GENETIC MAPPING OF POTENTIAL QTLS ASSOCIATED WITH DROUGHT TOLERANCE IN WHEAT

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ABSTRACT

Water shortage is a major constraint to crop production in many parts of the world. Drought tolerance in wheat may be improved by manipulating and detecting quantitative trait loci (QTLs) related to drought tolerance. In the present project microsatellite markers were used for mapping QTLs for photosynthesis, cell membrane stability and relative water content in wheat. An F2 population derived from the intraspecific cross, Chakwal-86 (drought tolerant cultivar) x 6544-6 (drought sensitive genotype) was grown under drought stress in a hydroponic system. The correlation analysis revealed that net photosynthetic rate positively correlated with stomatal conductance, transpiration rate and cell membrane stability. Relative water content also positively correlated with cell membrane stability. Composite interval mapping detected one QTL for net photosynthesis, one for relative water content and two for cell membrane stability on the 2A wheat chromosome. These QTLs may be used to tailor drought tolerant wheat through marker assisted selection or genetic engineering.

Keywords: QTLs, drought, wheat, cell membrane stability, net photosynthesis, relative water content

Footnote: This work is a part of my PhD thesis research

INTRODUCTION

Hexaploid Triticum aestivum is the most widely grown wheat species as it is a staple food for about 40% of the world population. However, its yield is low in drought prone areas of the world. Plants suffer drought when water supply to the roots is less than optimum level. Due to considerable change in the world climate and rainfall patterns caused by global warming as a result of excessive fuel burning (Goyal, 2004; Kim and Byun, 2009), the arid area of the world is increasing rapidly. More than 50% of the global area under wheat cultivation is affected by drought stress (Rajaram, 2001; Ashraf, 2010). Drought adversely affects a variety of physiological and biochemical processes of plants causing disruption in chlorophyll pigments and production of reactive oxygen species/oxidants which damage photosynthetic as well as other cellular membranes (Kidambi, 1990; Ashraf and O’ Leary, 1996; Lawlor and Cornic, 2002; Peltzer et al., 2002; Praba et al., 2009). Lower net photosynthesis in turn decreases growth which leads to reduction in wheat biomass (Bhat and Rao, 2005; Ashraf and Foolad, 2007).

Genetic improvement for yield under drought conditions is possible and has been made to some extent in many drought stricken areas of the world by the exploration of morphological, anatomical and ecological adaptations of plants to drought environments (Cutler et al., 1977). An understanding of the genetic basis of physiological traits involved in drought tolerance and then manipulation through marker assisted breeding would make tremendous improvement in crop yield (Castonguay and Markhart, 1992). Improvement for drought-stress tolerance can also be made by the insertion of drought tolerant genes in new wheat cultivars (Budak et al., 2013). Therefore, there is a dire need to identify loci for the underlying genes involved in drought tolerance. This is possible through identification and genome mapping of QTLS involved in drought tolerance. A number of QTLS for commercially important traits in wheat and other crop plants (Saliba-Colombani et al., 2001; Li et al. 2006; Cutler et al. 2006; Welcker et al., 2007; Kiani et al., 2007a; Nalini et al., 2010; Wang et al., 2011; Bennet et al., 2012; Aprile et al., 2013; Elshafei et al., 2013;; Zhang et al., 2013; Mysza et al., 2014; Zhang et al., 2014) have been mapped. However, a little effort has been made to locate QTLS for drought resistance in wheat especially for the physiological traits such as net photosynthesis, relative water content, cell membrane stability etc. The present study was designed to identify and map drought tolerance conferring traits using SSR markers. The information generated would help to improve drought tolerance of wheat.

MATERIALS AND METHODS

An F2 wheat population derived from the biparental intraspecific cross of a drought sensitive
et al., 2011) however limited from gel pictures taken with the help of gel documentation system. Data of amplifications were scored and recorded from gel pictures taken with the help of gel documentation system.

**Polymerase Chain Reaction (PCR):** PCR was performed in 0.2 ml PCR tubes. The total volume of PCR mixture was 20µl, containing 4.8 µl of PCR (deionized) water, 2 µl of PCR buffer (Fermentas), 1.6 µl of MgCl$_2$ (Fermentas), 6.4 µl of 2.5mM dNTPs (Fermentas), 1.0µl each of 30ng/µl forward and reverse primer, 0.2 µl of 5 units/µl Taq polymerase (Fermentas) and 3 µl of 15ng template DNA. Amplification was performed in the Master Cycler Gradient, Eppendorf, Germany. The thermal cycler was programmed for 35 cycles of denaturation at 95°C for 1 min, annealing at 50-60°C (depending on individual primer) for 1 min and extension at 72°C for 1 min with an initial denaturation at 94°C for 5 min and final extension at 72°C for 10 min.

**SSR Analysis:** A total of 425 SSR primer pairs; 100 from the Xgwm, 100 from KSUM and 100 of the WMS series (Gene Link, USA) were assayed on the parent genotypes to detect polymorphic markers for genotyping the population. The polymorphic markers detected in the parents were used to assay the population.

**Statistical Analysis:** Phenotypic data of the F$_2$ population were subjected to correlation analysis after Dewey and Lu (1959) using MINITAB computer software. After scoring of gels, the genotypic data were analyzed by the statistical program MAPMAKER/EXP version 3.0 (Lander et al., 1987) to construct a genetic map. QTL cartographer v 2.5 (Wang et al., 2006) was used for detection/tagging of QTLs on the map by computing phenotypic data of each F$_2$ individual against its genotypic data.

**RESULTS AND DISCUSSION**

Since the 1980’s, accurate genetic maps of plant and animal species are being constructed using DNA markers. A lot of work in the last decade using DNA markers to construct genetic maps and tagging of QTLs has been reported in literature (Zhang et al., 2002; Zhang et al., 2005; Han et al., 2006; McCouch et al., 2002; Torada et al., 2006; He et al., 2007; Nalini et al., 2010; Cui et al., 2011; Wang et al., 2011) however limited work has been conducted on wheat. The present study was the first attempt to map QTLs for the physiological traits, net photosynthesis, cell membrane stability and relative water content in wheat related to drought stress.
The phenotypic data obtained from the F2 population showed normal frequency distributions for all traits with transgression (Fig 1). As the two parents were genetically diverse, transgressive segregation of the F2 progeny showed that both parents transmitted favorable alleles for each trait. Transgressive segregation results when high or low alleles for a trait disperse between parents and come together in the individuals of the F2 progeny (Prioul, et al., 1997).

In the present study association/correlation among the physiological traits studied showed that net photosynthesis positively correlated with stomatal conductance, transpiration rate and cell membrane stability while relative water content also positively correlated with cell membrane stability (Table 1). The correlation results reveal that these traits are linked together on the same chromosome which was also proved through detection of QTLs for these traits on the same linkage group on chromosome 2A.

Among the 425 SSR primer pairs assayed on the parent genotypes to detect potential polymorphic markers, 365 amplified the genomic DNA. Among the amplified, 23 loci were found polymorphic between the parent genotypes. A polymorphism of 5.41% was found between the parents. Wheat is reported to be low in polymorphism (Roder et al., 1998; Haudry et al., 2007). Kirigwi et al. (2004) studied polymorphism between two bread wheat genotypes and found 90 out of 700 SSR markers (10%) to be polymorphic. Because of the low polymorphism in wheat (Roder et al., 1998) relatively a few QTLs have been detected in this crop compared to other species. Twelve out of the 23 loci in the present study segregated co-dominantly in a 1:2:1 ratio, while 11 were dominant in 3:1 ratio. A total of 12 DNA markers were surveyed on the mapping population. Gel pictures showing some of the dominant and co-dominant segregating markers are given in Fig 2-4.

Table 1. Phenotypic correlation among the traits $P_n$ (net photosynthesis), $E$ (transpiration rate), $g_s$ (stomatal conductance), RWC (relative water content) and CMS (cell membrane stability) studied in 6 week old F2 population of the cross between the wheat cultivar, Chakwal-86 and the genotype 6544-6.

<table>
<thead>
<tr>
<th></th>
<th>RWC</th>
<th>$P_n$</th>
<th>$E$</th>
<th>$g_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_n$</td>
<td>0.08</td>
<td>0.158</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E$</td>
<td>0.062</td>
<td>0.158</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$g_s$</td>
<td>0.113</td>
<td>0.266**</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>CMS</td>
<td>0.18*</td>
<td>0.187*</td>
<td>0.06</td>
<td>0.18*</td>
</tr>
</tbody>
</table>

*, **,  = significant at 0.05, 0.01 and 0.001 levels respectively

Figure 1. A.
Frequency distribution/histogram of CMS (cell membrane stability) studied in 6 week old F2 population of the cross between the drought resistant wheat cultivar, Chakwal-86 and the drought sensitive genotype 6544-6. White arrows indicate mean values of the trait in parent 1 (Chakwal-86) while the black arrows indicate mean value of the trait in parent 2 (6544-6).

Eighty percent of the SSRs used in study amplified more than one amplicon. SSRs are said to amplify more than one band in wheat because of its hexaploid origin, as its genes were evolved from fusion of A, B and D genomes (Buteler et al., 1999). Linkage analysis through the MAPMAKER/EXP resulted in linkage of 4 SSR loci on a linkage group on 2A wheat chromosome. The total map length was 87.5 cM. The map distance between markers was approximately 10-15 cM. It was in harmony with best distance for QTL.
detection which is considered to be 10 cM (Kearsey, 1998). The results of linkage analysis along with genotypic and phenotypic trait data were imported to the software “QTL cartographer V 2.5”. QTLs were identified by finding association between marker genotype and trait (phenotype) using SMA (single marker analysis), IM (Interval mapping) and CIM (composite interval mapping) method. The final genetic map constructed is shown in Fig 5. Four markers associated with three physiological traits were identified by CIM method (Table 2). The algorithm used by the computer softwares to detect QTLs is based on LOD (Log of the odds) ratio. Generally a LOD score of ≥2.4 is suggested to avoid false positives while detection of QTLs (Lander and Botstein, 1989). In the present study, QTLs for \( P_n \), CMS and RWC with LOD score ≥ 2.4 were detected. However, Nalini et al. (2010) reported QTLs obtained with low stringency (LOD ≥ 2.0) may be because higher LOD score leaves out some potential QTLs. The present findings may be useful in marker-assisted breeding of drought tolerant wheat. The loci detected may also be helpful to find the genes involved in drought tolerance. These genes will be helpful to tailor drought resistant varieties of wheat through transgenic approaches.

![Histogram of RWC](image1.png)

**Figure 1. B**
Frequency distribution/histogram of RWC (relative water content) studied in 6 week old F\(_2\) population of the cross between the drought resistant wheat cultivar, Chakwal-86 and the drought sensitive genotype 6544-6. White arrows indicate mean values of the trait in parent 1 (Chakwal-86) while the black arrows indicate mean value of the trait in parent 2 (6544-6).

![Histogram of Pn](image2.png)

**Figure 1. C**
Frequency distribution/histogram of Pn (net photosynthetic rate) studied in 6 week old F\(_2\) population of the cross between the drought resistant wheat cultivar, Chakwal-86 and the drought sensitive genotype 6544-6. White arrows indicate mean values of the trait in parent 1 (Chakwal-86) while the black arrows indicate mean value of the trait in parent 2 (6544-6).
Figure 1. D
Frequency distribution/histogram of E (transpiration rate) studied in 6 week old F$_2$ population of the cross between the drought resistant wheat cultivar, Chakwal-86 and the drought sensitive genotype 6544-6. White arrows indicate mean values of the trait in parent 1(Chakwal-86) while the black arrows indicate mean value of the trait in parent 2 (6544-6).

Figure 1. E
Frequency distribution/histogram of gs (stomatal conductance) studied in 6 week old F$_2$ population of the cross between the drought resistant wheat cultivar, Chakwal-86 and the drought sensitive genotype 6544-6. White arrows indicate mean values of the trait in parent 1(Chakwal-86) while the black arrows indicate mean value of the trait in parent 2 (6544-6).

Fig. 2. Parental survey with the primers (starting from left) Xgwm 311-2D, 617-6A, 497-2A, WMS 169, and KSUM-119 showing polymorphism. P1= Chakwal-86, P2= 6544-6, M= DNA ladder
Fig 3. Population survey with primer Xgwm 372-2A showing parents, Chakwal-86 (P1) and 6544-6 (P2) along with 10 individuals from the F₂ population. M= DNA ladder.

Fig 4. Population survey with primer Xgwm 497-2A showing parents, Chakwal-86 (P1) and 6544-6 (P2) along with 10 individuals from the F₂ population. M= DNA ladder.

Fig 5. Genetic map of wheat chromosome 2A showing position of QTLs for net photosynthesis (Pₙ), cell membrane stability (CMS) and relative water content (RWC).
Table 2. QTLs detected for net photosynthetic rate ($P_n$), cell membrane stability (CMS), stomatal conductance ($g_s$) and relative water content (RWC) through Composite Interval Mapping from the F$_2$ population of a cross between drought tolerant cultivar, Chakwal-86 and drought sensitive genotype, 6544-

<table>
<thead>
<tr>
<th>Trait</th>
<th>QTL</th>
<th>Nearest marker</th>
<th>QTL position</th>
<th>LOD score</th>
<th>R$^2$ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_n$</td>
<td>QPn2AC</td>
<td>KSUM-119</td>
<td>26.5 cM</td>
<td>2.5</td>
<td>17</td>
</tr>
<tr>
<td>CMS</td>
<td>QCMSa2AC</td>
<td>KSUM-119</td>
<td>27.1 cM</td>
<td>2.6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>QCMSb2AC</td>
<td>Xgwm 497</td>
<td>4.4 cM</td>
<td>2.7</td>
<td>7</td>
</tr>
<tr>
<td>RWC</td>
<td>QRWC2AC</td>
<td>KSUM-119</td>
<td>27 cM</td>
<td>2.7</td>
<td>19</td>
</tr>
</tbody>
</table>

QTLs are defined by “Q” followed by the acronym and “a” or “b” are to differentiate more than one QTL for the same trait while 2A stands for the chromosome number. QTLs detected by IM are designated by an “M” while QTLs detected through CIM are designated by “C”. QTLs detected by Interval Mapping and Composite Interval Mapping were all at probability $P<0.001$.

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