QTL markers associated with Low temperature tolerance in winter wheat

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Abstract

Low temperature tolerance (LT) is an important agronomic trait in winter wheat that determines the plants ability to cope with below freezing temperatures. To identify genomic regions, which determine the level of LT tolerance in hexaploid wheat, F2:3 and F2:4 populations produced from crossing between winter type tolerant parent Mirnovoskaya 808(LT50 =-20 oC) and spring type, susceptible parent, Pishtaz (LT50 =-7 oC) were analyzed. The levels of LT tolerance for these populations were evaluated using artificial freeze test LT50, the temperature at which 50%of plants were killed by LT stresses. The molecular analyses were assessed using 170 SSR primer pairs and 22 AFLP primer combinations. The result of phenotypic analysis showed continuous distribution of trait values (LT50 =-3 to -23 oC) which is in agreement with the distribution of trait expected for a polygenic and quantitatively inherited trait. The relationship between LT tolerance (LT50) and genotypic data was analyzed using single marker analysis, interval mapping and composite interval mapping methods. Three detected QTLs for spring parent, Pishtaz, with partial dominant effects and three detected QTLs of winter parent, Mirnovoskaya 808, with over dominant effects. 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 theses results do confirm the effectiveness of both parents for this characteristic.
Key words: Low-temperature tolerance, LT50, Triticum aestivum, QTL mapping.

1 Introduction

Freezing tolerance, the ability of plants to survive subfreezing temperatures, is the major component of winter survival (Figures1 and 2) and important characteristic necessary for optimum seed yield of winter wheat varieties [10 and 17]. Freezing tolerance may be assayed in field or in controlled conditions. The correlation between field survival and laboratory studies of freezing tolerance were reported between 0.77 and 0.92 [13]. Another important factor is acclimation ability, which is the

ability of plants to increase its freezing tolerance, or survive at lower temperatures, after a period of cold-temperature treatment [10]. A strong correlation between cold acclimation and freezing tolerance in winter type wheat has been noticed [12]. Cold acclimation is associated with several physiological and biochemical alterations in the plants. Changes in plants during acclimation include increases in soluble sugars, proteins, amino acids and organic acids, accumulation of osmolytes and protective proteins as well as modification of membrane lipid composition and alterations in gene expression [6, 8, and 11]. In wheat cold induced genes have been isolated and characterized and there is a high correlation between the expression of some of these genes and the development of freezing tolerance, which appear to be up-regulated by low temperature [1, 4, 14, and 15]. Freezing tolerance is also strongly correlated with the capacity for maintaining high photosynthesis during cold acclimation, because it is indispensable to ensure an energy source during cold acclimation. Cessation of growth during cold acclimation is also necessary to reach the resistance [11and15]. During 1st stage of cold acclimation, the water content of prehardened plants decreases and soluble sugars and free proline of leaves increase [10]. The genetic regulation of freezing tolerance and winter hardiness is complex in most or all crop species [5, 7, and 18]. The reported gene action for freezing tolerance has varied from recessive to partially dominant in winter wheat [3], largely additive [2], to partially dominant [9], in alfalfa and partially recessive in potato [16]. In recent years, molecular markers, as useful complementary tools for classical breeding methods, were used in selection programs for quantitative traits such as freezing tolerance. Various molecular markers were developed and applied for mapping QTLs. The objective of the present study was to identify SSR and RAPD markers linked to cold resistance genes in wheat.

2 Material and methods

To identify genomic regions, which determine the level of LT tolerance in hexaploid wheat, F2:3 and F2:4 populations produced from crossing between winter type tolerant parent Mirnovoskaya 808(LT50 =-20 oC) and spring type, susceptible parent, Pishraz (LT50 =-7 oC) were analyzed. The levels of LT tolerance for these populations were evaluated using artificial freeze test LT50, the temperature at which 50%of plants were killed by LT stresses. The molecular analyses were assessed using 170 SSR primer pairs and 22 AFLP primer combinations. The result of phenotypic analysis showed continuous distribution of trait values (LT50 =-3 to -23 oC) which is in agreement with the distribution of trait expected for a polygenic and quantitatively inherited trait. The relationship between LT tolerance (LT50) and genotypic data was analyzed by single marker analysis, interval mapping and composite interval mapping methods, using Win QTL Cartographer 2.5 [19] and LOD=2.5.

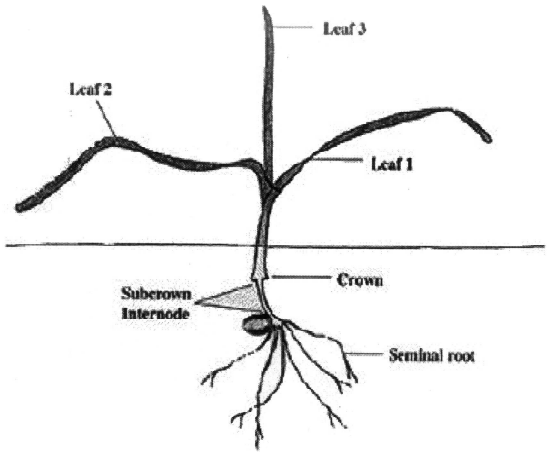

Figure1. The shematique of winter cereal due to have a crown tissue underground is able to survive in a cold season.

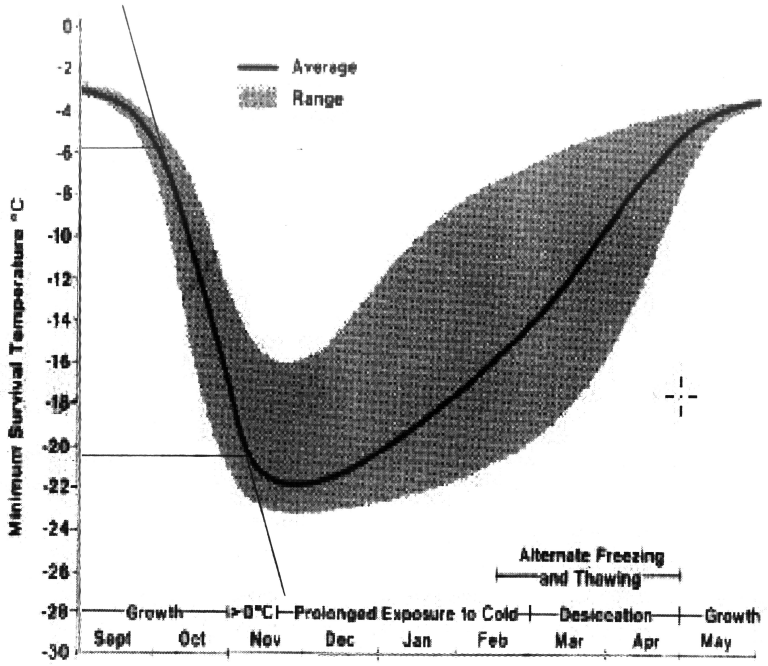

Figure2. Cold acclimation for winter wheat started from September to May.

3 Results and discussion

3.1 Phonotypical evaluation

LT_{50} values for parental lines along with 178 $F_{2:3}$ genotypes derived from a cross between them were shown in Figure 3 as a frequency distribution for 11 temperature levels. $F_{2:3}$ genotypes showed continuous distribution for this trait which revealed that there should be a polygenic inheritance for LT_{50}. Mean value for LT_{50} was -14.52 ^{o}C. More than 5% of families (about 10 families) had LT_{50} values less than that of susceptible parent Pishtaz, and more than 24% of families (about 44 families) on the other hand showed LT_{50} values more than that of tolerant parent Mirnovoskaya 808.

The frequency distribution of $LT_{50\ values}$ for parental lines along with 86 F2:4 genotypes derived from cross between them for 11 temperature levels as a frequency distribution was shown in Figure 4. As it shown in Figure 2, the distribution is continuous for this trait as well for the second year' s experiment. Mean value for LT_{50} was -14.56^{o}C. More than 14% of families (about 12 families) had LT_{50} values less than that of susceptible parent Pishtaz, and more than 8% of families (about 7 families) on the other hand showed LT_{50} values more than that of the tolerant parent Mirnovoskaya 808. In Figure 5 the mean values of LT_{50} for parental lines and 85 pair of $F_{2:3}$ and $F_{2:4}$ genotypes derived from crosses between them shown as mean frequency distribution at 11 freezing temperatures. As it,s shown the mean value for this trait was -14.07 ^{o}C. More than 6% genotypes (about 8 genotypes) had LT_{50} values less than that of susceptible parent Pishtaz, and more than 4% of families (about 6 families) on the other hand showed LT_{50} values more than that of the tolerant parent Mirnovoskaya 808.

3.2 Molecular evaluation

The molecular analyses were assessed using 170 SSR primers pair and 22 AFLP primers

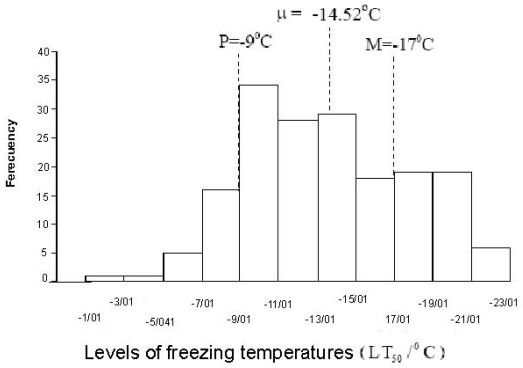

Figure3. The frequency distribution for $F_{2:3}$ populations at 11 freezing temperatures.

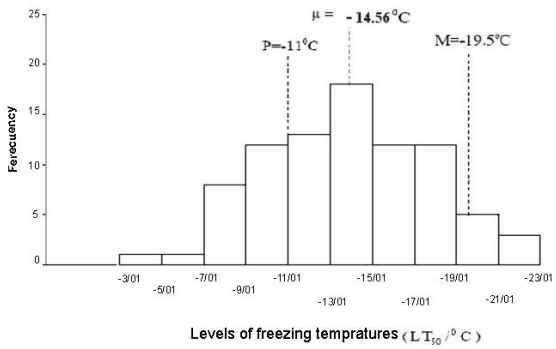

Figure4. The frequency distribution for $F_{2:4}$ populations at 11 freezing temperatures.

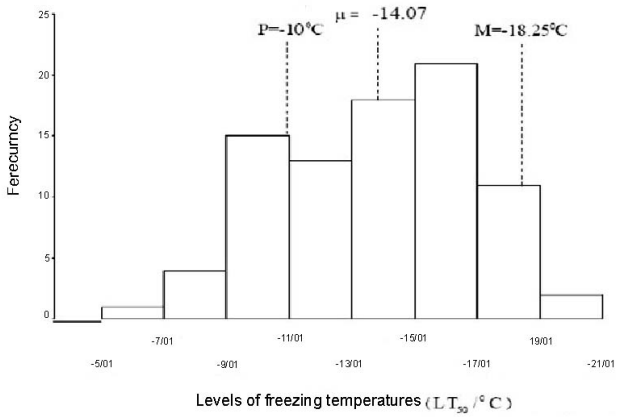

Figure5. The frequency distribution for the mean of $F_{2:3}$ and $F_{2:4}$ populations at 11 freezing temperatures.

combinations. Linkage groups of SSR and AFLP markers for wheat and the position of QTLs which controlling cold tolerance in the linkage group 1 shown in Figure 6

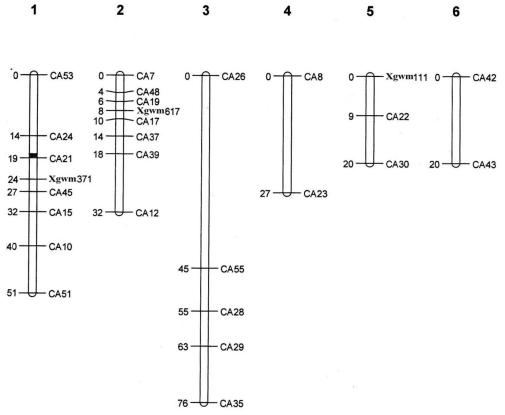

Figure6. Linkage groups of SSR and AFLP markers for wheat and the position of QTLs which controlling cold tolerance in the linkage group 1.

The relationship between LT tolerance (LT_{50}) and genotypic data was analyzed using single marker analysis, interval mapping and composite interval mapping methods. Three detected QTLs for spring parent, Pishtaz, with partial dominant effects and three detected QTLs of winter parent, Mirnovoskaya 808, with over dominant effects. 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 theses results do confirm the effectiveness of both parents for this characteristic (Tables1and 2 and Figures 8-12).

Table1. Molecular markers related to cold tolerance in a population derived from a cross between Mirnovoskaya 808 and Pishtaz

Marker	Chromosome	b_0	b_1	F(1,n-2)	P-value
CA24	5B	14/054	-1/633	12/876	0.000
CA21	5B	14/110	-1/866	16/749	0.000
Xgwm371	5B	14/140	-1/763	15/392	0.000
CA45	5B	14/102	-1/675	14/365	0.000
CA15	5B	14/150	-1/641	12/470	0.000
CA10	5B	14/086	-1/252	7/843	0.006
CA51	5B	14/252	-1/830	15/169	0.000
CA27	-	13/769	-1/364	6/692	0.010
Xgwm 397	4A	14/328	-1/800	14/203	0.000
Xgwm 174	5D	14/288	-1/356	5/601	0.019

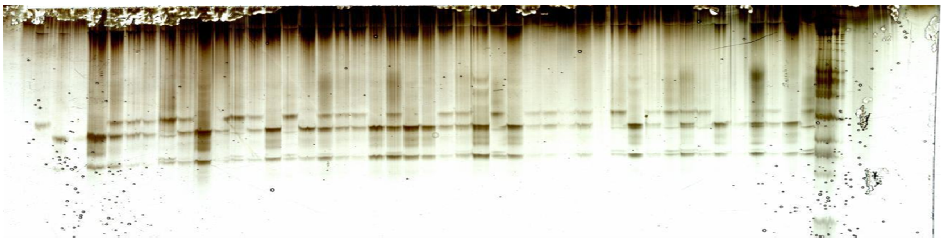

Figure7. Electrophoretcal gel related to the DNA analysis in a group of 45 F_2 genotypes using single microsatellite marker (*Xgwm* 60-7A).

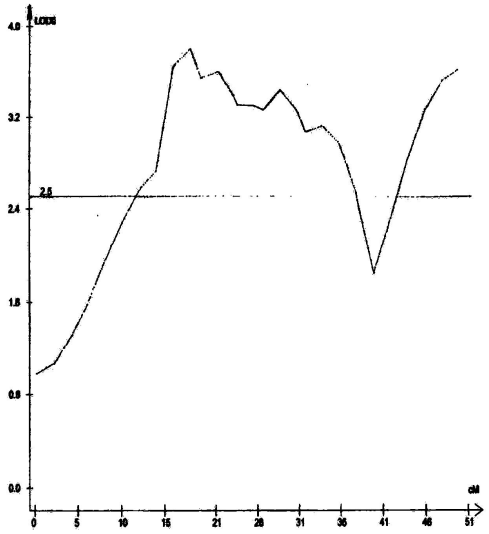

Figure8. Curve using single marker analysis for cold tolerance in the first year experiment.

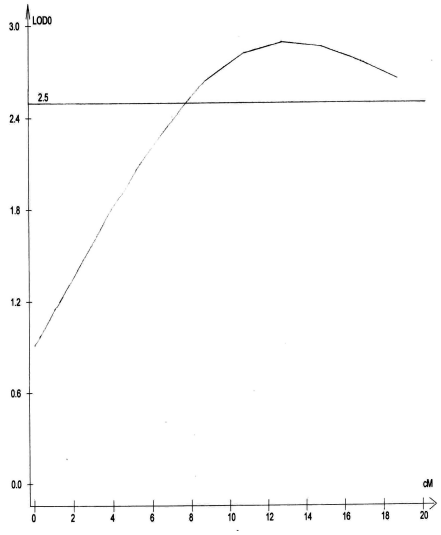

Figure9. Curve using single marker analysis for cold tolerance in the second year experiment.

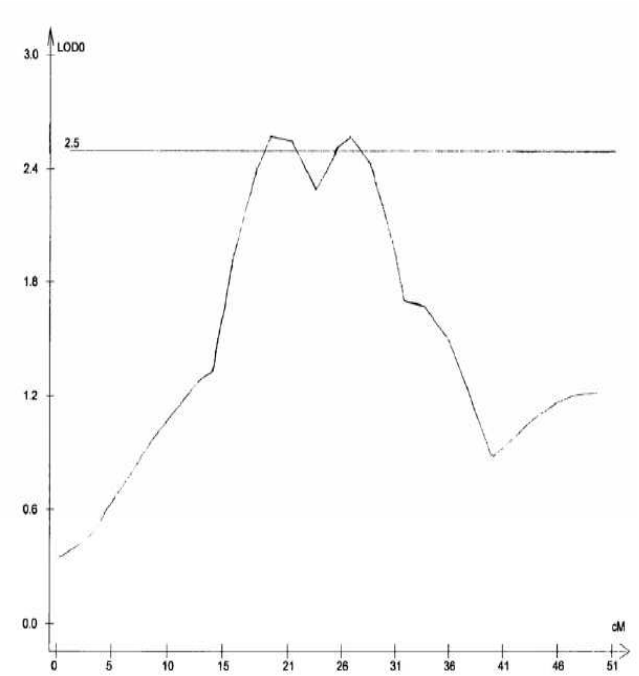

Figure10. Curve using single marker analysis for cold tolerance in wheat.

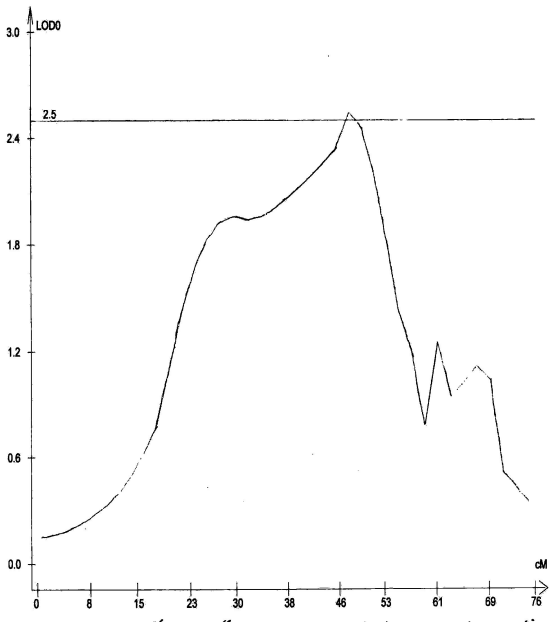

Figure11. Curve using single marker analysis for cold tolerance based on two years mean.

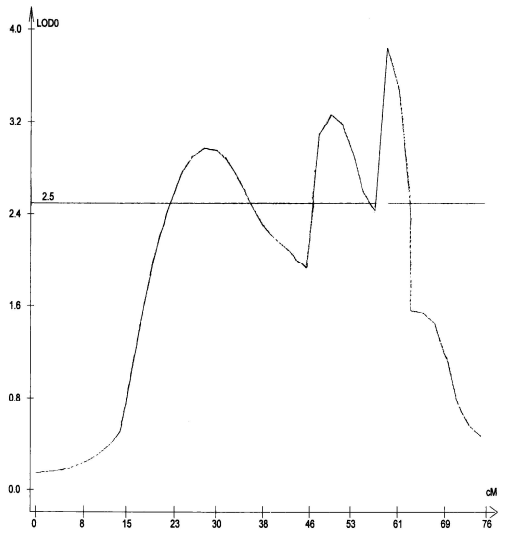

Figure12. Curve using composite interval mapping for cold tolerance based on two years mean.

Table2. Linkage group, distance, chromosome no., LOD, additive and dominant effects and variance percentage for explaining of phenotypical variance of cold tolerance.

Method	QTL	Linkage group	Dist.	Chromosome	LOD	Additive effect	Dominant effect	Variance
SIM	1	1	*Xca*21-*Xca*24	5B	3.78	-2.05	-0.13	%11.3

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