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Full Length Research Paper

Mapping of QTLs for frost tolerance and heading time using SSR markers in bread wheat

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Selection for complex genetic traits, such as frost tolerance, can be simplified in plant breeding programs when linked markers were detected. The use of microsatellite markers for tagging and mapping important genes or QTLs is a goal in wheat genetic projects. In this study, 200 microsatellite markers were studied and after parental assay, 41 polymorphic SSR markers were used for genotyping of 280 F2 individual plants derived from F1 generation of wheat cross (Norstar× Zagros). The progeny of individual F2 plants were used as F2:3 families for the assessment of LT50 and heading time. Single marker analysis revealed that seven markers with total of 27% of phenotypic variance determination linked to LT50 and five markers linked to the heading time. Two markers that were located on 2B and 5A chromosomes affected both LT50 and heading time significantly. It was assumed, therefore, that some closely linked QTLs or QTLs with pleiotropic effects govern both traits simultaneously, as the LT50 of F2:3 families were significantly correlated with the heading time of F2:3 families, Thus, it is concluded that later heading time is associated with the higher level of frost tolerance in wheat.

Key words: Bread wheat, frost tolerance, heading time, QTL mapping, single marker analysis, SSR.

INTRODUCTION

Abiotic stresses are crucial barriers for crop production and the yield potential of any specific cereal variety is rarely maximized due to the restriction imposed by abiotic stresses.

It is recognized, however, that different responses of varieties to variable environmental circumstances are genetically based. The genetic control of responses to abiotic stresses such as cold stress is complex (Chun et al., 1998) and most of the variation seems to be controlled by QTLs (Limin and Fowler, 1993; Snape et al., 2001). The frost tolerance of winter wheat is governed by polygenes (Brule-Bable and Fowler, 1988; Galiba et al., 2001). At least 10 chromosomes of bread wheat are involved in the frost tolerance (Cahalan and Law, 1979; Sutka, 1981; Sutka, 2001). The homeologous group 5 chromosomes are most frequently implicated (Sutka and Kovacs, 1985). Sutka (1994) in the study of monosomic and substitution analysis portended that, although there were many chromosomes controlling frost tolerance, the 5A and 5D chromosomes were more effective than others. Major genes affecting this trait (Fr1) and vernalization requirement (VrnA1) were reported to be on the long arm of chromosome 5A (Galiba et al., 1995; Vagojfavli et al., 2000; Sutka, 2001). According to Galiba et al. (1995) and Sutka et al. (1999), VrnA1 and Fr1 loci are linked and located on the distal portion of the long arm of 5A. The location of VrnD1-Fr2 on chromosome 5D has also been established (Sutka et al., 1999; Snape et al., 2001). Also Efremova et al. (2004) found that the presence of frost resistance genes homeoallelic to the known genes Fr1 and Fr2 on 5A and 5D chromosomes in the common wheat were possible. Yazdi-Samadi et al. (2006) in the study of reciprocal substitution analysis of freezing resistance in wheat found that 5A, 5D, 3B and

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4D chromosomes were carrying freezing resistance genes. The group 5 chromosomes are most frequently involved in both vernalization response and frost tolerance (Galiba et al., 1995; Storlie et al., 1998). Netsvetaev and Netsvetaeva (2004) proved the significant association of frost tolerance with homeologous chromosome regions 1 and 6 of the A, B, and D wheat genomes that were encoded gliadin storage proteins.

Heading time in wheat is governed by three major factors such as vernalization requirement, photoperiod sensitivity and narrow-sense earliness. Generally wheat cultivars are divided into two types (winter and spring growth habit) depending on their need for cold temperature to initiate heading (Kato et al., 1999). Shindo et al. (2003) established the importance of several loci on the 2B chromosome governing heading time in wheat. Other loci that had significant effect on heading time were located on chromosomes 2B, 5B, 7A and 5A (Kato et al., 1999; Kato et al., 2002; Shindo et al., 2003). Acording to Worland (1996) and Snape et al. (2001), there is close association between the location of frost tolerance genes and the genes controlled the flowering time. Fowler et al. (2001) were concluded that, low temperature tolerance is dependent on a highly integrated regulatory mechanism and in this respect vernalization requirement, photoperiod sensitivity and earliness controlled and delayed heading time by postponing the transition from the vegetative to the reproductive phase.

During the last decade, development of DNA markers has resulted in the construction of genetic maps for the main cereal species including bread wheat (Borner et al., 2002). Determining the location of genes and QTLs controlling complex characters such as frost tolerance and heading time can be possible by using marker mediated techniques (Snape et al., 2001). Although the low level of polymorphism in wheat for genetic markers, specially RFLP markers, has restricted the genetic mapping (Roder et al., 1998; Stephenson et al., 1998; Kato et al., 1999; Gupta et al, 2002; Somers et al., 2004), recent studies have reported higher polymorphism with microsatellites than any other marker systems (Akkaya et al., 1992; Korzun et al., 1999; Manifesto et al., 2001; Borner et al., 2002; Song et al., 2002). This characteristic of microsatellites has made them a useful marker in the genetics and breeding of bread wheat (Bryan et al., 1997; Korzun et al., 1999).

To be useful for screening of winter hardiness, a test should have a high repeatability. It should be simple, rapid and highly correlated with field survival index. Nowadays, LT50 is regarded as a promising test in this respect (Fowler et al., 1981; Fowler et al., 1999; Mahfoozi et al., 2001). LT50 is defined as the lowest test temperature at which 50% of the plants survive after freezing.

This study was conducted to detect SSR markers linked to frost tolerance and localization of effective QTLs that were involved in controlling frost tolerance and heading time.

MATERIALS AND METHODS

Mapping population

The mapping population was prepared by crossing selected parents. One of the parents (P1) was the Norstar variety, the most winter hardy cultivar, that is used as a standard winter hardy wheat in many research programs with LT50 = -22.25°C (Limin and Fowler, 1983; Fowler et al., 1999). The second parent (P2) was Zagros, a cultivar with spring growth habit which is one of the most frost sensitive varieties with LT50 = -3.5°C. Norstar was used as the maternal parent. After crossing, the F2 seeds from 70 F1 plants were obtained and used for making F2 generation. The F2 seeds were vernalized at 2 ± 0.5°C for five weeks and the vernalized seedlings were transplanted to a greenhouse with the temperature of 20°C and 14/10 (D:N) photoperiod. Two hundred eighty F2:3 families were also obtained and used for freezing assay.

Freezing test

Frost tolerance was assessed according to Fowler et al. (1981) and Mahfoozi et al. (2001). A set of 12 test temperatures that ranged from -3 to -25°C with an increment of two degrees was used. For each test temperature, five plants from each F2:3 families were used (60 plants for each family). After imbibation of the seeds (Fowler et al., 1993), germinated seeds were transplanted to a controlled greenhouse under the temperature of 20°C and 14/10 (D:N) photoperiod. When the growing seedlings reached to 3 - 4 leaf stage, or 13 - 15, in Zadoks scale (Zadoks et al., 1974), plants were transferred to growth chamber for cold acclimation. Acclimation was undertaken for 35 days at 2 ± 0.5°C, and 14/10 (D:N) photoperiod. After acclimation period, crowns were prepared according to Fowler et al. (1993) and tested in a programmable freezer. Rate of decrease in temperature was 2°C/h until -17°C and thereafter was 8°C/h until -30°C (Mahfoozi et al., 2001). After freezing, samples were transferred to 4°C incubator and then the thawed crown regions were replanted in the controlled chambers at 20°C and 14/10 (D:N) photoperiod. After three weeks LT50 was recorded for every family. The final LT50 was reported after probit transformation.

Microsatelite analysis

Nuclear DNA was extracted from wheat young leaves of individual F2 plants according to Saghai-Maroof et al. (1984), with small modifications. After qualifying the DNA with 0.8% agarose gel electrophoresis and spectrophotometer, DNA samples were diluted to 15 ng/µl concentration. Wheat microsatellites were chosen from Roder et al. (1998), Gupta et al. (2002), Song et al. (2002), Somers et al. (2004), McIntosh et al. (2005) and www.grain genes.org.

Two hundred microsatellites were tested for polymorphism between parents using 1 kb ladder number VIII of corn (Fermentas company), and 41 polymorphic SSR were used for genotyping of the F2 population. Polymerase chain reaction (PCR) was performed according to Hoisington et al. (1994). PCR products were then detected with Sequi-Gen GT system from BIO-RAD. Silver staining was undertaken according to CYMMIT laboratory protocols (Hoisington et al., 1994). Mapping of the genes as QTLs was performed using Kosambi mapping function (Kosambi, 1944), with the MAPMANAGER-QTX20 (Chmielewicz and Manly, 2002) software, and for other statistical operations the SPSS 10.05, and Excel softwares were used.

Field experiment

The F2:3 families were planted in field (2006) in a randomized

Table 1. QTL analyses of SSR markers for LT50 in the Norstar×Zagros wheat cross.

Character	Marker	Likelihood Ratio	% Variance accounted	P value	Additive effect	Dominance effect
LT50	Xgwm174	8.8	3	0.01	0.02	0.04
	Xgwm292	7.9	3	0.01	-0.01	0.01
	Xgwm148	9	4	0.01	0.03	0.2
	Xgwm357	12.1	5	0.002	0.05	-0.1
	Xgwm666	6.5	2	0.03	0.04	0.02
	Xgwm499	7.4	6	0.02	0.03	0.1
	Xcfa2190	7.3	3	0.02	0.07	0.01

Table 2. QTL analyses for the Heading time in the Norstar×Zagros wheat cross.

Character	Marker	Likelihood Ratio	% Variance accounted	P value	Additive effect	Dominance effect
Heading time	Xgwm148	8.3	13	0.015	1.11	3.3
	Xgwm617	8.5	18	0.014	-1.68	3.8
	Xgwm149	11.4	13	0.003	1.23	3.1
	Xbarc180	13.2	14	0.0013	-0.72	3.7
	Xgwm666	8.2	9	0.02	0.82	2.8

complete block design (RCBD) with two replications and thereafter, the days to heading was measured. This experiment was performed to reveal any probable correlation between this trait and LT50 and to discover possible common QTLs governing both traits.

RESULTS

Heading time and LT50

The mean of time from transplanting the vernalized seedling to time of heading for Zagros and Norstar were 64 and 84 days respectively. For the F2:3 families it was 66.27 ± 0.38. The mean LT50 of the two parents of the mapping population were -3.5 ± 0.43 for Zagros, and -22.25 ± 0.53 for Norstar, respectively. The mean of LT50 was -8.03 ± 0.284 for F2:3 families. The range of LT50 for this generation was -1.5 to -24.2.

Single marker analysis

Forty-one SSR markers were found to be polymorphic between parents and thus were used for marker analysis. These markers were located on 12 wheat chromosomes. Single marker analysis (SMA) with marker regression approach (Kearsey and Hyne, 1994; Hyne and Kearsey, 1995) showed that Xgwm174 and Xgwm292 on 5D, Xgwm148 on 2B, Xgwm357 on 1A, Xgwm666 on 5A-7A, Xcfa 2190 on 5A and Xgwm499 on 5B were linked to LT50 (Table 1).

Additionally, QTL analysis for the heading time in F2:3 generation showed that Xgwm148 on 2B, Xgwm149 on 4B, Xgwm666 on 5A-7A and two marker loci including Xgwm617 and Xbarc180 on 5A were linked to heading time (Table 2).

DISCUSSION

There was a significant correlation between frost tolerance (LT50) and heading time in F2:3 ($r = -0.56^{**}$) families. Thus families with later heading time were also more frost tolerant. Apparently, some closely linked QTLs or QTLs with pleiotropic effects govern these characters. According to our results two markers were significant in both traits (Tables 1 and 2). Brule-Bable and Fowler (1988) stated the possibility of genetic linkage between cold hardiness and late maturity (winter growth habit). According to Limin and Fowler (2002) increasing the life cycle increase the vegetative phase, all of which extend the time that low-temperature tolerance (LT) genes are more expressed. The differences of days to heading mostly resulted from the differences in the transition time to enter the reproductive phase during vernalization saturation.

Analysis of QTLs for LT50 showed that two QTL from 5D, two QTL from 5A, one QTL from 2B, one QTL from 5B and one major QTL from 1A chromosome were linked to LT50. Except the QTL linked to Xgwm292 marker locus, the others had positive additive effect. This shows that the frost resistance increases alleles inherited from frost resistant parent of norstar which possess positive dominance effect, indicating that the resistance alleles are dominantly inherited (Table 1). However, based on

our results (Table 1), we recommend the use of the most effective QTLs with the least p value in the marker assisted selection programs concerning LT50.

Sutka (1981, 2001) reported the importance of 2B, 5A, 5B and 5D chromosomes on the frost tolerant using chromosome substitution line. Sutka and Kovacs (1985), Sarma et al. (1998), Storlie et al. (1998), Sutka et al. (1999), and Galiba et al. (2001), confirmed the key role of 5A chromosome in controlling frost tolerance. Snape et al. (2001) outlined the role of 5A and 5D chromosomes on the flowering time and frost tolerance. Leonova et al. (2003) and Toth et al. (2003) reported some QTLs on 5B chromosome that were located near Vrn-B1 and had important role on frost tolerance and flowering time. Baga et al. (2006) based on 594 SSR markers and 380 AFLP markers in two doubled haploid mapping populations using single marker analysis (SMA) and simple interval mapping (SIM) analysis, revealed that there is a major QTL on 5A chromosome, a minor QTL on 1D chromosome and the major QTL of both populations located near Vrn-A1 locus. Chun et al. (1998) based on genetic studies of antifreeze proteins and their correlation with winter survival in wheat showed that plants with chromosome substitutions of 1A, 5A, 5B and 5D exhibited much higher antifreeze activity than the other substitution lines and therefore were more frost tolerant. The correlation of lower LT50 values and increased proline content in the advance generations derived from F2 in common wheat was documented by Dorffling et al. (1997) and they concluded that both of the increased frost tolerance and proline content were heritable traits. According to Galiba et al. (1992), although the genes controlling osmoregulation and proline content were primarily located on chromosomes 5A and 5D, the contribution of other chromosomes such as 1A and 2D cannot be ignored.

QTL analysis for the heading time in F2:3 indicated one major QTL on 2B, one QTL on 4B, one QTL on 5A/7A and two QTL on 5A chromosomes for this trait. According to our results, QTLs inherited from both parents are important in increase of the heading time. All the detected QTLs were dominantly inherited. The heading time of winter wheat, winter survival and delayed development in autumn are important breeding aims for winter wheat production and for adaptability in a particular environment. In order to facilitate traditional breeding efforts, use of informative SSR markers is recommended. Worland et al. (1996) also stated the possible effect of 5A chromosome on earliness. Kato et al. (1999), Snape et al. (2001) and Borner et al. (2002) reported the role of 2B chromosome on time of flowering of wheat. Kato et al. (2002) established the effect of 5A chromosome on ear emergence time. Shindo et al. (2003) detected some QTLs on 2A, 2B, 5B, 6D, 7A and 7D chromosomes in relation with heading time.

Single marker analysis approach was preferred by some researchers for QTL mapping. This method has the advantage of simplicity and certain situation supplies equivalent or more powerful result than a comparable two or more marker tests (Coffman et al., 2003).

Conclusions

Some of the primers in this study were associated with both heading time and frost tolerance, which is the indication of the pleiotropic effect or linkage of the respective QTLs. These QTLs may contribute to the regulatory system that control the activation period of frost tolerance genes and play an important role on developmental regulation (Limin and Fowler, 2002). Therefore, with a genotype with late heading, the activity of frost tolerance genes are longer and the level of expression of these structural genes are greater. However, in order to obtain more detailed results it is suggested to use further SSR primers in the future studies for saturating the chromosome intervals.

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REFERENCES

Akkaya MS, Bhagwat AA, Cregan PB (1992). Length polymorphisms of simple sequence repeat DNA in soybean. Genetics 132: 1131-1139.

Baga M, Chodaparambil SV, Limin AE, Pecar M, Fowler DB, Chibbar RN (2006). Identification of quantitative trait loci for low temperature tolerance in cold-hardy winter wheat. Plant and Animal Genome XIV Conference, San Diego, Ca, p. 353.

Borner A, Schumann E, Furste A, Coster H, Leithold B, Roder MS, Weber WE (2002). Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L) . Theor. Appl. Genet. 105: 921-936.

Brule-Babel AL, Fowler DB (1988). Genetic control of cold hardiness and vernalization requirement in winter wheat. Crop Sci 28: 879-884.

Bryan GJ, Collins AJ, Stephenson P, Orrey A, Smith JB, Gale MD (1997). Isolation and characterization of microsatellites from hexaploid bread wheat. Theor. Appl. Genet. 94: 557-563.

Cahalan G, Law CN (1979). The genetic control of cold resistance and vernalization requirement in wheat. Heredity 42: 125-132.

Chmielewicz KM, Manly KF (2002). Software for genetic mapping for mendelian markers and quantitative trait loci. Roswell Park Cancer Institute.

Chun JU, Yu XM, Griffith M (1998). Genetic studies of antifreeze proteins and their correlation with winter survival in wheat. Euphytica 102: 219-226.

Coffman CJ, Doerge RW, Wayne ML, McIntyre LM (2003). Intersection test for single marker QTL analysis can be more powerful than two marker QTL analysis. BMC Genetics 4: 1-10.

Dorffling K, Dorffling H, Lesselich G, Luck E, Zimmermann C, Meltz G, Jurgens HU (1997). Heritable improvement of the frost tolerance in

winter wheat by invitro selection of hydroxylprolin- resistant prolin overproducing mutants. Euphytica 93: 1-10.
Efremova TT, Laikova LI, Arbuzova VS, Popova OM (2004). Effect of the 5R(5A) alien chromosome substitution on the growth habit and winter hardiness of wheat. Russ. J. Genet. 40: 810-812.
Fowler DB, Berton G, Limin AE, Mahfoozi S, Sarhan F (2001). Photoperiod and temperature interactions regulated low temperature-induced gene expression in barely. Plant Physiol. 127: 1676-1681.
Fowler DB, Gusta LV, Tyler NJ (1981). Selection of winterhardiness in wheat.III.Screening methods. Crop Sci 21: 896-901.
Fowler DB, Limin AE, Ritchie JT (1999) Low-temperature tolerance in cereals: model and genetic interpretation. Crop Sci. 39: 626-633.
Galiba G, Kerepesi I, Vagujfalvi A, Kocsy G, Cattivelli L, Dubcovsky J, Snape JW, Sutka Y (2001). Mapping of genes involved in glutathione, carbohydrate and COR14b cold induced protein accumulation during cold hardening in wheat. Euphytica 119: 173-177.
Galiba G, Quarrie A, Sutka J, Morgonov A, Snape JW (1995). RFLP mapping of vernalization(Vrn1)and frost resistance(Fr1)genes on chromosome 5A of wheat. Theor. Appl Genet. 90: 1174-1179.
Galiba G, Simon-Sarkadi L, Kocsy G, Salgo A, Sutka J (1992). Possible chromosomal location of genes determining the osmoregulation of wheat. Theor. Appl. Genet. 85: 415-418.
Gupta PK, Balyan HS, Edwards KJ, Isaac P, Kurzun V, Roder M, Leroy P (2002). Genetic mapping of 66 new microsatellite (SSR) loci in breed wheat. Theor. Appl. Genet. 105: 413-422.
Hoisington D, Khairallah M, Gonzalez-de-Leon D (1994). Laboratory protocols:CIMMYT applied Molecular Genetics Laboratory. Second Edition. Mexico, D.F.:CIMMYT.
Hyne V, Kearsey MJ (1995). QTL analysis: further uses of 'marker regression. Theor. Appl. Genet. 91: 471-476.
Kato K, Miura H, Sawada S (1999). Detection of an earliness per se quantitative trait locus in the proximal region of wheat chromosom 5AL. Plant Breed 118: 391-394.
Kato K, Miura H, Sawada S (2002). Characterization of QEet.ocs-5A.1, a quantitative trait locus for ear emergence time on wheat chromosome 5AL. Plant Breed 121: 389-393.
Kearsey MJ, Hyne V (1994). QTL analysis: a simple ' marker-regression' approach. Theor. Appl. Genet. 89: 698-702.
Korzun V, Roder MS, Wendehake K, Pasqualone A, Lotti C, Ganal MW, Blanco A (1999). Integration of dinucleotide microsatellites from hexaploid bread wheat into a genetic linkage map of durum wheat. Theor ApplGenet 98: 1202-1207.
Kosambi DD (1944). The estimation of map distances from recombination values. Ann. Eugen 12: 172-175.
Leonova E, Pestsova E, Salina E, Efremova T, Roder M, Borner A (2003). Mapping of the Vrn-B1 gene in *Triticum aestivum* using microsatellite markers. Plant Breed. 122: 209-212.
Limin AE, Fowler DB (1983). Cold-Hardiness response of sequential winter wheat tissue segments to differing temperature regimes. Crop Sci 32: 838-843.
Limin AE, Fowler DB (1993). Inheritance of cold hardiness in *Triticum aestivum* x synthetic hexaploid wheat crosses. Plant Breed 110: 103-108.
Limin AE, Fowler DB (2002). Developmental traits affecting low-temperature tolerance response in near-isogenic lines for the vernalization locus Vrn-A1 in Wheat (*Triticum aestivum* L.em Thell.). Ann of Bot 89: 579-585.
Mahfoozi S, Limin AE, Fowler DB (2001). Influence of vernalization and photoperiod responses on cold hardiness in winter cereals. Crop Sci 41: 1006-1011.
Manifesto MM, Schlatter AR, Hopp HE, Suarez EY, Dubcovsky J (2001). Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. Crop Sci. 41: 682-690.
McIntosh RA, Devos KM, Dubcovsky J, Rogers WJ, Morris CF, Apples R, Anderson OD (2005). Catalogue of gene symbols for wheat: 2005 supplement."http://wheat.pw.usda.gov".
Netsvetaev VP, Netsvetaeva OV (2004). Revealing hereditary variation of winter hardiness in cereals. Russ. J. Genet. 40: 1239-1244.
Roder M, Korzun V, Vendehake K, Plaschke J, Tixier MH, Leroy P, Ganal M (1998) A Microsatellite map of wheat. Genetics 149: 2007-2023.
Saghai-Maroof MA, Soliman K, Jorgensen RA, Allard RW (1984). Ribosomal DNA spacer- length polymorphisms in barley : Mendelian inheritance, chromosomal location, and population dynamics. PNAS 81: 8014-8018.
Sarma RN, Gill BS, Sasaki T, Galiba G, Sutka J, Laurie DA, Snape JW (1998). Comparative mapping of the wheat chromosome 5A Vrn-A1 region with rice and its relationship to QTL for flowering time. Theor. Appl. Genet. 97: 103-109.
Shindo C, Tsujimoto H, Sasakuma T (2003). Segregation analysis of heading traits in hexaploid wheat utilizing recombinant inbred lines. Heredity 90: 56-63.
Snape JW, Sarma R, Quarrie SA, Fish L, Galiba G, Sutka J (2001). Mapping genes for flowering time and frost tolerance in cereals using precise genetic stocks. Euphytica 120: 309-315.
Somers DJ, Isaac P, Edwards K (2004). A high density microsatellite consensus map for wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 109: 1105-1114.
Song QJ, Fichus EW, Cregan PB (2002). Characterization of trinucleotide SSR motifs in wheat. Theor. Appl. Genet. 104: 286-293.
Stephenson P, Bryan G, Kirby J, Collins A, Devos K, Busso C, Gale M (1998). Fifty new microsatellite loci for the wheat genetic map. Theor. Appl. Genet. 97: 946-949.
Storlie EW, Allan RE, Simmons MK (1998). Effect of the Vrn1-Fr1 interval on cold hardiness levels in near-isogenic wheat lines. Crop Sci. 38: 483-488.
Sutka J (1981). Genetic studies of frost resistance in wheat. Theor. Appl. Genet. 59: 145-152.
Sutka J (1994) Genetic control of frost tolerance in wheat (*Triticum aestivum* L.). Euphytica, 77: 277-284.
Sutka J (2001). Genes for frost resistance in wheat. Euphytica 59: 145-152.
Sutka J, Kovacs G (1985). Reciprocal monosomic analysis of frost resistance on chromosome 5A in wheat. Euphytica 34: 367-370.
Sutka J, Galiba G, Vagujfalvi A, Gill BS, Snape JW (1999) Physical mapping of Vrn-A1 and Fr1 genes on chromosom 5A of wheat using deletion line. Theor. Appl. Genet. 99: 199-202.
Toth B, Galiba G, Feher E, Sutka Y, Snape W (2003). Mapping genes affecting flowerjng time and frost resistance on chromosome 5B of wheat. Theor. Appl. Genet. 107: 509-514.
Worland AJ (1996). The influence of flowering time genes on environmental adaptability in European wheats. Euphytica 89: 49-57.
Yazdi-Samadi B, Dashti H, Nabipour A, Sarrafi A (2006). Reciprocal substitutions analysis of freezing resistance in wheat (*Triticum aestivum* L.). Plant Breed. 125: 217-220.
Zadoks JC, Chang TT, Konzak CF (1974). A decimal code for growth stages of cereals. Weed Res. 14: 415-421.