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RESEARCH ARTICLE

Identification of QTLs Associated with Cold Tolerance in Wheat (*Triticum aestivum* L.)

Gorji AH*, R Hajianfar and B Rostamforody

Department of Plant Breeding, Faculty of Agriculture, Boroujerd Branch, Islamic Azad University, Boroujerd, Iran

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***Corresponding Address:**
Gorji AH
amirhgorji@yahoo.com

ABSTRACT

To identify genomic regions, which determine the level of LT tolerance in hexaploid wheat, F2:3 populations produced from the cross spring-type, susceptible parent, Zagros (LT50 =-6ºC) and winter-type tolerant parent Norstar (LT50 = -20.7ºC) were examined. The result of the phenotypic analysis showed continuous distribution of trait values (LT50 = -1 to -23ºC). The relationship between LT tolerance (LT50= Low temperature for 50% killed) and genotypic data was analyzed using composite interval mapping, interval mapping and single marker analysis methods. Twenty SSR from 170 SSR and from 22 combinations AFLP, ten combinations between parents were polymorphic and in total, 75 loci were polymorphic. For all the loci, the deviations from the expected Mendelian ratio were evaluated using the chi-square goodness-of-fit test. Because of deviation from segregation may affect the recombination coefficients, very vague markers, were excluded from analysis. Thus, from 75 markers, 27 markers on six linkage groups with an average distance of 8 cM between adjacent markers were assigned and approximately 224 cM of the wheat genome was covered.

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INTRODUCTION

Securing high and stable crop yields is one of the primary tasks of agricultural production. To ensure high yield stability the ideal genotype should carry favourable alleles at many, possibly all, stress tolerance loci. The recent advances in the genetic and molecular understanding of stress responses have led to the identification of a great number of single loci, quantitative trait loci (QTLs) and genes related to stress tolerance. A higher LT tolerance is also provided by short day conditions, which delay flowering during the winter months (Limin and Fowler, 2006). In addition to vrn-1, genetic and cytogenetic studies have associated at least 15 out of 21 different pairs of chromosomes with LT tolerance in wheat (Sutka, 1994). On group 5 chromosomes, frost-tolerance (Fr) loci have been identified in close proximity to the vrn-A1, vrn-B1 and vrn-D1 loci (Tóth *et al*., 2003). On chromosome 5A ^{m} of diploid T. monococcum, two Fr loci, Fr-A1 and Fr-A2, are involved in cold-induced expression of genes associated with LT tolerance (Vágújfalvi *et al*., 2005). The Fr-A2 ^{m} locus carries a cluster of CBF genes (Miller *et al*., 2006), which encode transcriptional factors with important roles in the activation of LT stress responses in plants (Thomashow *et al*., 2001). Expression of certain CBF genes located to the Fr-A2 region in hexaploid wheat correlates with increased LT tolerance (Vágújfalvi *et al*., 2005).

MATERIALS AND METHODS

Material: To identify genomic regions, which specify the level of LT tolerance in hexaploid wheat, F2:3 populations produced from crossing between winter- type tolerant parent Norstar and spring-type, susceptible parent, Zagros was analyzed. The levels of LT tolerance for this population were rated using artificial freeze test LT50, the temperature at which 50% of plants were killed by LT stresses. The molecular analyses were assessed using 172 SSR primer pairs and 20 AFLP primer compositions. The relevance between genotypic data and LT tolerance (LT50) was analyzed by single marker analysis, interval mapping and composite interval mapping methods, using Win QTL Cartographer 2.5 [19] and LOD=2.5.

In this study, winter cultivar parent Norstar as cold tolerant and cold-sensitive cultivar Zagros as the population parents for the production of F2:3 were used. Phenotypic assessment using LT50features in Boroujerd Azad University was performed. Parents and 180 progeny seeds were sown in pots in the greenhouse. Temperature of at least 50% of plants to maintain their life, as tolerance to cold stress was recorded. DNA extraction using a

parental leaves and individuals F2 in 5 to 10 leaf stage with method Dellaporta *et al.* (1983) was performed. Quantity and quality DNA samples, by Spectrometry method and agarose gel 0/8% was determined.

Method: After determining the concentration of DNA samples, new samples with the same concentration of 10 mg in microliter prepared and in polymerase chain reaction, for SSR primer, were used. For the molecular analysis of SSR and the AFLP primers were used. For the SSR, parents polymorphic using 172 primer pairs SSR from Xgwm series were examined. AFLP based on the method of Vos *et al.* (1995) was performed and genomic DNA with Mse I and Pst I were digested. Selective amplification step using primers Pst I + A and Mse I + CT was performed. The resulting product from using polymerase chain reaction by denaturing 6% polyacrylamide gels was separated and by silver nitrate staining method were detected.LT50 values by proposed method Limin & Flower (2006) were determined .SSR and AFLP bands scoring by method based on Lander *et al.* (1987) was conducted. For discover relationships between genotypic and phenotypic data in order to locate putative QTLs, single marker analysis using software (Wang *et al*., 2007) Cartographer 2.5 Win QTL and Lod=2.5 was performed.

RESULTS

The result of phenotypic analysis showed continuous distribution of trait values (LT50 =-3 to -23ºC) which is in agreement with the distribution of trait expected for a polygenic and quantitatively inherited trait. LT50 values for parental lines along with 180 F2:3 genotypes derived from a cross between them were shown in Figure 2 as a frequency distribution for 11 temperature levels. Mean value for LT50 was -14.32ºC±3.22. More than 5% of families had LT50 values less than that of susceptible parent, and more than 25% of families on the other hand showed LT50 values more than that of tolerant parent .The molecular analyses were assessed using 170 SSR primers pair and 22 AFLP primers combinations. The relationship between LT tolerance (LT50) and genotypic data was analyzed using single marker analysis, interval mapping and composite interval mapping methods. Twenty SSR from 170 SSR and from 22 AFLP, ten combinations between parents were polymorphic and in total, 75 loci were polymorphic. For any position, deviation from the expected according to Mendelian ratios through the chi-square goodness-of-fit test was performed. Because of deviation from Scattering may affect the recombination coefficients, very vague markers (P<0/01), ago preparation continuity maps were excluded from analysis. Thus, from 75 markers, 27 markers on six linkage groups with an average distance of 8 cM between adjacent markers were assigned and approximately 224 cM of the wheat genome covered. The position SSR markers on chromosome 4A, 5B and 5D were identified (Table 1). Because the detected QTLs located on the 5B and 7D chromosomes and other ones which were linked to AFLP markers were inherited in both parents; therefore these results do confirm the effectiveness of both parents for this characteristic.

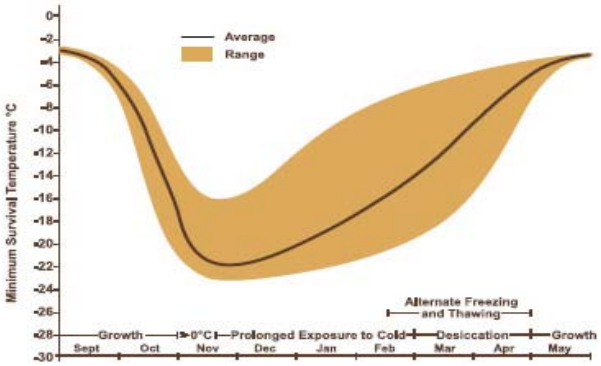

Fig. 1: Changes in winter hardiness of Norstar Winter Wheat over period of September through May.

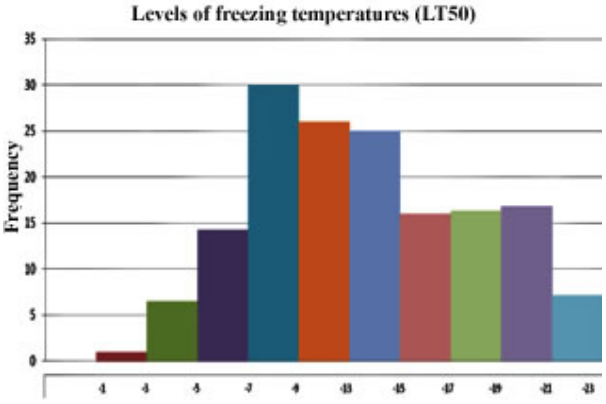

Fig. 2: The frequency distribution for F 2:3 populations at freezing temperatures

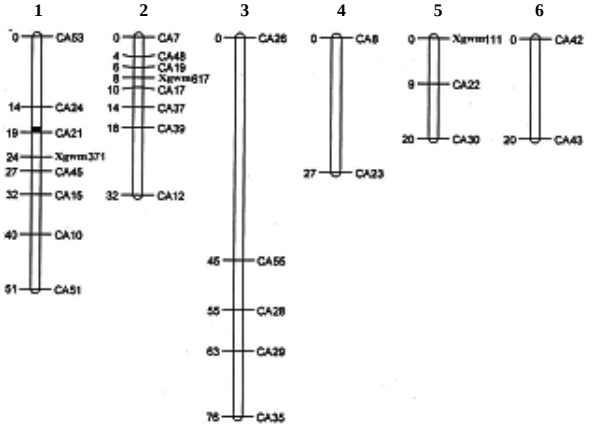

Fig. 3: Linkage groups of AFLP and SSR markers for wheat and the position of QTLs which controlling cold tolerance in the linkage groups

DISCUSSION

One of the goals of most breeding programs worldwide is to maintain resistance to low temperatures in the commercial varieties at its existing level (Braun & Saulesku, 2002). Resistance to low temperatures is a variety-specific wheat trait. It is not constant and plants acquire it as they prepare for the winter and go through the process of hardening (GALIBA *at al*., 2000). The

Table 1: Molecular markers related to cold tolerance in a population derived from a cross between Norstar and Zagros

Marker	Chromosome	b0	b1	F(1,n-2)	P-value
CA10	5B	14/081	-1/251	7/841	0.007
CA15	5B	14/151	-1/640	12/471	0.000
CA21	5B	14/111	-1/867	16/748	0.000
CA24	5B	14/053	-1/632	12/877	0.000
CA27	-	13/768	-1/363	6/691	0.010
CA45	5B	14/101	-1/674	14/364	0.000
CA51	5B	14/251	-1/831	15/160	0.000
Xgwm371	5B	14/142	-1/762	15/391	0.000
Xgwm 397	4A	14/327	-1/801	14/202	0.000
Xgwm 174	5D	14/281	-1/356	5/601	0.018

distribution of markers among the 21 chromosomes on the genome was uneven, with the D genome showing less polymorphism than the A and B genomes as generally seen in mapping projects (Röder *et al*., 1998). However, a few maps for hexaploid wheat have reported genome sizes in excess of 3,500 cM. For example, Quarrie *et al*. (*2005*) mapped 567 markers to generate a genome map of 3,522 cM and a map reported by Sourdille *et al*. (2003) contained 659 markers and was 3,685 cM in length. In barley, a vernalization locus, *Vrn-H3*, has been mapped to chromosome 1H (Takahashi and Yasuda, 1971). Recently, an *AP1*-like gene was identified in the proximity of the barley *Vrn-H3* region (Von Zitzewitz *et al*., 2005). A photoperiod locus, *Ppd-H2*, is also located on chromosome 1H in barley (Laurie *et al*., 1995). The role of photoperiod in LT tolerance has been demonstrated in barley, where the expression profile of *TaVRT-1* orthologue and length of vegetative phase are altered by short and long days (Fowler *et al*., 2001). Photoperiod response is also an important factor determining LT tolerance level in *Arabidopsis* (Alonso-Blanco *et al*., 2005). The major LT-tolerance locus identified on the 5A chromosome coincided with the position of frost resistance locus *Fr-A2* mapped proximal to *vrn-A1* in diploid wheat (Vágújfalvi *et al*., 2005) and orthologous LT-tolerance locus in barley (Francia *et al.*, 2004). In both diploid wheat and barley, a cluster of *CBF* genes is associated with the *Fr-2* locus (Francia *et al*., 2004; Miller *et al*., 2006). The existence of multiple LT-induced pathways has been demonstrated in *Arabidopsis* by transcriptome analysis, where at least 28% of LT-induced genes are activated independently of *CBF* (Fowler and Thomashow, 2002). The results show that, tolerance to cold is the quantitative trait and thus affected by environmental conditions.

Conclusion

With regard to various sources, the effectiveness of the of additive and dominance effects of genes in controlling cold tolerance has been reported, thus, in the identification of QTLs with additive and dominance effects associated with cold tolerance and markers related to this QTLs in selection and breeding programs for this trait can be helpful. Also use from candidate markers and genes associated with cold tolerance that have been identified on quintet chromosomes can accelerate molecular research are concerned.

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