wang and Zeng, 2006; Zeng et al., 1999; Zeng et al., 2005).

The true value of QTL associations for crop improvement still is unknown. Inherent problems in QTL experiments, such as finite sample sizes and phenotypic data accuracy, limit the QTL that can be detected by investigators. Further, these problems cause overestimation of the effects of detected QTL (Wang and Zeng, 2006), while failing to detect other QTL of equal effect for any given population (Beavis, 1994). Additionally, most plant QTL detection is conducted in populations developed for genetic mapping or QTL detection, and often these populations are considerably different from elite breeding populations.

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Chapter 2

Quantitative trait loci and epistasis for crown freeze tolerance in the Kanota x Ogle hexaploid oat mapping population

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Abstract

Winter hardiness is a complex trait, and poor winter hardiness limits commercial production of winter oat. Crown freeze tolerance is the most important factor conferring winter hardiness, and controlled crown freeze tests correlate well with field winter hardiness. The objective of this study was to identify quantitative trait loci for crown freeze tolerance in the Kanota × Ogle recombinant inbred line (RIL) mapping population and to examine their relationship with other winter hardiness component traits. One hundred thirty five RILs were evaluated for crown freeze tolerance in a controlled environment. Previously published molecular marker and linkage map information was used for crown freeze tolerance QTL detection. Seven QTL and four complimentary epistatic interactions were identified that accounted for 56% of the phenotypic variation. Ogle contributed alleles for increased crown freeze tolerance at three loci, while Kanota contributed alleles for increased crown freeze tolerance at four loci. All loci where Kanota alleles increased crown freeze tolerance showed complementary epistasis for decreased crown freeze tolerance with the QTL near UMN13. Two of the major QTL identified were in the linkage groups associated with a reciprocal translocation between chromosomes 7C and 17 which previously was associated with spring growth habit in oat. The results confirm the importance of the chromosomes involved in the reciprocal 7C-17 translocation in winter hardiness component traits.

Key words: *Avena byzantina*, *Avena sativa*, frost tolerance, multiple interval mapping, epistasis, quantitative trait loci

Introduction

Low levels of winter hardiness limit the area of commercial winter oat (*Avena* species) production in much of North America and Europe. Cereal winter hardiness is controlled by several quantitative traits including crown freeze tolerance, vernalization and photoperiod responses, heading date and plant height (Fowler et al. 1999). Crown freeze tolerance is the most important winter hardiness trait (Olien 1967), and Marshall (1965) developed a crown freezing procedure that predicted winter field survival. Selection for improved crown freeze tolerance using this protocol led to cultivars, germplasm lines, and populations with improved winter hardiness (Marshall and Kolb 1982; Livingston et al. 1992; Livingston et al. 2004). Photoperiod and vernalization responses combined with heading date act as freeze stress avoidance mechanisms which delay growth of freeze sensitive reproductive tissues until warmer temperatures arrive. Plant height tends to be correlated with these traits, as plants that flower later have more time to grow taller.

An intergenomic reciprocal translocation associated with winter field survival and crown freeze tolerance was identified by Santos et al. (2006). This work indicated chromosomes where crown freeze tolerance genes were located, but more specific chromosomal regions have yet to be identified in oat. This contrasts with other winter cereals because quantitative trait loci (QTL) or genes for freeze tolerance have been identified in diploid wheat (*Triticum monococcum*) (Vagujfalvi et al. 2003), bread wheat (*T. aestivum* L.) (Limin and Fowler 2002; Toth et al. 2003; Fowler and Limin 2004; Kobayashi et al. 2005), and barley (*Hordeum vulgare* L.)(Hayes et al. 1993; Pan et al.

1994; Francia et al. 2004). Almost all of these QTL for freeze tolerance are also linked to QTL for other winter hardiness component traits, such as vernalization response or heading date.

The 'Kanota' × 'Ogle' recombinant inbred line population has been studied by several researchers in oat (Siripoonwiwat et al. 1996; Holland et al. 1997; Barbosa-Neto et al. 2000; Wight et al. 2003). The cultivar Kanota is a facultative winter type released in the early 1920's (Salmon and Parker 1921). It does not have the 7C-17 translocation as is typical of *A. byzantina* C. Koch fall-sown oat (Jellen and Beard 2000). Ogle is an improved spring oat released in Illinois in 1980 (Brown and Jedlinski 1983). Ogle has poor freeze tolerance and has the 7C-17 translocation typical of *A. sativa* L. spring-sown oat (Jellen and Beard 2000). The difference in freeze tolerance between the parents, and the large quantity of molecular marker and related QTL data accumulated in previous research, make this population useful for identifying QTL for crown freeze tolerance. Identification of crown freeze tolerance QTL would provide a tool for improving winter hardiness though marker assisted selection. This approach is particularly suitable for a low heritability trait that is sporadically expressed. An additional benefit would likely come from an enhanced understanding of the relationships among different winter hardiness component traits. The objective of this study was to identify quantitative trait loci for crown freeze tolerance in the Kanota × Ogle RIL mapping population.

Materials and Methods

Phenotypic Evaluation

Seed of 135 recombinant inbred lines (RILs) from the cross between Kanota and Ogle were provided by Dr. Howard Rines of the USDA-ARS in St. Paul, Minnesota. Crown freeze tolerance data were collected on all 135 RILs, but five lines were dropped from the subsequent QTL analysis based on questions as to their legitimacy (Wight et al. 2003).

The experimental design was a sets within replications design with 15 random sets of 10 entries within each of four complete replications over time. The full complement of 135 RILs plus seven entries of one parent and eight entries of the alternate parent were grown for five weeks in a 9 m^2 growth chamber in the Southeastern Plant Environment Laboratory at North Carolina State University. The chamber was illuminated for a 12 h photoperiod with photosynthetic photon flux density of 300 μmol m^{-2} s^{-1} with a day temperature of 13 °C and night temperature of 10°C. Five seeds of each entry were 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.) and lightly watered daily with a complete nutrient solution. At the five leaf stage, plants were transferred to a hardening growth chamber for a three week cold hardening treatment. The hardening chamber held a constant 2°C with a 12 hour photoperiod of photosynthetic photon flux density of 300 μmol m^{-2} s^{-1}. Plants were watered with a complete nutrient

solution (Livingston 1991) three times per week, and watered with tap water on alternate days.

After hardening, plants were removed from the nursery tubes and soil was washed off the roots with ice water. Roots were trimmed to 0.5 cm in length and crowns were trimmed to five 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 super cooling 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 -1.5°C for 36 hours to induce second phase cold hardening (Livingston 1996). Subsequently, the freezer temperature was decreased to -5°C at a rate of -1°C per hour. The freezer was held at -5°C for 3 hours and then raised to 2°C at a rate of 2°C per hour. Within each replication the entries were assigned to entry sets of 10 entries. The same 10 entries were frozen in each of five sponges. The five sponges representing each 10-entry group were placed on five different shelves in the freezer.

After the crowns and sponges thawed, the roots were removed from the crowns by trimming with scissors, and the crowns were planted in 50 by 30 cm plastic flats filled five cm deep with moist Metromix 200. The flats were returned to the growth chamber in the Southeastern Plant Environment Laboratory. After three weeks of re-growth, recovery for each crown was visually measured on a scale of 0-10 (0=dead, 10=no freeze damage).

The data were analyzed using the MIXED procedure of SAS (Littell et al. 1996) with the Satterthwaite option for calculating degrees of freedom. Heritability was estimated for the population excluding parental checks using an all random effects (entry, replication, set, and sponge) model following the method described by Holland et al. (2003), but adjusted for the differences in experimental design. Entries (including parents) were then considered a fixed effect and the LSMEANS statement generated entry LSmeans (means). The DIFF option (α=0.05) was used to test for transgressive segregation among entry means. Procedure UNIVARIATE was used to check for a normal distribution of entry means. Correlations of entry means for crown freeze tolerance, measured in this experiment, with heading date and height of vernalized and non-vernalized entries measured in a previous study by Holland et al. (1997) were estimated with the CORR procedure.

QTL Detection

The Kanota × Ogle genetic linkage map published by Wight et al. (2003) consisted of a framework 286 markers selected and mapped from a pool of over 1000 markers genotyped in the population. This framework map and the corresponding genotypic data were used for map based QTL detection using Windows QTL Cartographer V.2.5 (Wang et al. 2006). Multiple interval mapping (MIM) was used to test for the presence of QTL and epistatic interactions, and to estimate their effects. The MIM QTL search was initiated with no initial model. The likelihood ratio for the presence of a QTL was calculated every

1 cM. The Schwarz (1978) Bayesian Criterion, the default option, was used as the penalty function to prevent over fitting the model (Zeng et al. 1999; Basten et al. 2004).

Some of the potential QTL identified in this study were in regions with limited genotyping of the framework markers, so four additional markers (Table 2.1) that mapped to nearby intervals and were genotyped on more than 80 percent of the RILs were inserted into the framework map (Wight et al. 2003). Mapmaker/EXP version 3.0 (Lander et al. 1987) was used to estimate the position of inserted markers on the map of Wight et al. (2003). Although the addition of these markers changed the map distance between adjacent markers as calculated by Mapmaker, the position of adjacent markers was not changed in the map. QTL detection was repeated using the augmented map and genotype data. The summary command was used to estimate the effects of the QTL and epistatic interactions.

Examination of Epistasis

Epistatic interaction between QTL was evaluated in SAS using the genotypes of the nearest markers to approximate the identified QTL. Two way interactions between UMN13 and each of four markers UMN433, BCD1968B, BCD1230B, or UMN5485 were tested. Only 66 of the RILs were genotyped at UMN433, therefore the genotypes at UMN433 for some of the remaining RILs were predicted based on flanking marker genotypes. For 40 RILs that were uniform for parental alleles at the flanking markers BCD1405 and OG41, which define a 20 cM region containing UMN433, the UMN433

genotype was predicted as the parental genotype. If an RIL did not have the alleles from the same parent at both flanking loci, the genotype at locus UMN433 was not predicted.

The GLM procedure was used to model the additive terms and the orthogonal epistatic interaction, and the LSMEANS statement with the PDIFF option was used to estimate and compare the means of the four different marker combination classes. Four marker combination classes are expected at two distinct loci with homozygous lines. Marker class means suggested possible complementary gene action so, duplicate or complementary gene action between each pair of loci was further evaluated using ANOVA with a coded dummy variable. If an RIL had the Kanota allele at UMN13 and the Ogle allele at the other locus (UMN433, BCD1968B, BCD1230B, or UMN5485) then it was coded 0, otherwise it was coded 1. For each pair of markers, crown freeze tolerance was modeled in the MIXED procedure using three potential models. First, a simple linear model with the two markers (modeling simple additive gene action), second a linear model with interaction (a typical test for epistasis), and third a model consisting of the coded variable (modeling duplicate or complementary gene action) were compared using the Akaike information criterion (Akaike 1969) calculated with the MIXED procedure to identify the regression model that best fit the gene action (Rawlings et al. 1998). Finally QTL estimates were re-estimated using MIM including the epistatic interaction terms between QTL near UMN13 and each of the four other QTL near UMN433, BCD1968B, BCD1230B, or UMN5485.

Results

Variance among entries for crown freeze tolerance was highly significant ($p<0.0001$). Recombinant inbred line entry means ranged from 3.04 to 7.13 with Ogle rated 4.33 and Kanota rated 5.81 (Figure 2.1). The distribution was not normal ($P<0.05$) and showed negative skewness. Eight lines were significantly less freeze tolerant than Ogle, and three lines were significantly more freeze tolerant than Kanota. The "immediate response" heritability was 52 ± 4% on an entry mean basis (Holland et al. 2003). Freeze tolerance line means did not show any significant correlation with the heading date, height, or vernalization responses measured by Holland et al. (1997), indicating that the freeze tolerance in this population is not caused simply by the difference in vernalization requirement between a winter and spring cultivar.

Seven QTL for crown freeze tolerance in seven different linkage groups and four epistatic interactions between QTL were identified (Table 2.2). Together, these main and epistatic effects accounted for 56.4 percent of the phenotypic variation in crown freeze tolerance (Table 2.2). Kanota contributed alleles that increased crown freeze tolerance at four QTL on linkage groups LG 24_26_34, LG 21+46_31+4, LG 3+38, and LG 11_41+20 which respectively accounted for 11.0, 7.5, 6.4, and 5.1 percent of the phenotypic variation. Ogle contributed alleles for increased crown freeze tolerance at three QTL on linkage groups LG 16_23, LG 25, and LG 22_44_18 and respectively accounted for 6.5, 6.4, and 3.0 percent of the phenotypic variation.

One epistatic interaction was detected between the QTL on LG 3+38 (near marker UMN433) and LG16_23 (near marker UMN13) using MIM. This interaction accounted for 6.7 percent of the freeze tolerance variation. Analysis of the four marker class means indicated that the interaction followed the pattern of complimentary epistasis for decreased freeze tolerance or duplicate gene epistasis for increased freeze tolerance (Table 2.3). The mean for RILs with the Ogle alleles at UMN433 and Kanota alleles at UMN13 was 4.37, and was significantly less ($P<0.001$) than the other three two-locus genotype group means that ranged from 5.12 to 5.24. These latter three class means were not significantly different from each other ($P>0.6$). Model comparison using the Akaike information criterion showed that the complimentary gene action model for decreased freeze resistance best fit the genetic action of these loci (Table 2.4).

The occurrence of additional epistatic combinations involving the QTL near UMN13 was investigated. The interactions between three marker pairs BCD1968B with UMN13, BCD1230B with UMN13, and CDO1090C with UMN13 all followed a similar pattern to that described above for UMN433 and UMN13. The interaction terms in the additive plus orthogonal interaction model were not significant in the three combinations (confirming the MIM results), but analysis of the two-locus genotypic means showed strong evidence of duplicate or complementary epistasis (Table 2.3). The coded regression modeling duplicate or complementary gene action was superior for all three pairs of markers (Table 2.4). The coded regression model was highly significant

($P<0.001$) for all marker pairs, hence each of these interactions was included in the MIM model (Table 2.2).

Notably, epistasis was not detected in combinations between the four loci, BCD1230B, UMN433, BCD1968B and CDO1090C, where Kanota donated alleles for increased freeze tolerance(Table 2.2). This suggested that the locus near UMN13 was involved in complementary gene interaction for freeze sensitivity with the four loci where Kanota donated alleles for freeze tolerance.

Discussion

Transgressive Segregation

Three of 130 Kanota × Ogle RILs (2.3%) were transgressive segregants with increased freeze tolerance compared with 20.2% in the 'Fulghum' × 'Wintok' population examined by Santos et al. (2006). Eight Kanota × Ogle RILs (6.2%) were significantly less freeze tolerant than Ogle, while no lines in the Fulghum × Wintok population demonstrated transgressive segregation for lower freeze tolerance (Santos et al. 2006). Ogle, a spring-sown oat, did contribute three freeze tolerance alleles to its progeny (Table 2.2). The inheritance of factors for improved winter hardiness from spring-sown parents is a relatively common phenomenon (Amirshahi and Patterson 1956; Murphy 1958). The RILs that were less freeze tolerant than Ogle can be attributed to alleles exhibiting complementary epistasis for low freeze tolerance donated by Kanota and Ogle (Table 2.2). When an RIL contained homozygous Kanota alleles at QTL 3 (near marker UMN13) and homozygous Ogle alleles at QTL 1, 2, 5, or 6, the epistatic interaction of those loci resulted in less crown freeze tolerance than when an RIL had Ogle alleles at both loci.

Freeze Tolerance QTL

Quantitative trait loci for freeze tolerance are commonly linked with other winter hardiness component trait QTL in winter cereals (Pan et al. 1994; Galiba et al. 1995; Toth et al. 2003). Correspondence of freeze tolerance QTL identified in this study with other QTL for oat winter hardiness component traits both confirmed the significance of the freeze tolerance QTL and contributed to the understanding of the relationship between

winter hardiness component traits. Most of the genomic regions identified as freeze tolerance QTL in this study were associated with vernalization response, heading date, or plant height by previous researchers (Holland et al. 1997; Siripoonwiwat et al. 1996). This supports the importance of these chromosomal regions in conferring winter hardiness.

The largest QTL effect in our study was near the locus BCD1968B on LG 24_26_34. Analysis of controlled environment vernalization response by Holland et al. (1997) identified this genomic region as the largest QTL affecting vernalization response in the Kanota × Ogle population (R^2=29%) The Kanota allele increased the heading date of vernalized plants by 4.4 days compared to the Ogle allele. In non-vernalized plants the Kanota allele increased heading date only 2.2 days compared to the Ogle allele, resulting in a vernalization response of 2.2 days. The Kanota allele also increased heading date in field evaluations (Siripoonwiwat et al. 1996). In a second mapping population from a cross of Ogle by the winter cultivar 'TAM O-301', the same pattern of vernalization response was observed at the corresponding map location. A syntenous region on wheat chromosome $5A^m$ in diploid *T. monococcum* contains the vernalization gene Vrn-A^m1 (Dubcovsky et al. 1998), and in hexaploid wheat this region is associated with linked vernalization and freeze tolerance genes (Galiba et al. 1995; Sutka et al. 1999). This genomic region seems to plays a key role in winter hardiness component traits throughout the *Pooideae* subfamily.

A second freeze tolerance QTL was located on LG 3+38 near UMN433 in a region associated with heading date. Holland et al. (1997) found the Kanota allele in this region

increased heading date in vernalized plants by 2.2 days (R^2=12%) and increased the heading date in non-vernalized plants by 1.4 days (Holland et al. 1997). The Kanota allele from this region increased heading date in field trials also (Siripoonwiwat et al. 1996), which indicated that the Kanota allele could be an allele for *per se* lateness.

The 7C-17 intergenomic reciprocal translocation was associated with oat winter hardiness and freeze tolerance (Santos et al. 2006), as well as classification of oat cultivars into *A. byzantina* or *A. sativa* categories (Zhou et al. 1999; Jellen and Beard 2000). The QTL on linkage groups 3+38 and 24_26_34 were in the linkage groups associated with this translocation (Fox et al. 2001). The combined effect of these two QTL on freeze tolerance (R^2=17.6%) was similar to the effect of the translocation on freeze tolerance (R^2=22%) reported by Santos et al. (2006). However, the likely breakage point for the translocation is at position 74 in LG 3+38 (Fox et al. 2001), almost 50 cM from the QTL on LG 3+38 identified in this study (Wight et al. 2003), so this QTL probably was not the genetic factor controlling crown freeze tolerance and winter field survival that Santos et al. (2006) associated with the translocation Furthermore, improved freeze tolerance in the Fulghum × Wintok population was inherited from the parent with the translocation, and in this experiment improved freeze tolerance was inherited from the parent without the translocation. While these QTL results confirm the importance of loci on chromosomes 7C and 17 in controlling oat winter hardiness traits, they do not identify the location of QTL on or near the translocation which Santos et al. (2006) associated with greater freeze tolerance.

The smallest QTL for increased freeze tolerance from Kanota was on LG 11_41+20 near marker CDO1090C. The Kanota allele in this region increased plant height in vernalization and field studies (Siripoonwiwat et al. 1996; Holland et al. 1997). A nearby QTL was identified where the Kanota allele increased heading date vernalization response (Holland et al. 1997). Phenotypically, increased vernalization response and height are associated with increased freeze tolerance and winter hardiness in winter cereals, and this QTL follows the same pattern.

The freeze tolerance QTL located on LG 21+46_31+4 near marker BCD1230B was the second largest QTL in the model. This region was not associated with other winter hardiness traits in previous QTL studies (Siripoonwiwat et al. 1996; Holland et al. 1997). There have been a limited number of QTL studies in oat, and the previous studies with this population used a smaller number of RILs (Siripoonwiwat et al. 1996; Holland et al. 1997). The increased power conferred by the full set of 130 RILs in this study likely contributed to the discovery of this QTL. An additional analysis of the data from this study that included only the 71 RILs used in previous QTL studies failed to identify the QTL near BCD1230B. It is possible that other winter hardiness QTL in the region have not been discovered. Finally, it is possible that there is a freeze tolerance QTL with no associated effects on flowering time or plant height at this location, but more evaluation of oat winter hardiness QTL would be needed to support this possible conclusion.

Kanota contributed alleles at three QTL for decreased freeze tolerance, and the QTL with the largest additive effect was located at position 0 of LG25 (Table 2.2). The Kanota allele in this region decreased plant height in the field and controlled environment vernalization experiments (Siripoonwiwat et al. 1996; Holland et al. 1997). LG 25 is the smallest linkage group with identified freeze tolerance QTL, and no corresponding portion of the Ogle × TAM O-301 map has been identified. Additionally, because the QTL mapped to position 0 on the LG, it was probably located outside the chromosomal region mapped by LG 25. This could account for the absence of a corresponding region in the Ogle × TAM O-301 population (Holland et al. 2002).

A minor QTL at which the Kanota allele decreased freeze tolerance was identified on LG 22_44_18 near marker UMN5485 (Table 2.2). Winter hardiness component traits were located in this region in both the Kanota × Ogle and Ogle × TAM O-301 populations. A locus was identified in the Kanota × Ogle population where the Kanota allele was associated with negative vernalization response for heading date (the Ogle allele had a positive vernalization response), and reduced plant height in the non- vernalized plants and field evaluations (Siripoonwiwat et al. 1996; Holland et al. 1997). Both phenotypes tend to be associated with lower freeze tolerance. Holland et al (2002) found a corresponding vernalization QTL in the Ogle x TAM O-301 population. Wight et al (2003) identified this region of LG 22_44_18 as being homoeologous to LG 24_26_34, where the largest QTL for freeze tolerance was found. Analysis of the interaction between these two possible homoeologous QTL suggested possible duplicate gene action (data not presented). These

may be similar genes for vernalization from homoeologous genomic regions. In wheat, homeologous genes for vernalization are found on wheat group 5 chromosomes (Snape et al. 2001). Because the QTL on LG 24_26_34 is syntenous with the group 5 chromosomes in wheat, we speculate that these two genomic regions for oat vernalization and freeze tolerance follow a similar pattern to the homeologous winter hardiness QTL on the wheat group 5 chromosomes.

The most complex QTL identified was on LG 16_23 near UMN13. Although this QTL did not have the largest additive effect (a= -0.21) it was the most important QTL identified in the study because of epistatic effects. Previous research showed the Kanota allele in this region reduced plant height in field and controlled environment studies (Siripoonwiwat et al. 1996; Holland et al. 1997). It decreased heading date in field trials and caused a negative heading date vernalization response (Siripoonwiwat et al. 1996; Holland et al. 1997). These results indicated the Kanota allele conferred a short, early phenotype that was not responsive to vernalization. It was similar to the QTL on LG 3_38 near UMN433 except that in the QTL on LG 16_23 Ogle donated the allele for greater freeze tolerance.

Analysis of the QTL interactions of the UMN13 locus revealed a complex network of epistatic interactions. Almost all the reduction in freeze tolerance conferred by the Kanota allele near UMN13 resulted from epistatic interactions with QTL where Ogle alleles decrease freeze tolerance (Table 2.3). Conversely, the Kanota alleles for additive

increases in freeze tolerance (QTL 1, 2, 5 and 6 in Table 2.2) increased freeze tolerance when the UMN13 locus was homozygous for Kanota alleles. In other words, the additive effect of the QTL arose from partitioning the effect of complementary gene action into the additive terms of the genetic model. This QTL near UMN13 showed the same pattern of epistasis with all of the QTL where Kanota provided the alleles for increased freeze tolerance (Table 2.3). Epistasis of this form has an important impact on marker assisted selection. Typical QTL detection studies use interval mapping or composite interval mapping to identify QTL and estimate their effects, often ignoring the epistatic effects. Using this study as an example, a breeder seeking to improve crown freeze tolerance would select for the Kanota allele at UMN433 and the Ogle allele at UMN13 (according to the additive model typically used in QTL studies) and expect an increase in freeze tolerance over that of Kanota. However the epistatic interaction would result in no increase in freeze tolerance over Kanota (Table 2.3). These results indicated that using QTL studies to identify potential targets for marker assisted selection without investigating epistasis is a risky proposition.

It was difficult to determine whether these loci exhibited duplicate gene action for increased freeze tolerance, or complementary gene action for decreased freeze tolerance because both types of epistatic interaction would produce the same pattern of phenotypes in this population. The QTL where alleles from Kanota increased freeze tolerance did not show the same pattern of duplicate gene interaction between each other. For example, there was no interaction between loci UMN433 and BCD1968B, and both these QTL have

different effects on vernalization (Holland et al. 1997). If both of these loci had duplicate gene action with UMN13, then we would expect duplicate gene interaction between them. Hence, we hypothesize that a gene from Kanota near UMN13 had a complementary epistatic interaction with the other QTL in a genetic or biochemical pathway where UMN13 is near the first gene in the pathway and the QTL near BCD1968B, BCD1230B, UMN433, and CDO1090C are controlled by the gene near UMN13. Future analysis of these QTL with crown histology studies (Livingston et al. 2005) could describe how and why these genes interact. Alternatively, mapping expression of oat EST sequences (Braütigam et al., 2005) homologous to known cereal winter hardiness genes may effectively characterize the function of some of the QTL identified in this study.

Unfortunately, MIM did not precisely model the kind of complementary epistatic interactions found in this population. Modeling duplicate or complementary gene action is difficult because the genetic effects can not be orthogonally divided between additive and epistatic genetic effects using MIM in QTL Cartographer. The method we used in SAS with coded dummy variables can model the genetic action, but it is not orthogonal to the QTL main effects, which makes building a multiple locus model problematic especially when one locus is involved in multiple interactions. Although we identified complementary gene action between several QTL pairs, we had to estimate these gene effects using the orthogonal model in MIM to build a multiple locus model.

In this population we had closely linked markers and could test for potential epistatic combinations using analysis of variance. The ANOVA provided good justification for including the epistatic terms, despite the fact that most of them had low LOD values in the MIM model (Table 2.2). It is reasonable to reduce the threshold for incorporating epistatic interactions between main effect QTL because there is not the same degree of multiple comparison problem as when searching for main effect QTL. This contrasts with the method of testing for interaction between all pairs of markers employed by EPISTACY (Holland 1998) where multiple comparisons cause a substantial problem. Furthermore, the potential consequences of identifying a QTL for marker assisted selection and then making a Type II error by not identifying epistatic interaction(s), are much more costly than making a Type II error in identification of additive effect QTL.

It was expected that many of the freeze tolerance QTL identified in this research were also associated with vernalization response (Holland et al. 1997) as many authors have reported tight linkage between freeze tolerance genes and vernalization genes in winter cereals (Pan et al. 1994; Storlie et al. 1998; Sutka et al. 1999; Toth et al. 2003; Kobayashi et al. 2005). While it is not possible to discern linkage from pleiotropic effect in this population, the similarity between these results and those from other winter cereals supports the linkage hypothesis for most of the QTL. It was unexpected that freeze tolerance was not correlated with any of the vernalization traits measured by Holland et al. (1997). The extensive epistasis between QTL likely explains much of the lack of phenotypic correlation. Similar complementary gene action was not detected for these

QTL in the vernalization experiment. Regions near UMN13 did not show any significant epistasis for any traits. Additionally, several genomic regions affecting freeze tolerance were not associated with QTL for vernalization traits; for example, the QTL on LG 21+46_31+4 near BCD1230B. The QTL on LG24_26_34 was the largest additive QTL for freeze tolerance and for heading date vernalization response (Holland et al. 1997) and this may have contributed to heading date vernalization response being the phenotypic trait with the closest correlation to freeze tolerance (P=0.07, r=0.22). Although the line mean correlations were not statistically significant, most of the freeze tolerance QTL were in genomic regions previously associated with winter hardiness component traits, illustrating the utility of QTL analysis in dissecting the genetic architecture of oat winter hardiness component traits.

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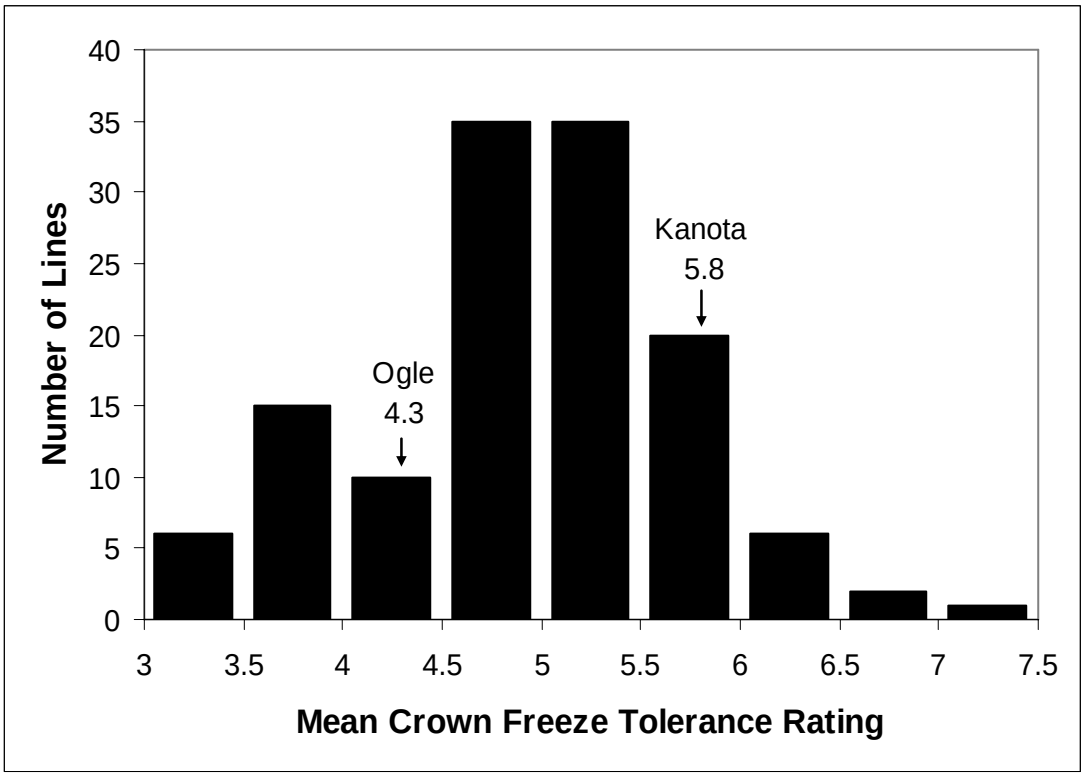

Figure 2.1. Frequency distribution of mean crown freeze tolerance ratings (0=dead, 10=no damage) of 130 recombinant inbred lines derived from a cross of 'Kanota' and 'Ogle' with parental checks.

Table 2.1. Molecular markers inserted into the genetic linkage map of Wight et al (2003).

Linkage Group	Position [cM]	Marker Name
3+38	5.82	OG41
11_41+20	63.47	UMN364A
16_23	85.99	WG466
16_23	90.19	CDO665C

Table 2.2. Quantitative trait loci associated with crown freeze tolerance. A population of 135 RILs was scored for crown freeze tolerance using a controlled crown freeze test. Map based QTL detection was conducted using multiple interval mapping in QTL Cartographer V 2.5.

QTL	Linkage Group[a]	Position [cM]	Nearest Marker(s)	MIM LOD[b]	Additive Effect[c]	R^2 %
1	**24**_26_34	41	BCD1968B	1.79	0.24	11.0
2	21+46_**31**+4	70	BCD1230B	1.31	0.22	7.5
3	**16**_23	111	UMN13	1.40	-0.21	6.5
4	**25**	0	waaccac273	1.24	-0.22	6.4
5	**3**+38	23	UMN433	1.17	0.19	6.4
6	11_41+**20**	61	CDO1090C	1.22	0.18	5.1
7	**22**_44_18	157	UMN5485	0.66	-0.15	3.0
5 × 3	Epistatic Interaction		UMN433 × UMN13	1.17	0.19	6.7
2 × 3	Epistatic Interaction		BCD1230B × UMN13	0.20[d]	0.09	2.3
1 × 3	Epistatic Interaction		BCD1968B × UMN13	0.16[d]	0.07	0.9
6 × 3	Epistatic Interaction		CDO1090C × UMN13	0.08[d]	0.05	0.6

[a]Linkage Group from Wight et al (2003), Number in bold is location of QTL on linkage group from O'Donoughue et al (1995).

[b]Multiple interval mapping (MIM) LOD score is not equivalent to composite interval mapping LOD score due to penalty function.

[c]Additive effect of a Kanota allele on crown freeze tolerance measured on a scale of 0–10

[d]Inclusion of this epistatic term is based on identification of interaction between these QTL in SAS.

Table 2.3. Marker class crown freeze tolerance means for combinations of UMN13 with UMN433, BCD1968B, BCD1230B, and CDO1090C. Loci are homozygous for either parent Kanota or Ogle.

	UMN433[a]		BCD1968B		BCD1230B		CDO1090C	
UMN13	Ogle	Kanota	Ogle	Kanota	Ogle	Kanota	Ogle	Kanota
Ogle	5.24	5.12	5.12	5.31	5.04	5.12	5.04	5.24
Kanota	4.37	5.17	4.39	5.22	4.34	5.02	4.36	5.04

[a]Genotype of UMN433 marker was estimated for 40 RILs uniform for flanking markers BCD1405 and OG41 that were not genotyped for UMN433. Marker UMN433 was directly genotyped in 66 RILs used in this analysis.

Table 2.4. Akaike information criterion (AIC) for alternate models of QTL interaction. A lower AIC value indicates a better model.

Marker Pair	Additive Model (No Interaction)	Additive Plus Interaction	Coded Regression[a]
BCD1968B × UMN13	221.1	219.5	218.1
BCD1239B × UMN13	146.6	144.3	143.0
UMN433[b] × UMN13	231.7	224.1	221.9
CDO1090C × UMN13	257.9	256.0	254.5

[a]Coded regression models complementary gene action for decreased freeze tolerance or duplicate gene action for increased freeze tolerance

[b]Genotype of UMN433 marker was estimated for 40 RILs uniform for flanking markers BCD1405 and OG41 that were not genotyped for UMN433. Marker UMN433 was directly genotyped in 66 RILs used in this analysis.

Chapter 3

An Intergenomic Reciprocal Translocation Associated with Oat Winter Hardiness Component Traits

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Abbreviations:

LSmean, least-squares mean

FN, Fulghum × Norline recombinant inbred line population

FW, Fulghum × Wintok recombinant inbred line population

RIL(s), recombinant inbred line(s)

T7C-17, intergenomic reciprocal translocation involving chromosomes 7C and 17

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 as well as fall and spring growth habit. The objective of this experiment was to use a population of 128 recombinant inbred lines derived from a cross of winter tender 'Fulghum' (non- T7C-17) with winter hardy 'Norline' (T7C-17) to validate the effect of T7C-17 on the winter hardiness component traits winter field survival and crown freeze tolerance, and evaluate the effects of T7C-17 on heading date, height, and vernalization and photoperiod responses. Crown freeze tolerance, vernalization response, and photoperiod response were evaluated in controlled environment studies. Heading date and plant height were evaluated in Kinston, North Carolina, and winter field survival was evaluated in five environments in the mountains of North Carolina and Virginia. The translocation segregated in a 2:1 ratio, not in the expected 1:1 ratio. The translocation was significantly correlated with crown freeze tolerance (r=0.72) and winter field survival (r=0.62). The heritability of crown freeze tolerance was 83% and the heritability of winter field survival was 76%. Field heading date was significantly correlated with translocation status (r=0.20). Plant height, vernalization response, and photoperiod response were not associated with the translocation. These results confirmed the importance of T7C-17 in conferring winter hardiness traits in winter oat.

INTRODUCTION

Low levels of winter hardiness limit the area of commercial winter oat production in much of North America. Winter hardiness in cereals is related to several quantitative traits including crown freeze tolerance, vernalization and photoperiod responses, heading date and plant height (Fowler et al., 1999). Crown freeze tolerance is the most important winter hardiness trait (Olien, 1967). Photoperiod and vernalization responses combined with heading date act as freeze stress avoidance mechanisms which delay growth of freeze sensitive reproductive tissues until warmer temperatures arrive (Snape et al., 2001). Consequently, winter field survival and crown freeze tolerance are often correlated with other winter hardiness component traits such as heading date, vernalization response, or photoperiod response 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. The non translocated form is prevalent in *A. byzantina* and certain accessions of *A. sterilis* (L.), the progenitor of domesticated *A. byzantina* and *A. sativa* (Jellen et al., 2004; Zhou et al., 1999). Avena byzantina The absence of the translocation has been associated with traditional winter type germplasm, while spring sown germplasm typically has T7C-17 (Jellen and Beard, 2000). The observation that traditional winter oats typically did not have T7C-17 suggested a relationship between the translocation and winter hardiness.

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 in

1960 (Patterson and Schafer, 1978). It is winter hardy (Livingston and Elwinger, 1995; Livingston et al., 2004) and does have 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). Although both lines are traditional winter cultivars, they both have T7C-17. Santos et al. (2006) found a highly significant association between translocation status and both crown freeze tolerance and field winter survival in a recombinant inbreed line (RIL) population derived from a cross of Fulghum and Wintok. The objective of this experiment was to use a RIL population derived from a cross of winter tender Fulghum (non- T7C-17) with winter hardy Norline (T7C-17) to validate the association of T7C-17 with the winter hardiness component traits winter field survival and crown freeze tolerance, and evaluate the association of the translocation with 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 winter tender Fulghum (normal karyotype) by winter hardy Norline (7C-17 translocation). Plants in the Fulghum × Norline (FN) 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 non-viable seed were 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/absence based on observation of multiple cells from root-tip meristems of three plants per RIL.

Controlled Crown Freeze Tolerance Test

The experiment was an incomplete blocks within complete replications design with 14 incomplete blocks of 10 entries within each of five complete replications over time. Each complete replication consisted of 120 RILs (eight lines were not included because of limited $F_{6:7}$ seed) plus seven entries of the parents 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 four days. Five seedlings of each entry were then planted 1.5cm deep in five adjacent 20cm long nursery tubes held in racks of 100 tubes. Plants were grown in Metromix 200 (Scotts-Sierra Horticultural Products Co.) and lightly watered daily with a complete nutrient solution. The plants were grown for five weeks 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 three week cold acclimation treatment. The hardening chamber held a constant 3°C with a 10 hour photoperiod of photosynthetic photon flux density of 300 μmol m^{-2} s^{-1}. While cold acclimating, plants were watered with a complete nutrient solution (Livingston, 1991) 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 0.5cm and crowns were trimmed to 5cm 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 hours to induce subzero acclimation (Livingston, 1996). Subsequently, the freezer temperature was by decreased 1°C per hour to -10°C. The temperature was held at -10°C for 3 hours and then raised by 2°C per hour 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 by sponge grid pattern within 50 by 30 cm plastic flats filled five cm deep with moist Metromix 200. The flats were returned to the growth chamber in the Southeastern Plant Environment Laboratory. After three weeks of re-growth, recovery for each crown was visually measured on a scale of 0-10 (0=complete plant death, 10=no freeze damage), double the scale used in Santos (2006).

Field Trials

Heading date and plant height were evaluated using a randomized complete block design with two replications. $F_{6:7}$ seed of each RIL plus the parents and Wintok as checks were planted on 23 October 2002 at the Cunningham Research and Education Center, Kinston, NC. Plots were 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 2002-03. 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. $F_{6:8}$ seed of the RILs plus five entries of the parental checks and Wintok was used. The experiment was planted in 16 September, 2003 at the Upper Mountain Research Station (elevation=895m) near Laurel Springs, NC, and 9 October 2003 at the Mountain Research Station (elevation=727m) near Waynesville, NC. Plots were hand planted with six grams 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. The following spring, field survival was estimated for each plot in March 2004 as the percent survival 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 VT College of Agriculture and Life Sciences Kentland Research Farm, near Blacksburg, VA. Plots in 2004-05 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 2004-05 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 December, 2004 (http://www.ncdc.noaa.gov). No winter damage was observed in Waynesville in either season, so the data were not included in 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 sub-plot factors. Seedlings for the non-vernalized treatment were germinated in moist paper towels for 4 days at 20°C. Seedlings with the vernalized treatment were germinated in moist paper towels for four weeks 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 $\mu mol\ m^{-2}\ s^{-1}$, and the long day treatment was simulated using a 2 hour mid-night interruption with low intensity incandescent lights. This provided long day stimulus to the plants, while minimizing the difference in photosynthetically

active radiation. After 42 days with differing photoperiod treatments, both chambers were increased to 16h 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 plot 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. Heritabilities were estimated for the population excluding checks using an all random effects model following the method described by Holland et al. (2003) 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 lsmeans (means) for the three translocation classes. Entries (including parents and checks) were then considered a fixed effect and the LSMEANS statement generated entry LSmeans (means). The DIFF option (α=0.05) was used to test for transgressive segregation. Correlations were estimated among freeze tolerance, winter field survival, heading date, and translocation status using the CORR procedure. Procedure GLM was used to regress winter field survival on crown freeze tolerance. The Univariate procedure was used to test normal distributions for winter field survival and crown freeze tolerance, for both the whole population and the two translocation classes.

RESULTS

Thirty nine FN lines had a non- T7C-17 karyotype and 81 lines had T7C-17. 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 1:3 ratio ($P=0.058$), but a 1:2 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 $7C^{17}$ (Figure 3.1) and was excluded from the analyses of the translocation classes, but included in analyses treating genotype as a fixed effect.

Crown Freeze Tolerance

Norline was significantly more freeze tolerant than Fulghum (Table 3.1). Five lines were significantly more freeze tolerant than Norline (4%), but no lines were significantly less freeze tolerant than Fulghum as the measured freeze tolerance of Fulghum was not significantly different from zero. Distribution of crown freeze tolerance means was bimodal (Figure 3.2). However, dividing the population by translocation status produced two populations with normal distribution for crown freeze tolerance (Figure 3.2). Translocation status was highly significant with an additive effect of 1.4 (Table 3.1). The heritability of crown freeze tolerance was 83±2%. Translocation status was significantly correlated with crown freeze tolerance $r=0.72$ (Table 3.2).

Winter Field Survival

Environment mean winter field survival ranged from 44 percent at Laurel Springs 2004-05 to 100 percent at Waynesville in 2003-04 and 2004-05. The results from Waynesville were

not included in further analyses. Three environments had differential winter field survival (Table 3.1). 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 by 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 combined had a bimodal distribution when examining the full population (Figure 3.3). However the distribution of winter field survival was normal for the non-T7C-17 class (Figure 3.3), but the population of RILs with T7C-17 was not normally distributed being skewed toward greater field survival. Line mean correlation between winter field survival and translocation status was very highly significant (r=0.61) (Table 3.2). Mean crown freeze tolerance was very highly significantly correlated with winter field survival (Table 3.2, Figure 3.4).

Heading date was normally distributed with a heritability of 81±17 % (Table 3.1). The additive effect of the translocation was to increase heading date by 0.74 days. 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. So, it may be more accurate to state that lines uniform for T7C-17 had a heading date 1.5 days 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 freeze tolerance and winter field survival genes.

Very significant genotype, vernalization, photoperiod, genotype by vernalization, and genotype by photoperiod effects were detected in the controlled environment vernalization and photoperiod responses study (data not shown). However, translocation status did not have a significant effect on either vernalization or photoperiod responses. 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 3.1) indicating dominant gene effects. The winter field survival and crown freeze tolerance genes from Norline appeared to show dominant gene action, while heading date genes from Fulghum appeared dominant. However, orthogonal linear contrasts modeling additive and dominant gene action 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. An 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 $7C^{17}$ (Figure 3.1). This line had normal fecundity and could not be identified as abnormal based on phenotype. It had intermediate crown freeze tolerance (Table 3.1) and was significantly different from both parents. The

comparative winter field survival of the nullisomic line seemed to vary with environment. In Laurel Springs 2004-05, 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 3.1). The nullisomic RIL had a relatively late heading date equal to Norline. It was significantly shorter than either parent with a height of 75cm compared to 112cm for Fulghum and 108cm for Norline. Identification of genetic factors using nullisomic lines can be complicated by the deficiency of non-target genes, but the data indicated that chromosome $7C^{17}$ carries genes for increased crown freeze tolerance, increased winter field survival, and increased height.

DISCUSSION

Phenotypic Data

The distribution of RIL freeze 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 freeze tolerance heritability of 83% was greater than the 67% reported by Santos et al. (2006). The greater heritability in this population likely enabled the identification of a bimodal distribution with two normal subpopulations, in contrast to the Fulghum × Wintok (FW) population, wherein the larger error variance resulted in a more normal crown freeze tolerance distribution (Santos et al., 2006).

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

× Wintok population may have already been present in Norline. This would account for our observation of a greater percentage of lines that were more freeze tolerant than Wintok, and a lesser percentage of transgressive segregants for freeze tolerance. Fulghum is an ancestor of Norline (Marshall, 1992), so it is possible that some of the alleles that Fulghum carried for increased freeze tolerance were inherited by Norline.

The asymmetric distribution of transgressive segregants in both populations suggested that there may have been epistatic interaction leading to greater crown freeze tolerance. Wooten et al. (submitted) found an asymmetric distribution of transgressive RILs for crown freeze tolerance in a cross of 'Kanota' × 'Ogle'. They identified complementary gene action among QTL for crown freeze tolerance leading to a greater number of RILs with decreased freeze tolerance. In the FW and FN populations, the number of transgressive segregants indicated that there could be complementary gene interaction for increased crown freeze tolerance. Because all of the RIL that were more crown freeze 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 translation resulting in increased freeze tolerance. It is also possible the segregation distortion of the translocation caused the asymmetric distribution of transgressive segregants. QTL mapping in these populations could determine if these transgressive segregants were the results of epistasis or additive gene action, and such QTL could be excellent targets for marker-assisted selection to improve crown freeze tolerance.

The great variation between the selection environments for winter field survival illustrated the difficulties in conducting field evaluations for winter hardiness and the need for indirect selection methods such as cytogenetic and molecular markers, or crown freeze 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 FW population during the 1998-1999 season at Laurel Springs were intermediate between the results from the two years of this study (Santos et al., 2006). These results confirmed the findings of Livingston and Elwinger (1995) that Laurel Springs is an excellent location for winter field survival testing, and support the relevancy and repeatability of the winter field survival findings of Santos et al. (2006).

In this study, Fulghum had significantly less winter field survival than Norline and 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 Winterhardiness Nursery. It appears that while Norline had a greater crown freeze tolerance than Wintok as measured with this protocol, there was little difference between the winter field survival of the two lines. In the FN population it appeared that genes for increased crown freeze tolerance conferred greater field survival. In the FW population, there was an additional genetic factor conferring greater winter field survival on Wintok and its progeny that did not increase crown freeze tolerance to the same extent. When considering the number of lines that were transgressive segregants for increased crown freeze tolerance in the two populations, we speculate that the genes for increased crown freeze tolerance Norline may have inherited from Fulghum may not increase winter field survival to the same degree as they increase crown freeze 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 FW population.

Translocation Effects

Santos et al. (2006) reported a similar segregation distortion for T7C-17; however they found a ratio closer to1:3 rather than the 1:2 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 QTL affecting photoperiod or vernalization responses were associated with regions of segregation distortion (Holland et al., 2002). However, the molecular markers from linkage groups in the region of T7C-17 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 supported this theory because the translocated portion of chromosome 7CL was on chromosome 17^{7C} in the nullisomic line. The 1:2 segregation ratio found in this population suggested there might have been a non-viable 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 non-functional 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 non translocated 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 was 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* and some *A. sterilis* 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 freeze tolerance was almost exactly the same in the FW (Santos et al., 2006) and FN populations. However, in this experiment 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 FN compared to 20.5% in the FW population (Santos et al., 2006). These similar T7C-17 effects on crown-freeze tolerance and different T7C-17 effects on winter field survival seemed to indicate that while Norline and Wintok had the same genes for crown freeze 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 freeze tolerance, but the same translocation effect, Norline likely had genes for increased freeze 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 freeze tolerance were developed from a cross between Norline and Wintok (Livingston et al., 2004) and some of these were significantly more winter hardy than Norline and Wintok (Livingston, UOWHN unpublished data, 2004-2006). These results from crosses of Norline and Wintok support the hypothesis that Wintok had a gene for winter field survival associated with T7C-17 but not present in Norline, while Norline had genes not present in Wintok for crown freeze tolerance that were not associated with T7C-17. QTL mapping of winter field survival and crown freeze 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 freeze 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 FW population (Santos et al., 2006). This indicated that the freeze tolerance and winter field survival genes on T7C-17 are at least partially dominant. Dominant or partially dominant alleles for increased crown freeze tolerance or winter survival may allow for 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 suggested that the earliness genes from Fulghum near the translocation were likely 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 freeze 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.

CONCLUSION

In a RIL population derived from Fulghum × Norline, the T7C-17 had a large effect on the winter hardiness component traits winter field survival and crown freeze tolerance, a minor effect on field heading date, and no significant effect on height or vernalization and photoperiod responses. Comparing these results with those of Santos et al. (2006) indicated that Norline and Wintok have similar genes for crown freeze tolerance on T7C-17, but Wintok has additional gene(s) for field winter survival associated with T7C-17.

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Table 3.1. Population mean and extremes, parental phenotypes, translocation class means, additive translocation effect, and heritabilities for oat winter hardiness component trait from a recombinant inbred line population derived from a cross of 'Fulghum' × 'Norline'.

	Crown Freeze Tolerance	Heading Date	Percent Winter Field Survival			
				Laurel Springs	Blacksburg	Combined
	0-10†	Day of the year	Laurel Springs 2004	2005	2005	
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
Non-translocation 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

Table 3.1. (Continued)

*, **, *** Designate significant at P< 0.05, 0.01 and 0.001, respectively, or indicate level of significant difference between population extreme and most similar parent

† 0=complete plant death, 10= no freeze damage

‡ Additive Translocation Effect calculated by (mean of translocation class – mean of non-translocation class)/2

Table 3.2. 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.

	Winter Field Survival	Crown Freeze Tolerance	Heading Date	Translocation Status
Winter Field Survival	1	0.73***	0.46***	0.61***
Crown Freeze Tolerance	0.73***	1	0.23**	0.72***
Heading Date	0.46***	0.23**	1	0.20*
Translocation Status	0.61***	0.72***	0.20*	1

*, **, *** Designate significant at P< 0.05, 0.01 and 0.001, respectively

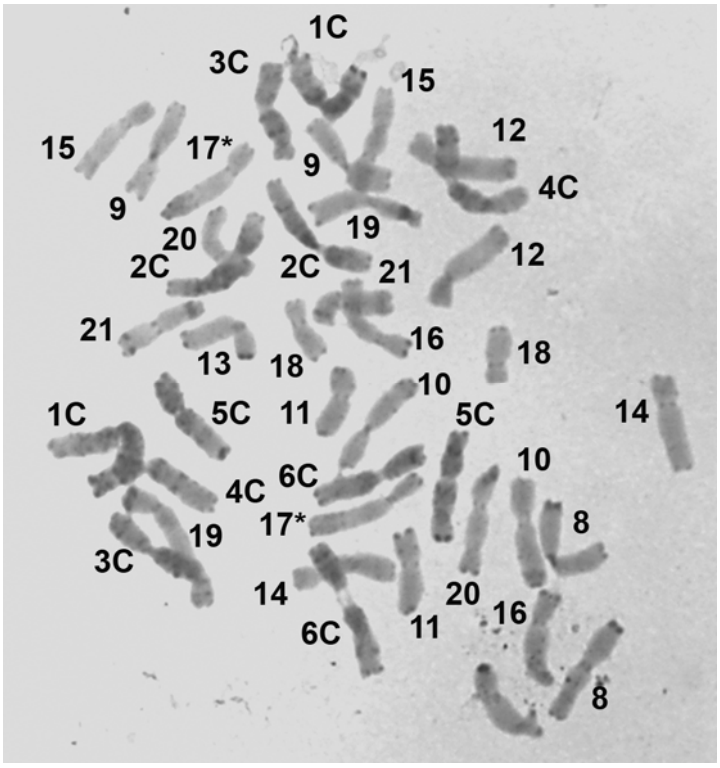

Figure 3.1. C-banded somatic chromosomes from chromosome $7C^{17}$ nullisomic oat line. Chromosomes are labeled with their number and 17* identifies chromosome 17^{7C}.

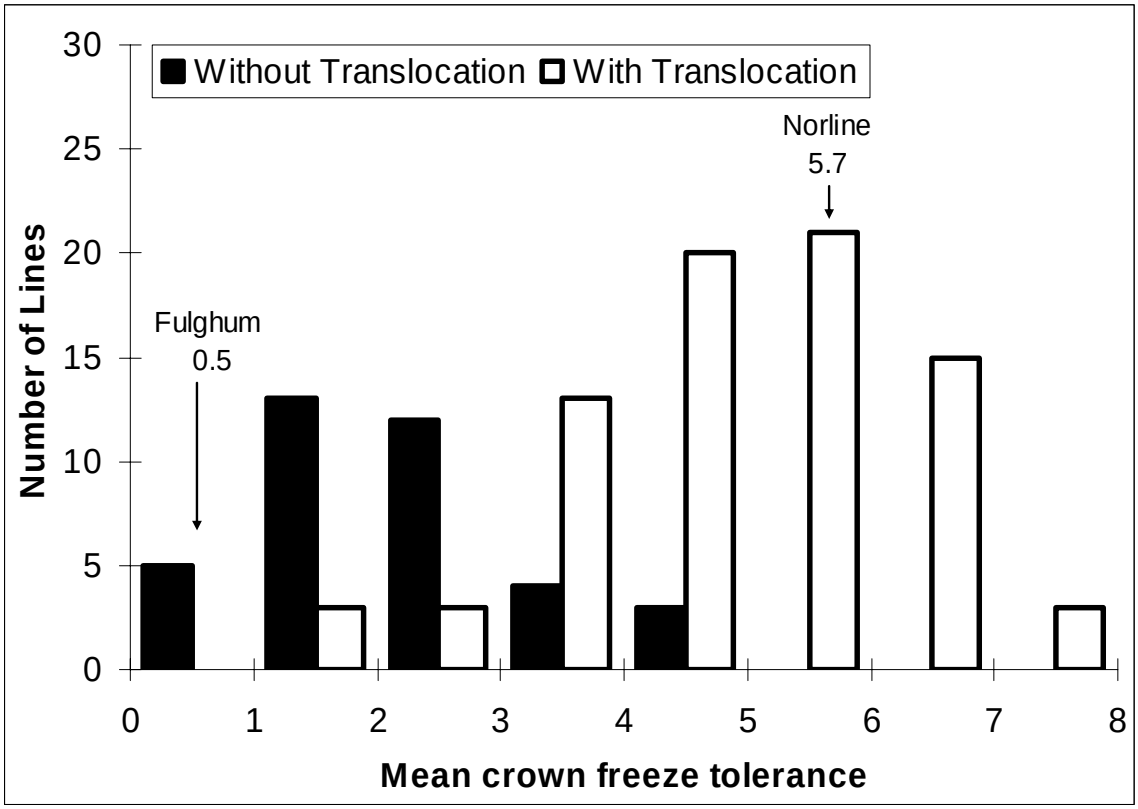

Figure 3.2. Frequency distribution of crown freeze tolerance line means for homogeneous translocation classes. A population of 116 oat recombinant inbred lines and their parents winter tender 'Fulghum' and winter hardy 'Norline' were evaluated for crown freeze tolerance on a scale from 0-10; a rating of 0=complete plant death, and a rating of 10= no freeze damage.

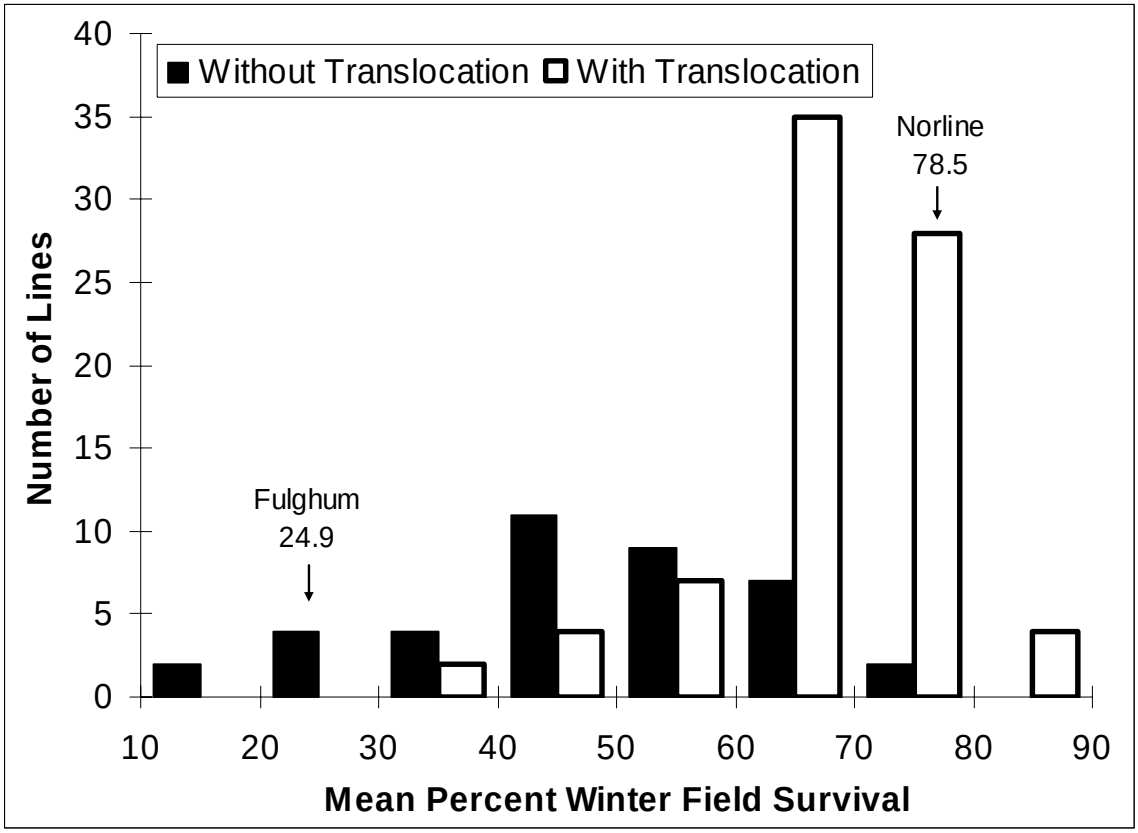

Figure 3.3. Frequency distribution of winter field survival line means for homogeneous translocation classes. A population of 120 oat recombinant inbred lines and their parents winter tender 'Fulghum' and winter hardy 'Norline' were evaluated for winter field survival in three environments.

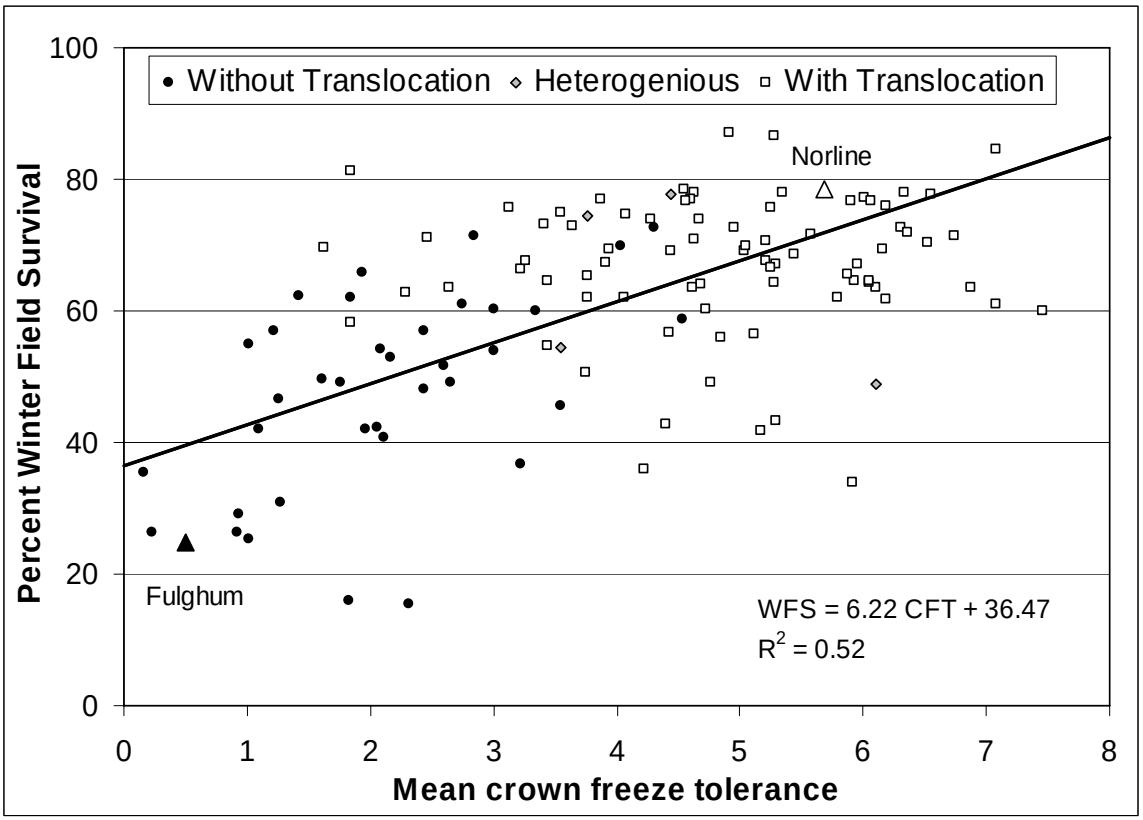

Figure 3.4. Scatter plot and regression of winter field survival vs. crown freeze tolerance for a population of 120 oat recombinant inbred lines and their parents winter tender 'Fulghum' and winter hardy 'Norline'. WFS=winter field survival, and CFT=crown freeze tolerance.

Chapter 4

Quantitative Trait Loci and Epistasis for Oat Winter Hardiness Component Traits

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Abbreviations:

LSmean, least-squares mean

FN, Fulghum × Norline recombinant inbred line population

FW, Fulghum × Wintok recombinant inbred line population

KO, Kanota × Ogle recombinant inbred line population

LOD, Log of the odds

QTL, quantitative trait locus or loci

RIL(s), recombinant inbred line(s)

T7C-17, intergenomic reciprocal translocation involving chromosomes 7C and 17

ABSTRACT

Winter hardiness is a complex trait and poor winter hardiness limits commercial production of winter oat (*Avena* species). The objective of this study was to identify Quantitative Trait Loci (QTL) for the winter hardiness component traits: winter field survival, crown freeze tolerance, heading date, plant height and vernalization response in a recombinant inbred line population derived from a cross of winter-tender cultivar Fulghum by winter-hardy cultivar Norline. Crown freeze tolerance, vernalization response, and photoperiod response were evaluated in controlled environment studies. Heading date and plant height were evaluated over two seasons in Kinston, North Carolina, and winter field survival was evaluated in five environments over two seasons in the mountains of North Carolina and Virginia. A partial genetic linkage map of regions believed to affect winter hardiness was developed using RFLP and SSR markers. Multiple interval mapping and EPISTACY were used to identify QTL. Most QTL were located on linkage groups FN3, FN22 and FN24 which corresponded to regions carrying winter hardiness QTL in other oat populations. QTL were identified for all traits except photoperiod response, and epistatic interactions were identified for winter field survival, crown freeze tolerance, vernalization response and plant height. Major QTL for winter field survival (R^2=35%) and crown freeze tolerance (R^2 =52%) were identified on linkage group FN3 which was associated with an intergenomic reciprocal translocation between chromosomes 7C and 17. These results improved our understanding of the interaction of winter hardiness traits by identifying QTL associated with winter hardiness.

INTRODUCTION

Poor winter hardiness limits winter oat production in much of North America. Cereal winter hardiness is a complex trait comprising several quantitative traits including crown freeze tolerance, vernalization and photoperiod responses, heading date and plant height (Fowler et al., 1999). Crown freeze tolerance is the most important winter hardiness trait (Olien, 1967). In addition photoperiod and vernalization responses combined with *per se* lateness act as freeze stress avoidance mechanisms which delay growth of freeze sensitive reproductive tissues until warmer temperatures arrive. Plant height tends to be correlated with these traits because plants that flower later have more time to grow taller.

There are relatively few reports of QTL for oat winter hardiness component traits. QTL for vernalization and photoperiod responses have been identified in spring × winter oat mapping populations (Holland et al., 1997; Holland et al., 2002), but no QTL for winter hardiness traits have been reported in winter × winter oat. Santos et al. (2006) found a reciprocal translocation (T7C-17) associated with crown freeze tolerance and winter field survival which identified chromosomes where crown freeze tolerance genes were located. More specific chromosomal regions have yet to be identified in winter oat. This contrasts with other winter cereals because QTL or genes for winter hardiness component traits have been identified in diploid wheat (*Triticum monococcum*) (Vagujfalvi et al., 2003; Yan et al., 2003), common wheat (*T. aestivum* L.) (Fowler and Limin, 2004; Kobayashi et al., 2005; Limin and Fowler, 2002; Toth et al., 2003), and barley (*Hordeum vulgare* L.) (Francia et al., 2004; Hayes et al., 1993; Pan et al., 1994).

Quantitative trait loci for different winter hardiness component traits, e.g. vernalization and freeze tolerance, are linked in almost all of these species.

'Fulghum' is a traditional winter oat cultivar derived from the land race 'Red Rustproof' in the late 19th century (Coffman, 1977). It has limited 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 in 1960 (Patterson and Schafer, 1978). It is winter-hardy (Livingston and Elwinger, 1995) and does have T7C-17 (Jellen and Beard, 2000). Norline and 'Wintok' are winter-hardy checks in the Uniform Oat Winter Hardiness Nursery and have similar winter hardiness, but differ for winter field survival in some environments (Livingston and Elwinger, 1995). Although both lines are traditional winter cultivars, they both have T7C-17. Santos et al. (2006) found a highly significant association between translocation status and both crown freeze tolerance and winter field survival in a recombinant inbred line (RIL) population derived from a cross of Fulghum and Wintok.

Identification of QTL for winter hardiness component traits would provide a tool for improving winter hardiness though marker assisted selection. This approach is particularly suitable because winter hardiness is sporadically expressed. An additional benefit would come from an enhanced understanding of the relationships among different winter hardiness component traits. The objective of this study was to identify QTL for the winter hardiness component traits: winter field survival, crown freeze tolerance, heading date, plant height and vernalization response in a recombinant inbred line population derived from a cross of Fulghum × Norline.

MATERIALS AND METHODS

Plant Material

The experiment was conducted using a population of 129 F_6 derived RILs developed from the cross of winter-tender Fulghum (non 7C-17 translocation) by winter-hardy Norline (7C-17 translocation). Plants 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.

Controlled Crown Freeze Tolerance Test

The experiment was an incomplete blocks within complete replications design with 15 incomplete blocks of 10 entries within each of five complete replications over time. Each complete replication consisted of 129 RILs plus seven entries of the parents Fulghum and Norline and 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 four days. Five seedlings of each entry were then planted 1.5cm deep in five adjacent 20cm long nursery tubes held in racks of 100 tubes. Plants were grown in Metromix 200 (Scotts-Sierra Horticultural Products Co.) and lightly watered daily with a complete nutrient solution. The plants were grown for five weeks 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 three week cold acclimation treatment. The hardening chamber held a constant 3°C with a 10 hour photoperiod of photosynthetic photon flux density of 300 μmol m^{-2} s^{-1}. While

cold acclimating, plants were watered with a complete nutrient solution (Livingston, 1991) three times per week and watered with deionized water on alternate days.

Crowns were prepared and frozen at -10°C as described in Santos et al. (2006) with a change in experimental design. 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 within each replication. One of the five congruent sponges from each incomplete block was placed on each shelf in a 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 50 by 30 cm plastic flats filled five cm deep with moist Metromix 200. The flats were returned to the growth chamber in the Southeastern Plant Environment Laboratory. After three weeks of re-growth, recovery for each crown was visually measured on a scale of 0-10 (0=complete plant death, 10=no freeze damage).

Field Trials

Heading date and plant height were evaluated using a randomized complete block design with two replications. $F_{6:7}$ seed of each RIL plus the parents and Wintok as checks were planted on 23 October 2002 at the Cunningham Research and Education Center, Kinston, NC. Plots were 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 2002-03. 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 measured 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. $F_{6:8}$ seed of the RILs plus five entries of the parental checks and Wintok was used. The experiment was planted on 16 September, 2003 at the Upper Mountain Research Station (895m elevation) near Laurel Springs, NC, and 9 October 2003 at the Mountain Research Station (727m elevation) near Waynesville, NC. Plots were hand planted with six grams 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 Springs 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 percent survival 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 Tech College of Agriculture and Life Sciences Kentland Research Farm (530m approximate elevation), near Blacksburg, VA. Plot size in 2004-05 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 2004-05 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 December, 2004

(http://www.ncdc.noaa.gov). No winter damage was observed in Waynesville in either season, so the data were not included in analysis of winter field survival.

Photoperiod and Vernalization Responses

Photoperiod and vernalization responses were evaluated in a growth chamber experiment 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 sub-plot factors. Seedlings for the non-vernalized treatment were germinated in moist paper towels for 4 days at 20°C. Seedlings with the vernalized treatment were germinated in moist paper towels for four weeks 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 $\mu mol\ m^{-2}\ s^{-1}$, and the long day treatment was simulated using a 2 h mid-night interruption with low intensity incandescent lights. This provided long day stimulus to the plants, while minimizing the difference in photosynthetically active radiation. After 42 days with differing photoperiod treatments, both chambers were increased to 16 h photoperiod to facilitate flowering. Temperature was held at 25/20°C photoperiod/darkness. Each plot consisted of two plants grown in the same pot and the days to flowering was recorded as the number of days from seedling emergence to panicle exertion for each plant. The mean of the two plants for each pot was used for ANOVA.

Molecular Markers

Plant tissue was harvested from young tillers of each parent F_6 plant in addition to the parents Fulghum and Norline, and the check cultivars Wintok, Kanota, and Ogle. DNA was extracted according to the large-scale DNA extraction protocol (CIMMYT, 2005). Briefly, DNA was extracted from ground frozen tissue using a CTAB extraction buffer, precipitated with isopropanol and washed with ethanol solutions. Suitable quantities of DNA were extracted for SSR markers from the initial plant samples, but the extraction was repeated using tissue harvested from 7-10 seedlings grown from $F_{6:7}$ seed of each RIL to extract enough DNA to complete RFLP analysis.

Sixty-two oat and barley simple sequence repeat (SSR) primers polymorphic in cultivated oat (Holland et al., 2001; Jannink and Gardner, 2005; Li et al., 2000; Pal et al., 2002) were evaluated for polymorphism in Fulghum and Norline. Twenty two showed possible polymorphism between the parents. These primers were evaluated in the population and checks. All SSR screening methods were described in Srnić et al. (2005) except that PCR amplifications were performed in a 10µl total volume containing 1X PCR buffer with 2.0mM Mg^{++}, 0.25mM dNTPs, 0.4pmol forward primer, 1.5pmol reverse primer, 1.5pmol M13-labeled primer, 0.2µg/µl BSA, 0.75U Taq polymerase, and 50ng genomic DNA.

RFLP probes chosen had previously been associated with the 7C-17 translocation (Fox et al., 2001), vernalization, or photoperiod response in oat (Holland et al., 1997; Holland et al., 2002). These probes were provided by the Graingenes probe repository or by Dr. Howard Rines USDA-ARS, St. Paul, Minnesota. Probes were amplified from

plasmids using PCR (CIMMYT, 2005), but without the use of mineral oil. Probe DNA was then purified using a QIAEX II agarose gel extraction protocol (Qiagen Iinc. Valencia, CA). Probes were P32 labeled using a random prime labeling kit (Invitrogen, Carlsbad, CA) and unincorporated P32 was removed with a sephadex G50 (Sigma-Aldrich Corp, St. Louis, MO) gravity column.

Twenty micrograms of DNA per sample were digested with 60 units of restriction enzyme in the appropriate buffer with the addition of 0.1mg/ml of BSA (Promega Corp. Madison, WI). After digestion, the DNA was loaded into a 1% agarose gel in 1x TBE and electrophoresed for 24h at 35V. DNA was transferred to Hybond-XL membrane (GE Healthcare, Little Chalfont, Buckinghamshire, England) using the neutral Southern blotting technique described in the Hybond manual. DNA was fixed on the membranes by baking at 70°C for 2h. Prehybridzation, hybridization, and membrane washing were conducted as described in Qi et al. (2003), and membranes autoexposed X-ray film for 2-21 days at -70°C. Parental polymorphism screens were conducted using Fulghum and Norline DNA digested with *Bam*HI, *Dra*lI, *Eco*RI, *Eco*RV, and *Hin*dIII. Putatively codominant polymorphisms were preferentially selected for population genotyping.

Data Analysis

The phenotypic data were analyzed using the MIXED procedure of SAS (Littell et al., 1996) with the Satterthwaite option for calculating degrees of freedom. For the vernalization and photoperiod study, the days to flowering data were transformed with the natural logarithm function to normalize the error variance. Heritabilities were estimated for the population excluding checks using an all random effects model

following the method described by Holland et al. (2003), but adjusted for the differences in experimental designs. Entries (including parents and checks) were then considered a fixed effect and the LSMEANS statement generated entry LSmeans (means). The DIFF option (α=0.05) was used to test for transgressive segregation. Vernalization and photoperiod responses were calculated as the difference between LSmeans for each genotype in the different treatments. Correlations were estimated among phenotypic traits using the CORR procedure. LSmeans for each RIL were combined with genotypic data, and single factor analyses for each marker were conducted for each trait using proc GLM. The SAS program EPISTACY (Holland, 1998) was used to check for epistasis.

Mapmaker/EXP version 3.0 (Lander et al., 1987) was used to construct a genetic linkage map using a minimum LOD score of 4 for grouping markers, and the Kosambi map function. Linkage groups were assigned names according to their likely homology with linkage groups in the Kanota × Ogle map (KO) (Wight et al., 2003) or Ogle × 'TAM O-301' map (OT) (Portyanko et al., 2001) based on SSR loci or multiple RFLP probes. A region of one linkage group was associated with chromosome 7C and T7C-17 using the marker data from a line in this population that was identified as nullisomic for chromosome $7C^{17}$ (Wooten et al., submitted b).

Multiple interval mapping (MIM) was used to test for the presence of QTL and epistatic interactions between main-effect QTL, and to estimate their effects (Kao et al., 1999). The MIM QTL search was initiated with no initial model. The likelihood ratio for the presence of a QTL was calculated every 1 cM. The Schwarz (1978) Bayesian Criterion, the default option, was used as the penalty function to prevent over fitting the model (Basten et al., 2004; Zeng et al., 1999). Epistatic interactions and corresponding

QTL detected with EPISTACY were manually added to the MIM model, but the interaction and QTL were then removed from the MIM model if the interaction was not significant according to the information criterion. This allowed for the inclusion of non-significant main-effect QTL in the MIM model, but only if the term was involved in a significant interaction. The nature of significant epistatic interactions were explored using procedure GLM in SAS. Epistatic interactions were modeled using the nearest loci and the LSmeans statement generated class means for the four marker class combinations.

RESULTS

Genetic Linkage Map

Thirty-four polymorphic loci were successfully screened in the population and 20 loci were linked in seven linkage groups at a LOD score of 4.0 for a linkage map with a total length of 129 cMorgans (Figure 4.1). Three linkage groups, FN3, FN22 and FN24, were similar to linkage groups 3+38, 22_44_18, and 24_26_34 in the Kanota × Ogle (KO) map (Wight et al., 2003), and one linkage group, FN11, was similar to group 11 in the Ogle × TAM O-301 (OT) map (Portyanko et al., 2001). The linkage groups that could not be aligned with previous oat maps were numbered FN41, FN42, and FN43 to avoid confusion with previously numbered oat linkage groups. The largest linkage group, FN3, included RFLP probes that were associated with T7C-17, or the linkage groups associated with chromosomes 7C and 17 in the KO map (Fox et al., 2001; Wight et al., 2003), as well as two SSR loci that were not previously mapped in oat. The RIL nullisomic for chromosome $7C^{17}$ confirmed that linkage group FN3 included the translocation region. The breakpoint between chromosome 7C and the translocation was between loci HVM20 on chromosome 7C and AM270s_1 on the translocation

Segregation distortion was detected for several markers. All markers on linkage group FN3 had significantly more Norline alleles than predicted from a 1:1 segregation ratio, but none differed significantly from a 2:1 segregation ratio. The unlinked marker AM31, the marker CDO1523 on group FN24 and the markers Astaveb and Avenin on group FN42 and all showed segregation distortion with more Fulghum alleles than expected.

Phenotypic Data

Five of the six traits measured had estimated heritabilities greater than 70% (Table 4.1). Although the photoperiod and genotype main effects and photoperiod by genotype effect were significant in ANOVA, the heritability of photoperiod response was low (h^2=25%), so the data are not presented. Fulghum and Norline were significantly different from each other for winter field survival, crown freeze tolerance, vernalization response and heading date, but not for plant height (Table 4.1). Winter field survival and crown freeze tolerance showed a bimodal distribution, while heading date and vernalization response were normally distributed. Plant height was not normally distributed because several lines had very short height giving the population a long tail. Transgressive segregants were identified for increased crown freeze tolerance, vernalization response, heading date and plant height, and for decreased vernalization response and plant height. One of the RILs was nullisomic for chromosome 7C[17] and was not included in the population for QTL detection. Winter field survival was significantly correlated with crown freeze tolerance and heading date, but not vernalization response or plant height (Table 4.2). Heading date was significantly correlated with all of the other traits. Vernalization response was not correlated with crown freeze tolerance.

Quantitative Trait Loci

QTL for all traits were identified and epistatic interactions were identified for all traits except heading date (Table 4.3, Figure 4.1). The inclusion of significant epistatic interactions between non-significant main-effect QTL complicates discussion of main-

effect QTL. Such QTL will not be considered main-effect (or additive) QTL even though they are included in the model due to their significant epistatic interactions. Four main-effect QTL were identified for winter field survival, plus two epistatic interactions. At three of the four main-effect QTL, the Fulghum alleles decreased winter field survival as expected, and two of those were on linkage group FN3. Examination of the interaction between AM2 and CDO1523 showed that the non-parental RILs (RILs with Fulghum alleles as one locus and Norline alleles at the other) had better performance than either parental genotype as indicated by the negative sign of the interaction. The interaction between UMN433 and CDO1523 followed a "classical" pattern for epistasis. If a line had the Fulghum alleles at UMN433 it had winter field survival near 53.4% regardless of the CDO1523 alleles. Mean winter field survival of recombinant inbred lines with Norline alleles at UMN433 varied depending on the alleles at CDO1523. Lines with Norline alleles at CDO1523 were slightly more freeze tolerant than those with Fulghum alleles. Fulghum alleles at UMN433 masked the effect of alleles at CDO1523. This was a complicated situation because the QTL near CDO1523 was involved in two different epistatic interactions.

One major QTL on linkage group FN3 was identified for crown freeze tolerance accounting for 46% of the phenotypic variation (Table 4.3). In addition, three minor additive QTL were identified. Fulghum alleles at the QTL near HVM20 and BCD1261 on FN3 and near CDO504 on FN22 decreased freeze tolerance while Fulghum alleles near Astaveb on FN42 increased crown freeze tolerance. The epistatic interaction between the QTL near HVM20 and Rast1_4 behaved as described by the sign of the interaction. However the interaction between HVM20 and CDO504 showed a "classical"

pattern of epistasis. Fulghum alleles at HVM20 masked expression of alleles at CDO504. Among RILs that had Norline alleles at HVM20, those with Norline alleles at CDO504 had significantly greater crown freeze tolerance than those with Fulghum alleles at CDO504.

Two main-effect QTL and two epistatic interactions were identified for vernalization response. Fulghum alleles at the larger main-effect QTL exhibited lower vernalization response than Norline alleles, as expected from the parental responses to vernalization. At the smaller main-effect QTL on linkage group FN3, the Fulghum alleles were more sensitive to vernalization. The two epistatic interactions which each involved a locus at either end of FN3 accounted for a greater percentage of variation than the two main-effect QTL (29% vs. 18%). Both epistatic interactions had a negative sign, because the non-parental combinations had greater vernalization response than the parental types. For both interactions, the marker class mean with the greatest vernalization response had Fulghum alleles at the locus on linkage group FN3 (either UMN433 or UMN220B) and Norline alleles at the other loci (either CDO1461A or CDO549). This shows that linkage group FN3 had QTL with a large but complex effect on vernalization response, and that the alleles at QTL on FN3 did not affect vernalization phenotype as expected.

Three QTL with no epistatic interactions were identified for heading date (Table 4.3). The QTL near CDO1319 on linkage group FN3 and near CDO484B on linkage group FN43 both had additive effects in the expected direction. Fulghum alleles at the unlinked locus CDO187SSR increased heading date. Two QTL were identified for plant

height with an epistatic interaction between them. Because the parents had little difference in plant height, there was no expected direction for the Fulghum alleles.

The portion of the phenotypic variation explained by the QTL (the sum of the R^2 values) did not approach the heritability for any trait (Table 4.1, Table 4.3). Although we were able to identify several major QTL for the winter hardiness traits, there likely were other QTL in regions of the genome not covered with molecular markers.

DICUSSION

Linkage Map

The RFLP mapped in this population targeted specific regions of the oat genome that were believed to carry winter hardiness component genes based on previous research. Most of these loci mapped to three linkage groups that were obviously homologous to the linkage groups 3+38, 22_44_18, and 24_26_34 in the Kanota × Ogle map (Wight et al., 2003). In contrast, we used all available polymorphic SSR markers regardless of genome location, so the SSR markers were not as likely to be in genomic regions of interest and tended to map into the small linkage groups.

Segregation distortion was found for several markers, especially those in linkage group FN3. Segregation distortion seems a common phenomenon in oat (Portyanko et al., 2001; Wight et al., 2003) and the prevalence of Norline alleles in the linkage group FN3 was expected because of segregation distortion causing a preponderance of translocation types (Santos et al., 2006; Wooten et al., submitted b). The observed segregation distortion with a preponderance of Fulghum alleles was more difficult to explain. It is possible that one or both of the regions near Astaveb and CDO1523 had genes that are homoeologous to a critical gene hypothesized to be on or near the T7C-17 (Wooten et al submitted b; Jellen and Beard, 2000). No RILs were found which had Norline alleles at both Astaveb and CDO1523 loci and did not have T7C-17 (Fulghum translocation type). This observation supported the theory that critical genes reside on the translocation in Norline, but the critical genes are in different locations in Fulghum, possibly near Astaveb or CDO1532. The position of a QTL near Astaveb (Table 4.3) where Fulghum alleles increase winter field survival and crown freeze tolerance suggests that this

hypothesized critical gene(s) is, or is linked to, a gene or genes for winter hardiness component traits.

Linkage group FN3 included parts of both chromosomes 7C and 17 and T7C-17, but the length of the linkage group was much shorter than in the Kanota × Ogle map (Wight et al., 2003). The T7C-17 was expected to reduce recombination and that may explain part of the small size of the linkage group FN3. However the Kanota × Ogle population also segregates for T7C-17, so it was not expected that linkage group FN3 in this map would be much shorter than in the KO map. Contrasting this were linkage groups FN22 and FN24 where the FN map expanded portions of reduced recombination in the KO map. All of the markers on linkage group FN22 mapped near position 100 on group 22_44_18 in the KO map, and all of the makers on linkage group FN24 mapped near position 41 in the linkage group KO 24_26_34 (Wight et al., 2003). Wight et al (2003) noted that Kanota and Ogle differ for several translocations in addition to T7C-17 and the difference in recombination between the KO and FN maps could have resulted from additional translocations segregating in the KO population.

The nullisomic $7C^{17}$ line allowed us to place the breakpoint of T7C-17 between loci HVM20 and AM270s_1. The polymorphic band produced with the HVM20 primers was not found in any of four attempts to amplify the HVM20 primers in DNA from the nullisomic line. Further, a monomorphic band was produced using the HVM20 primers on the nullisomic line in all four attempts which confirmed that the PCR amplification was successful with the DNA from the nullisomic line. The AM270s_1 primers produced the Norline allele in the nullisomic line so that locus likely was located on the

translocated part of 7CL on chromosome 17^{7C}. The identified translocation breakpoint also delineated the confidence intervals for most QTL on linkage group FN3 (Figure 4.1).

Phenotypic Data

All traits showed continuous variation with most having near normal distributions. The bimodal distributions of the winter field survival and crown freeze tolerance traits were explained by major genes for each trait found on linkage group FN3. These accounted for 30% of the variation in winter field survival and 46% of the variation in crown freeze tolerance. This was similar to the results of Santos et al. (2006) who reported that T7C-17 status accounted for 22% of the variation in crown freeze tolerance and 27% of the variation in winter field survival in a RIL population derived from Fulghum × Wintok. Clearly there were major genes on or near T7C-17 which conferred increased winter field survival and crown freeze tolerance and caused a bimodal distribution for these two traits. The significant correlation between crown freeze tolerance and winter field survival was expected and is the basis for the crown freeze tolerance protocol. The lack of significant correlations between vernalization response and crown freeze tolerance or winter field survival was unexpected as many authors have reported a correlation between these traits in small grains (Doll et al., 1989; Mahfoozi et al., 2001). This relationship in oat may not be as clear (Wooten et al. submitted a). Field heading date was correlated with all of the other traits measured. The much lower correlation between heading date and crown freeze tolerance compared to the correlation with winter field survival suggested that genes increasing heading date and field survival do not increase crown freeze tolerance to the same degree. Late heading date was likely

a freeze stress avoidance mechanism (Prasil et al., 2004), not a stress tolerance mechanism.

Quantitative Trait Loci

Many of the QTL identified in this study were associated with multiple winter hardiness component traits (Figure 4.1, Table 4.3). Many authors have reported tight linkage between winter hardiness traits in winter cereals (Kobayashi et al., 2005; Pan et al., 1994; Storlie et al., 1998; Sutka et al., 1999; Toth et al., 2003). While it is not possible to discern linkage from pleiotropic effect in this population, the similarity between these results and those from other winter cereals supports the linkage hypothesis for most of the QTL. Because most of the markers mapped in this population previously were associated with winter hardiness component traits, there were some similarities between the QTL identified in this study and those identified previously.

Linkage group FN3 had QTL for all of the traits measured in this study, however the configuration of vernalization QTL was contrary to the expected configuration. Typically QTL for increased freeze tolerance are linked to QTL for increased vernalization response. However, on linkage group FN3, Fulghum alleles increased vernalization response, but reduced crown freeze tolerance and winter field survival. Santos et al (2006) found an association between T17-7C and the traits crown freeze tolerance and winter field survival. Our results support the importance of this genomic region, with two additive QTL accounting for 52% of the variation for crown freeze tolerance and two additive QTL accounting for 35% of the variation in winter field survival in the region of the translocation. However, a previous QTL study in a spring ×

winter crosses did not identify QTL for freeze tolerance associated with the markers in the corresponding region of linkage group FN3 (CDO1319-BCD1261) (Wooten et al. submitted a). However a QTL for freeze tolerance was found near UMN433, which is at the end of the linkage group. Additionally, a QTL for plant height was identified in the region of T7C-17, but translocation status was not associated with plant height (Wooten et al., in submitted b). QTL for freeze tolerance were identified in the KO population for the markers on the ends of linkage group FN3, UMN220 (on KO LG 24_26_34) and UMN433 (on KO 3+38) (Wooten et al. submitted a). These QTL were also identified as vernalization response and heading date QTL (Holland et al., 1997; Siripoonwiwat et al., 1996). Further, the QTL near UMN220 in the KO population was involved in several epistatic interactions (Holland et al., 1997), possibly similar to its epistatic interaction in the FN population (Table 4.3). These results demonstrate the importance of examining winter hardiness component traits in a winter oat population, rather than a spring × winter mapping population. The QTL in this region that were previously identified with freeze tolerance in a winter × spring mapping population, were associated with vernalization response in this winter × winter population, but not freeze tolerance. Conversely, the QTL that were associated with crown freeze tolerance in a winter oat populations, were not identified in the winter × spring mapping populations, and these are the QTL with the largest effects.

Linkage groups FN22 and FN24 were both easily aligned with portions of the linkage groups in the KO populations and carried similar QTL to those found in the KO and OT mapping populations. Linkage group FN22 carried QTL for winter field survival, crown freeze tolerance, and vernalization response. In contrast to the situation on FN3,

the QTL on FN22 had the expected additive effect where the lines having the Fulghum alleles at the QTL were more similar to Fulghum for all three traits, indicating coupling phase linkage for alleles that decrease vernalization response, crown freeze tolerance, and winter field survival at QTL on this linkage group. This configuration of linked freeze tolerance, vernalization response, and winter field survival QTL is common across winter cereal species (Galiba et al., 1995; Kobayashi et al., 2005; Pan et al., 1994). The markers on linkage group FN22 were associated with vernalization response in both the KO and OT populations (Holland et al., 1997; Holland et al., 2002). Linkage group FN24 had QTL for plant height as well as epistatic interaction for crown freeze tolerance and winter field survival. In the KO and OT mapping populations this region carried a major QTL for vernalization response and heading date, as well as epistatic interactions for vernalization response (Holland et al., 1997; Holland et al., 2002; Siripoonwiwat et al., 1996).

Comparing the QTL configuration on FN3 with FN22 and FN24 (and the typical arrangement of winter hardiness component traits in winter cereals) showed the arrangement on FN3 was unusual. The major freeze tolerance QTL in FN3 were not previously identified in other oat populations, and was linked in repulsion to vernalization response QTL. It appeared that the T7C-17 created an unusual arrangement in this region that greatly increased the most important winter hardiness traits (winter field survival, and crown freeze tolerance) even when linked in repulsion to genes for vernalization response. Wooten et al. (submitted b) noted that T7C-17 had nearly identical effects for freeze tolerance in the FN population and the FW population examined by Santos et al. (2006), but the translocation had a substantially larger effect on

winter field survival in the FW population. The linkage of vernalization response QTL in repulsion in this population provided a good explanation for these observations. We suspect that the vernalization response QTL near T7C-17 were linked in coupling with freeze tolerance QTL in the FW population. The Wintok vernalization response alleles should increase winter field survival without much increase in crown freeze tolerance fitting the observed results for the translocation effect on crown freeze tolerance and winter field survival in the FN and FW populations (Wooten et al., submitted b; Santos et al., 2006). Unfortunately, the translocation likely disrupted the recombination in the region, and caused segregation distortion in both populations, complicating linkage mapping and identification of QTL in this region. Further QTL studies with crosses between lines that do not segregate for the translocation should be informative in precisely mapping the winter hardiness QTL in this region.

The linkage group FN11 corresponded to part of the linkage group OT11. The linkage group OT11 did have both main-effect QTL and epistatic interactions for heading date without vernalization response in the OT population (Holland et al., 2002), but the region in the OT map that had these QTL was approximately 30 cM away from the mapped position of Rast1_4 (Jannink and Gardner, 2005; Portyanko et al., 2001). It was unknown whether these are the same QTL because the FN map does not cover the region of OT11 which carries the winter hardiness component trait QTL.

Several QTL and epistatic interactions were identified on the small linkage groups of unknown location. The loci on FN42 had a QTL where Fulghum alleles increased winter field survival and crown freeze tolerance. The Astaveb loci is in a region where both the OT map and the corresponding region of the KO map had QTL for heading date

(Holland et al., 1997; Holland et al., 2002; Jannink and Gardner, 2005; Siripoonwiwat et al., 1996). The Avenin locus maps to OT8 (Jannink and Gardner, 2005), but it is approximately 60 cM away from the location of the nearest winter hardiness component trait QTL identified by Holland et al (2002). We suspect this region did not correspond to the QTL we mapped in the FN population. The epistatic interaction for winter field survival involving AM2 on FN41 could not be associated with any previous QTL studies because neither SSR marker on FN41 has been mapped to linkage groups in the KO or OT mapping populations. The QTL for heading date near CDO484B on linkage group FN43 was difficult to explain. There were several QTL for heading date traits near CDO484 on KO 24_26_34 (Holland et al., 1997), but this region was mapped as CDO484A which was in the linkage group FN24. These regions may be homoeologous to FN24, but there were too few markers to make that determination.

Three unlinked markers were identified as main-effect QTL, or were involved in epistatic interactions. The Fulghum alleles at loci CDO187SSR caused late heading date in the FN population. In the KO population the marker CDO187 was identified as a QTL for heading date (Siripoonwiwat et al., 1996), and these data seem to fit the same pattern. A QTL for vernalization response was identified near the marker CDO549 on linkage group 13 in the KO population, but unlike the FN population it was not associated with epistasis (Holland et al., 1997). No QTL for winter hardiness component traits have been associated with the RFLP probe CDO1461 in either the KO or OT populations.

CONCLUSION

Significant variation was observed for the winter hardiness component traits winter field survival, crown freeze tolerance, heading date, plant height and vernalization and photoperiod responses. QTL were identified for all traits except photoperiod response. Multiple QTL for related winter hardiness component traits were primarily identified on three linkage groups. The linkage group FN3 had major QTL for winter field survival (R^2=35%) and crown freeze tolerance (R^2 =52%). These QTL were in the region of the T7C-17 which was previously associated with winter hardiness traits. Surprisingly, vernalization response QTL alleles were linked in repulsion phase to the QTL alleles for increased winter hardiness on linkage group FN3. Linkage groups FN22 and FN24 had QTL alleles for decreased crown freeze tolerance, winter field survival, heading date, or vernalization response linked in coupling phase. These results identified the major QTL associated with winter hardiness component traits near T7C-17 which illustrated the importance of this genomic region to winter hardiness.

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Table 4.1. Population mean and extremes, parental phenotypes, and heritabilities for oat winter hardiness component traits from a recombinant inbred line population derived from a cross of 'Fulghum' × 'Norline'.

	Winter Field Survival	Crown Freeze Tolerance	Vernalization Response	Heading Date	Plant Height
	Percent	0-10†	ln(days to flowering)	Day of the year	cm
Population Mean	60.6	4.0	0.60	118.8	118
Population Min.	15.5^{ns}	0.2^{ns}	0.22*	109.5^{ns}	55***
Population Max.	87.0^{ns}	7.5***	0.95*	128.0***	155***
Fulghum	24.9±10.5	0.5±0.6	0.36±0.06	110.9±1.5	108±3
Norline	78.5±10.5	5.7±0.6	0.82±0.06	122.5±1.5	112±3
Heritability (%)	76±4	83±2	71±6	80±17	76±4

*, **, *** Designate significant at P< 0.05, 0.01 and 0.001, respectively, for significant difference between population extreme and most similar parent

† 0=complete plant death, 10= no freeze damage

Table 4.2. Correlation of winter hardiness component traits from a population of 131 oat recombinant inbred lines derived from a cross of Fulghum and Norline.

	Winter Field Survival	Crown Freeze Tolerance	Vernalization Response	Heading Date	Plant Height
Winter Field Survival	1	0.73***	0.06	0.41***	-0.07
Crown Freeze Tolerance	0.73***	1	-0.09	0.20*	-0.12
Vernalization Response	0.06	-0.09	1	0.36***	-0.15
Heading Date	0.41***	0.20*	0.36***	1	-0.22*
Plant Height	-0.07	-0.12	-0.15	-0.22*	1

*, **, *** Designate significant at P< 0.05, 0.01 and 0.001, respectively

Table 4.3. Quantitative Trait Loci (QTL) for the winter hardiness component traits winter field survival (WFS), crown freeze tolerance (CFT), vernalization response (VR), heading date (HD), and plant height (PHT) evaluated in a RIL population derived from a cross of winter-tender Fulghum and winter-hardy Norline.

Trait	QTL	Linkage Group	Position (cM)	Nearest Marker(s)	MIM LOD	Additive Effect†	R^2 %
WFS	1	FN3	22	HVM20	5.52	-9.7	30.7
WFS	2	FN3	47	UMN433	0.4	-2.3	4.5
WFS	3	FN22	16	AM102	2.6	-5.1	7.5
WFS	4	FN24	0	CDO1523	0	0.8	0
WFS	5	FN41	5	AM2	0	0.7	0
WFS	6	FN42	5	Avenin	0.5	2.3	1.1
WFS	1 x 4	Epistatic Interaction		UMN433 x CDO1523	0.6	2.5	1.0
WFS	4 x 5	Epistatic Interaction		CDO1523x AM2	0.9	-2.9	3.5
CFT	1	FN3	20	HVM20	8.2	-1.2	45.8
CFT	2	FN3	35	BCD1261	0.6	-0.26	6.7
CFT	3	FN22	0	CDO504	1.0	-0.29	3.0
CFT	4	FN11	0	Rast1_4	0	0.07	0
CFT	5	FN42	3	Astaveb	1.0	0.29	2.3

Table 4.3. (Continued)

CFT	1 x 3	Epistatic Interaction		HVM20 x CDO504	0.7	0.24	2.7
CFT	1 x 4	Epistatic Interaction		HVM20 x Rast1_4	0.9	-0.28	4.1
VR	1	FN3	0	UMN220B	0	-0.002	0
VR	2	FN3	43	UMN433	0.26	0.030	3.4
VR	3	FN22	0	CDO504	2.1	-0.070	13.9
VR	4	Unlinked		CDO1461A	0	-0.007	0
VR	5	Unlinked		CDO549	0	-0.023	0
VR	2 x 4	Epistatic Interaction		UMN433 x CDO1461A	1.2	-0.073	16.8
VR	1 x 5	Epistatic Interaction		UMN220B x CDO549	1.3	-0.072	13.5
HD	1	FN3	12	CDO1319	0.77	-1.0	4.3
HD	2	FN43	0	CDO484B	1.34	-1.3	9.6
HD	3	Unlinked		CDO187SSR	0.33	0.6	2.1
PHT	1	FN3	24	AM270S_1	0.4	2.1	1.8
PHT	2	FN24	0	CDO1523	1.3	4.5	11.1
PHT	1 x 2	Epistatic Interaction		AM270S_1 x CDO1523	0.6	-3.0	5.4

† Additive effect of a single Fulghum allele, equivalent to (mean of RILs homozygous for Fulghum alleles – mean of RILs homozygous for Norline alleles)/2

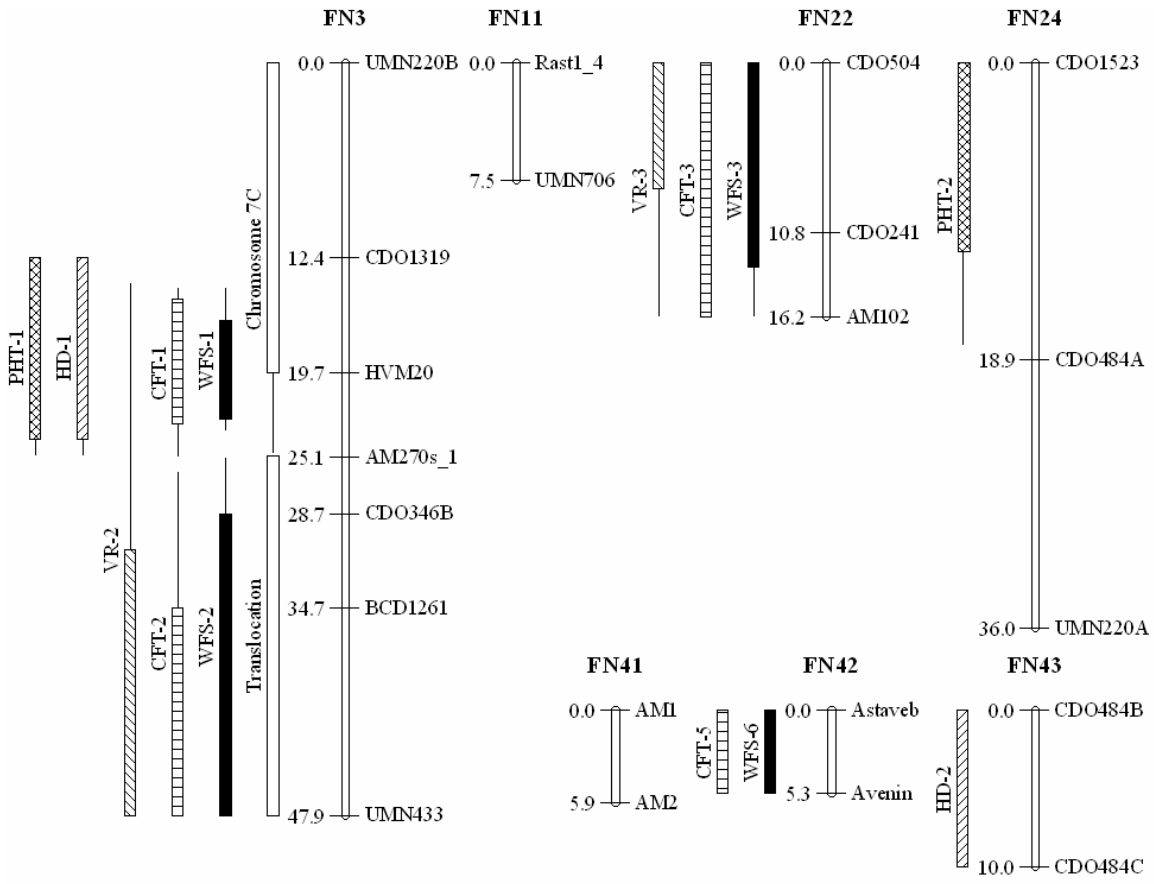

Figure 4.1. Linkage map from Fulghum × Norline recombinant inbred line population with quantitative trait loci for winter hardiness component traits. Locus marker names are shown on right side of linkage groups, with absolute position in centimorgans on the left. Approximate locations of main-effect quantitative trait loci are indicted with shaded bars representing the equivalent of a one LOD support interval and extending lines representing the equivalent of a two LOD support interval. QTL are labeled with trait abbreviation winter field survival (WFS), crown freeze tolerance (CFT), vernalization response (VR), heading date (HD), and plant height (PHT), and the QTL number for each trait (Table 4.3). The portions of linkage group FN3 that represent chromosome 7C and the translocated region are identified with labeled QTL bars.