

Additional Markers Added to the Fulghum/Norline Genetic Map and QTL Scan for Additional Winter Hardiness Traits in *Avena sativa* L.

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Abstract

Avena sativa, or Common Cultivated Oat, has the poorest winter hardiness among the small grain cereals. Marker-assisted selection for improved winter survival in oat is difficult, as the number of SSR markers available in this species is limited. The objectives of this research were to increase the number of SSR markers on the Fulghum x Norline recombinant inbred population genetic map, and to scan for QTL associated with winter hardiness component traits, including winter field survival, crown freezing tolerance, vernalization response, and heading date. SSR markers were developed from Kanota and Ogle genomic DNA libraries that were enriched for eight microsatellite motifs. New primers were evaluated for amplification, reproducibility, and polymorphism in 11 oat lines. SSRs showing high-quality polymorphism between Fulghum and Norline were subsequently examined in 139 recombinant inbred lines. Phenotypic data for winter hardiness component traits in the population were obtained in field and controlled chamber experiments. All previously mapped markers and new SSR markers were evaluated and QTL identified. Marker loci on linkage group FN3 accounted for multiple QTL associated with winter hardiness component traits. The markers found to be associated with winter hardiness will further assist breeders in selecting for winter hardy oats.

Introduction

Winter hardiness is a complex trait that encompasses tolerance to all adverse winter variables such as freezing temperatures, soil heaving, and reduction in vigor due to disease. Previous research with this Fulghum x Norline recombinant inbred population (Wooten et al, submitted) found QTL associated with winter hardiness and other component traits.

In the interim, additional SSR markers have become available for study. The objective of this research was to place additional markers on the Norline/Fulghum genetic map and identify QTL associated with the winter hardiness component traits: winter field survival, crown freezing tolerance, vernalization response, and heading date.



Figure 1: (Left) Different lines during growth phase of Crown Freeze Tolerance test (Right) Plants used as scoring standards in Crown Freeze Tolerance tests

Materials and Methods

Plant Material and Phenotypic Data:

- A Recombinant Inbred Population of 139 F₆-derived individuals was created by crossing winter hardy Norline by winter tender Fulghum.
- Heading Date (HD), and Plant Height (HT) data were collected over two seasons.
- Winter Field Survival (WFS) data were collected over two years at 3 locations with 5 replications per location.
- Crown Freezing Tolerance (CFT) data were collected using a freezing technique where the crown is extracted and frozen at -10 C°. After recovery, plants were scored on a scale from 0 to 10 (0 = dead and 10 = undamaged). Data were collected using a randomized incomplete block design with 5 reps and 5 plants per entry per rep.

Molecular Markers:

- Previously published SSR markers were evaluated in the RIL population along with new SSR markers developed by Dr. Joe Anderson USDA-ARS, Purdue University using cDNA libraries from Kanota and Ogle.
- RFLP probes, previously associated with the 7C-17 translocation, vernalization, and/or photoperiod response in oat, were evaluated in the RIL population.

Molecular Linkage Mapping:

- The population was scored with 20 RFLP probes and 48 SSR markers.
- Joinmap 3.0 was used to generate a linkage map of seven linkage groups with a total length of 172.1 cM.
- Linkage group names were assigned based on the previously mapped markers.
- Linkage groups that could not be assigned names were given the "unknown" designation.
- One RIL, nullisomic for chromosome 7C-17, was used to assign markers to chromosome 7C

Results and Discussion

- Major Winter Field Survival QTL (WFS 1, WFS 2) and Crown Freezing tolerance QTL (CFT 1, CFT 2) were found on linkage group FN3 (Figure 2, Table 1).

- This linkage group is associated with the 7C-17 translocation
- QTL for WFS (R²=40.6%) and crown freezing tolerance (R²=61.1%) were identified in or near the translocation on Linkage Group FN3
- These results support the finding of Santos et al. (2006)
- Major QTL near position 18 cM are likely on chromosome 7C and major QTL near position 24 cM are likely on the translocation.

- This was shown by using the RIL nullisomic for the 7C-17 translocation event
- Despite low LOD scores, it is thought that there are two QTL on linkage group FN3 because they span the translocation area.
- It is proposed that an "adaptive gene cluster" was created by the translocation event.

- The major QTL for WFS and CFT in this study are not in the same location as those found in the Kanota X Ogle population (Wooten et al., 2003).

- This is thought to be caused by the use of a winter oat X winter oat population, like the Fulghum/Norline RIL, instead of a spring X winter oat population, like the Kanota/Ogle RIL.

- One QTL for vernalization response was located on linkage group FN 22.

- Major QTL for WFS and CFT were also located in the same area of the linkage group FN22 (Figure 2, Table 1).

- This indicates coupled phase linkage, which is common across other winter cereals (Pan et al., 1994).

- QTL for vernalization and photoperiod response were identified in this region in the 'Kanota' x 'Ogle' and 'Ogle' x 'Tam O-301' populations (Holland et al., 1997; Holland et al., 2002).

- QTL for plant height (HT 1) was found to be located on linkage group FN24.

Table 1: Multiple Interval Mapping data for QTL discovered on Fulghum/Norline RIL.

Winter Field Survival					
QTL	Chrom.	Position	LOD	Effect	Effect (%)
WFS 1	FN3	18.11	3.1	-5.6503	20.4
WFS 2	FN3	24.41	2.56	-5.5724	20.2
WFS 3	FN22	10.5099	8.55	-7.0267	20.8
R2 Value	0.6147				

Crown Freezing Tolerance					
QTL	Chrom.	Position	LOD	Effect	Effect (%)
CFT 1	FN3	15.21	3.23	-0.5245	18.8
CFT 2	FN3	24.41	8.57	-1.0196	42.3
CFT 3	FN22	14.5099	3.37	-0.4052	5.1
R2 Value	0.6619				

Vernalization Effect					
QTL	Chrom.	Position	LOD	Effect	Effect (%)
VRN 1	FN22	14.5099	2.82	-0.0653	13.5
R2 Value	0.1348				

Plant Height					
QTL	Chrom.	Position	LOD	Effect	Effect (%)
HT 1	FN24	0.01	5.31	6.6468	20.7
R2 Value	0.2065				

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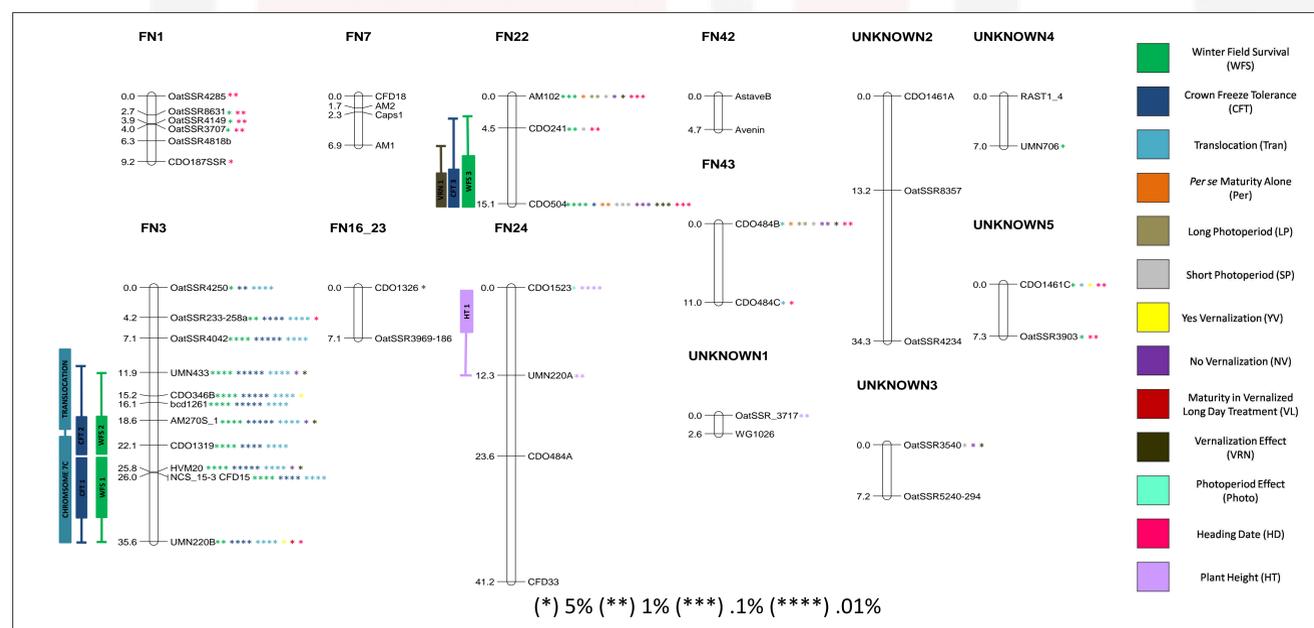


Figure 2: Fulghum/Norline Linkage map containing the known QTL and the Single factor analysis data from all the mapped markers