Mapping for Cold Tolerance in Two Recombinant Inbred Line Populations

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Summary

- Winter wheat production suffers economic losses due to cold and freezing injury every five years on average. The objectives of our research are to identify QTLs associated with resistance to cold in two winter wheat recombinant inbred populations.
- RIL populations Norstar/Centurk78 (86 lines) and Z0031/Karl (69 lines) are being used to map QTLs for cold tolerance.
- Initial screening has been done using a total of 1510 wheat SSR primers of the gwm and barc series. Parental polymorphism screens identify 197 polymorphic primers for the Norstar/Centurk78 population and 137 polymorphic primers for the Z0031/Karl population. One putative QTL has been identified in the Norstar/Centurk78 population near barc117 on chromosome 5A. Two putative QTLs have been identified in the Z0031/Karl population on chromosome 4B associated with the marker gwm368 and barc163.

Introduction

- Cold hardiness is a complex trait and is influenced by vernalization and photoperiod (Mahfoozi et al., 2000).
- From the plant’s early stages of growth, photoperiod and vernalization interact with temperature for expression of the low temperature tolerance genes and also affect the rate of phenological development (Mahfoozi et al.2001).
- Field screens for cold tolerance are subject to environmental variation. Therefore identification of molecular markers linked to cold tolerance will facilitate breeding as well as genetic studies into the mechanisms of cold tolerance.

Materials and Methods

Mapping population:

- The Recombinant inbred lines (RILs): 86 Norstar/Centurk78 RILs are F2:5 currently in F8, the Norstar and Centurk78 are the resistant parent. 69 Z0031/2*Karl BC1S8 lines were developed from a backcross between Karl, a high quality hard red winter wheat with good agronomic characteristics and the F1 between Karl and the synthetic hexaploid 2003Karl. Karl is the resistant parent.

Genotyping:

- DNA was extracted from 5-Toedlings using the procedure described by Dellaporta et al. (1983).
- SSR Marker analysis in wheat (Roder et al. 1998) was done using M13 tailed primers, some of the primers were labeled with DS33 Oye set to run on the ABI DNA Analyzer. SSR marker data for the M13 tailed primers was collected using the Global FR2 Analysis system and GENESCAN software (Li-cor Biosciences, Lincoln, Nebraska) and Gene Mapper V3.7 software was used to analyse data from ABI DNA Analyzer (Applied Biosystems, Foster City, CA).

Freeze testing under controlled conditions:

Figure 1: Snow covered fields

Figure 2: Plant material after the Freeze test

Freezing tests were conducted at the WSU wheat plant growth facility, in large walk in growth chambers that cool to -30°C. Three replications were conducted for each genotype. Winter wheat check cultivars Stephens & Elian plus population parents were included in each freeze test. Each genotype was evaluated in at least two replications.

- 10 day old seedlings at 3 leaf stage were hardened in a 1.4m2 growth chamber with 16h day length at a constant 4°C for 5 weeks.
- After the hardening period, the trays were flooded to have equal soil water content; the tops of the plants were removed to approximately 2cm above the soil surface. The artificial freeze test was conducted by dropping the temperature from 4°C to -10°C over a 24h period. Temperatures were then reduced by 1.5°C every 1.5hrs. A full set of genotypes was removed from the growth chamber at each of 8 temperatures during the freeze down.
- The temperature was monitored with a thermocouple thermometer probe, inserted into the soil. After the test the plants were placed in a growth chamber at 4°C. The temperature was raised to 13°C over 24h period. Plants were then placed in the greenhouse for re-growth.
- After 5 weeks the plants were scored for re-growth and classified as alive or dead.

Data Analysis:

- The LT50 value was calculated for each genotype in each freeze test according to Brule-Babel and Fowler (1989). Data were analyzed using analysis of variance in a genotypes within sets design.
- The linkage maps were constructed using MAPMAKER (Lander et al. 1993), single marker regression of the LSmean LT50 scores against each marker for each population and interval analysis for QTLs was conducted using GGENE (Nelson, 1997). Markers were considered to be associated with LT50 if the t-test for the difference between marker classes was significant at p<0.05.

Results & Discussion:

Norstar/Centurk78 population: From the preliminary data, the LT50 scores approximate a normal distribution. Norstar is the resistant parent and Centurk78 was used as the susceptible parent.

Figure 3: Frequency distribution of LT50 values for Norstar/Centurk78 population.

Table 1: Marker Regression report and putative QTLs for Norstar/Centurk78 population.

<table>
<thead>
<tr>
<th>Chrom</th>
<th>Locus</th>
<th>RSQ</th>
<th>LOD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>5A</td>
<td>barc117</td>
<td>0.1068</td>
<td>2.04</td>
<td>0.0026</td>
</tr>
<tr>
<td>5A</td>
<td>gwm186</td>
<td>0.0676</td>
<td>1.25</td>
<td>0.0183</td>
</tr>
<tr>
<td>6B</td>
<td>gwm518</td>
<td>0.0753</td>
<td>1.43</td>
<td>0.0115</td>
</tr>
</tbody>
</table>

Chromosome locations were determined based on previous genetic maps for these markers and will need to be verified.

- The primers used to screen the population covered 578bM of the 4200M which is 14% of the haploid size of the hexaploid wheat genome so future work will focus on saturating the areas covering the current putative QTLs. Also gaps in the current map on chromosomes 1D, 2A , 2B, 3D, 4B, 4D, 5D, 6B and 6D will be filled.

Z0031/Karl Population:

The LT50 were approximately normally distributed in this population. Karl is the resistant parent and Z0031 is the susceptible parent.

Figure 4: Frequency distribution of LT50 values for Z0031/Karl population

In this case we found 137 polymorphic markers of the 631 SSRs used to screen the parents. When the polymorphic markers were mapped using Mapmaker (Lander et. al.2001) we obtained 2 markers barc163 and gwm368 each accounted for 14% phenotypic variation and the QTLs had a LOD value between 1.5 and 2.0.

Table 2: Marker Regression report and the putative QTLs for Z0031/Karl population.

<table>
<thead>
<tr>
<th>Chrom</th>
<th>Locus</th>
<th>RSQ</th>
<th>LOD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>4B</td>
<td>gwm368</td>
<td>0.138</td>
<td>1.99</td>
<td>0.003</td>
</tr>
<tr>
<td>4B</td>
<td>barc163</td>
<td>0.098</td>
<td>1.45</td>
<td>0.0112</td>
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<tr>
<td>1D</td>
<td>barc229</td>
<td>0.144</td>
<td>1.59</td>
<td>0.0085</td>
</tr>
</tbody>
</table>

Chromosome locations were determined based on previous genetic maps for these markers and will need to be verified.

- The primers cover only 273cM which accounts for 6.5% of the total map size of hexaploid wheat, which is estimated as 4200cM. We have to add more markers to saturate the genome, specifically chromosomes 1A, 1B, 1D, 2A, 2D, 4A, 6D, 7A and 7D in order to locate significant QTLs for cold tolerance.

Future Work:

Target Region Amplification Polymorphism (TRAP) markers will be used to saturate the genomic regions consisting putative QTLs and to complete the map. Since all available SSR markers have been screened for Norstar/cenurk78 and Z0031/Karl populations.

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References:


Grain genes Database: http://www.pe.usda.gov/cgl/tgengenes