World Applied Sciences Journal 21 (8): 1181-1189, 2013
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2013.21.8.2866

Marker Assisted Selection for Heat Tolerance in Bread Wheat

[1]Shahab Sadat, [2]Khalil Alami Saeid, [3]Mohammad Reza Bihamta,
[4]Sepideh Torabi, [5]Seyed Ghasem Hoseini Salekdeh and [6]Gholam Abbas Lotfali Ayeneh

[1]Department of Plant Breeding,
Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of Agronomy and Plant Breeding,
Agriculture and Natural Resources University of Khuzestan, Mollasani, Iran
[3]Department of Agronomy and Plant Breeding, Faculty of Agronomy sciences,
College of Agriculture and Natural Resources- University of Tehran, Karaj, Iran
[4]Department of Plant Breeding, Science and Research Branch, Islamic Azad University, Tehran, Iran
Agricultural Biotechnology Research institute of Iran, Karaj, Iran
[6]Research Centre of Agricultural and Natural resources of Khuzestan-Ahvaz-Iran

Abstract: Due to difficulty of managing of heat tolerance through conventional phenotypic selection and the presence of several QTLs for such a polygenic trait with complex inheritance, Marker Assisted Selection (MAS) was considered as an efficient method for screening 25 bread wheat genotypes to heat stress. Fourteen SSR markers which were linked to some important traits including grain filling duration, HSI (Heat Susceptibility Index) grain filling duration, HSI_single kernel weight of main spike and HSI kernel weight under heat stress were selected from previous reports. After DNA extraction and running PCR products on 8% acrylamide gel and PeqGOLD MoSieve Agarose MS-1000 and staining procedure, data scoring and statistical analysis were performed. Constructed dendrogram using the Unweighted Pair-Group Method on Arithmetic mean (UPGMA) grouped genotypes in 3 clusters. Two genotypes were laid in a same cluster. One genotype was grouped in another cluster and the remaining 22 genotypes were laid in the third cluster. The results of field experiments from previous reports and the results of molecular screening for heat tolerance indicated that selected markers were able to screen bread wheat genotypes for heat tolerance.

Key words: Marker assisted selection · Heat stress · Wheat · Molecular markers

INTRODUCTION

The frequency of extreme events such as high temperatures is predicted to increase in a future warmer climate [1]. Heat stress severely restricts plant growth and productivity and is classified as one of the major abiotic adversities for many crops [2, 3, 4] particularly when it occurs during reproductive stages, which may lead to substantial yield loss in wheat [5]. The rising temperatures of the late phases of wheat development and particularly, from the beginning of heading and after anthesis, should be considered as an important factor limiting yield [6, 7, 8] in U.S. Southern Great Plains, Australia as well as many wheat-growing regions in China, India, the Middle East, Africa, some European countries and Iran. Heat stress at the time of anthesis until ripeness, reduces grain yield significantly. Gibson and Pulson [9] reported that heat stress (35°C) that began 10 days after anthesis and continued until ripeness, caused a reduction by 78% in grain yield and 63% in kernel number. Besides, it decreased the kernel weight by 29%. But heat stress that began 15 days after anthesis and continued until complete ripeness caused no effect on kernel number but a decease of 18% on kernel weight. Wardlaw *et al*. [10] reported that a global reduction in crops production of about 3-4 % occurs when the mean temperature increases by 1°C

Corresponding Author: Shahab Sadat, Department of Plant Breeding, Science and Research Branch, Islamic Azad University, Tehran, Iran. Tel: 06113348365- 9384715583.

above optimum. Although a variety of wheat cultivars that show improved yield stability under heat stress have been identified, [5, 11] the quantitative nature of heat tolerance and unpredictability of heat stress in the field makes it particularly difficult for breeders to effectively select for the trait. Due to difficulty of managing of heat tolerance through conventional phenotypic selection and the presence of several QTLs for a single target trait with complex inheritance, the selection of target traits can be achieved indirectly using molecular markers that are closely linked to underlying genes or that have been developed from the actual gene sequences. Theoretical-based simulation studies suggest that the effectiveness of marker-assisted selection (MAS) for polygenic traits can be greater than traditional trait-based selection [12, 13, 14, 15a, 15b]. In general, these studies agree that MAS efficiency is enhanced when markers are tightly linked (<5.0 cM) to quantitative trait loci (QTL), selection is performed in early generations prior to recombination between markers and QTL, large populations are used and selection is practiced on traits with low heritability. There are many markers along with the chromosomal location of the target genes reported from different scientists, currently in use for MAS applications in wheat at CIMMYT: High protein [16], Flour color [17], Grain hardness [18], Dough strength [19], Swelling volume [20], Fusarium head blight [21], Durable leaf and brown rust [22], stem rust [23, 24], Barley yellow dwarf [25], Crown rot [26], Resistance to Heterodera avenae [27a, 27b] and Boron tolerance [28]. Although a number of different marker systems are being used in genetic characterization of traits [29, 30], PCR based markers such as microsatellites (SSR) are the most popular in marker implementation due to their often codominant inheritance and their robustness, defined by repeatability and reliability as a PCR based marker system [31].

Accordingly, the aim of this study is to use Marker Assisted Selection for screening 25 bread wheat genotypes (including some cultivars and promising lines) for late seasonal heat tolerance.

MATERIALS AND MTHODS

Plant materials and DNA Isolation: Twenty five bread wheat genotypes including some common cultivars for cultivation in southern Iran w ith specific climate (late seasonal heat stress) along with some new promising lines for this climate were selected for the study. The seeds were provided by the Research Centre of Agricultural and Natural resources of Khuzestan-Iran. Total genomic DNA was extracted from fresh leaf material by appropriate modifications of the method described by Dallaporta *et al*. [32]. The quantity and quality of DNA were evaluated by spectrophotometery and 0.8% Agarose gel respectively.

Molecular Markers: Fourteen SSR markers (Table 1) were selected from previous reports [11, 33, 34]. These QTLs were adjacent to some important traits mainly grain filling duration, HSI[1] (Heat Susceptibility Index) grain filling duration, HSI_single kernel weight of main spike and HSI kernel weight under heat stress.

PCR Amplification and Running Conditions: PCR amplification was performed in a 25-µl reaction volume containing the following components: 20 ng of template DNA, 200 µM of each of the four dNTPs, 1X *Taq* polymerase buffer, 1 unit *Taq* polymerase, 2 mM MgCl2 and 0.25 µM of each of the two primers. All PCR components except for primers (Metabion-Germany) were provided by Fermentas Company. Amplifications were performed in a BioRad thermocycler programmed for 40 cycles. The amplification cycles followed by a Touch down PCR protocol recommended by the manufacturer to enhance the specificity of the initial primer-template duplex formation. At the beginning of the cycling stage, the annealing temperature was set 12°C above the T_m of the primers. In subsequent cycles the temperature was decreased 1°C per cycle (for 30 seconds) until it reached to annealing temperature (2 to 5°C below Tm). The remaining cycles were run on annealing temperature of the primers. The denaturation and extension were at 94°C for 30 seconds and 72°C for 1 minute respectively. After a pre-run of the gel (8%-denaturing) for an hour (until the temperature of the buffer reached at 60°C), 5 µl of the amplification products was mixed with an equal volume of formamide loading buffer (10 mM NaOH, 95 % Formamide, 0.05 % Bromophenol Blue, 0.05 % Xylene Cyanol) and was denatured for 2 min at 92°C in the thermocycler and placed on ice before being applied to the gel in TBE buffer [35]. The running conditions was 350-400V until the temperature was kept at 55°C [36]. In addition to acrylamide gel, PeqGOLD MoSieve Agarose MS-1000 - special agarose for separating small nucleic

[1]HSI = (1 - Xh/X)/(1 - Yh/Y), where Xh and X are the phenotypic means for each genotype under heat stressed and control conditions, respectively and Yh and Y are the phenotypic means for all lines under heat stressed and control conditions, respectively.

Table 1: Characteristics of fourteen en SSR markers for Marker Assisted Selection of 25 bread wheat genotypes for heat tolerance.

Marker name	Chromosome number	Reverse Primer	Forward Primer	QTL for	Reported by
gwm11	1B	GTGAATTGTGTCTTGTATGCTTCC	GGATAGTCAGACAATTCTTGTG	Grain-filling duration	[11]
Xcfd43	2D	CCAAAAACATGGTTAAAGGGG	AACAAAAGTCGGTGCAGTCC	Grain-filling duration	[33]
Xgwm356	2A	CCAATCAGCCTGCAACAAC	AGCGTTCTTGGGAATTAGAGA	HSI_single kernel weight of main spike	[33]
Xbarc137	1B	CCAGCCCCTCTACACATTTT	GGCCCATTTCCCACTTTCCA	Grain-filling duration	[33]
Gwm484	2D	AGTTCCGGTCATGGCTAGG	ACATCGCTCTTCACAAACCC	Grain-filling duration	[34]
Gwm293	5A	TCGCCATCACTCGTTCAAG	TACTGGTTCACATTGGTGCG	Grain-filling duration	[11]
gwm291	5A	AATGGTATCTATTCCGACCCG	CATCCCTAGGCCACTCTGC	HSI kernel weight	[33]
Gwm325	6D	TTTTTACGCGTCAACGACG	TTTCTTCTGTCGTTCTCTTCCC	HSI grain filling duration HSI kernel weight	[33]
Xgwm294	2A	GCAGAGTGATCAATGCCAGA	GGATTGGAGTTAAGAGAGAACCG	HSI_single kernel weight of main spike	[33]
Gwm268	1B	TTATGTGATTGCGTACGTACCC	AGGGGATATGTTGTCACTCCA	HSI kernel weight	[33]
Xwmc407	2A	CATATTTCCAAATCCCCAACTC	GGTAATTCTAGGCTGACATATGCTC	Grain-filling duration	[33]
Xcfa2129	1A	ATCGCTCACTCACTATCGGG	GTTGCACGACCTACAAAGCA	HSI grain filling duration HSI kernel number HSI kernel number HSI single kernel weight HSI_single kernel weight of main spike	[33]
Xgwm111.2	2B	ACCTGATCAGATCCCCATCG	TCTGTAGGCTCTCTCCGACTG	HSI kernel weight HSI kernel number HSI_kernel number of main spike	[33]
WMC527	3B	GCTACAGAAAACCGGAGCCTAT	ACCCAAGATTGGTGGCAGAA	HSI_kernel weight of main spike HSI kernel weight	[33]

acid fragments between 50 bp and 2000 bp and high gel strength for easy handling- (Peq Lab -Germany) was utilized to identify fragments of PCR products. With the concentration of 3.5% an appropriate separation of different fragment of PCR products with a size range of 20 to 2000 bp can be done easily.

Staining Procedure: The method described by Sanguinetti *et al*. [37] was slightly modified, with altered time for improved detection.

After electrophoresis, gels were submerged in a fixative solution containing 10% ethanol and 0.5% acetic acid, at room temperature (RT) for 20 minutes. The gels were then transferred to the 0.2% AgNO3 (analytical grade) for 30 minutes at room temperature. After a brief rinse with distilled water to eliminate the excess of silver ions, gels were developed in 3% NaOH containing 37% formaldehyde (5 ml/L) until bands became visible (brown). For Agarose gel staining, the gel was stained with EtBr after electrophoresis and the photograph of the gel was taken by a transluminator UVP. All staining chemicals were provided by Merck Company.

Data Scoring and Statistical Analysis: Detected Bands were coded in binary by giving character state (1) or (0) for presence or absence of bands in each genotype for subsequent statistical analysis. The generated data matrix was subjected to statistical analyses using NTSYS-pc version 2.0 software [38]. Genetic similarities were calculated using Dice similarity index as in Nei and Li [39] Dendrogram showing genetic relationships of the 25 genotypes were constructed using the unweighted pair-group method on arithmetic averages (UPGMA). For the dendrogram, the cophenetic coefficients between the matrix of genetic similarities and the matrix of cophenetic values were computed and the significance of cophenetic correlation was tested using Mantel matrix correspondence test [40].

RESULTS AND DISCUSSION

The selection of these fourteen SSR markers for screening of mentioned genotypes is mostly based on QTLs of mapping each yield component as a separate HSI. Although previous QTL studies have used a susceptibility index for measuring flooding tolerance in soybean [41] and drought tolerance in both hexaploid wheat [42] and durum wheat [43] but the approach of mapping each yield component as a separate HSI is unique and allows for the identification of QTL affecting single traits that would collectively contribute to overall yield stability and heat tolerance [33]. In view of this fact that heat shock comes above 15 days after anthesis, therefore it has no effect on kernel number but a considerable decrease in kernel weight. Accordingly the markers which were selected for this study had the highest influence on HSI_kernel weight of main spike, HSI_single kernel weight of main spike, Grain-filling duration and HSI grain filling duration. Considering that lower grain weight and altered grain quality result from heat stress during grain- filling [44], therefore some of the markers selected for this study were those with close proximity to this trait. Moreover grain filling duration has been widely used as a measurement of heat tolerance [45, 11, 34]. All 14 primers utilized for this study were polymorphic indicating their ability to assess mentioned

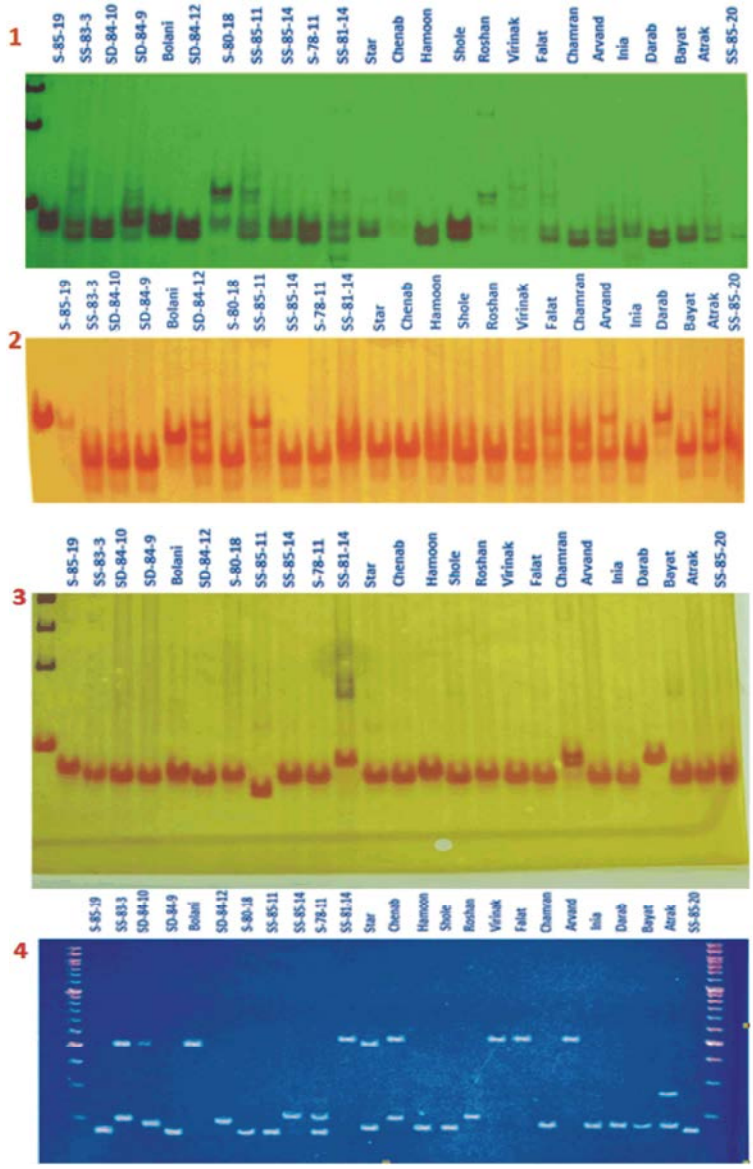

Fig. 1-4: Some gel photographs for the PCR products of using different markers and a 50 bp ladder for 25 bread wheat genotypes for screening to heat tolerance

bread wheat genotypes. Figures 1 to 4 show some photographs of the gels after staining by Silver nitrate and EtBr. In this study, a total of 75 alleles were detected among twenty five genotypes (Table 2). The highest belonged to marker gwm11 and Gwm293 with 9 alleles and the lowest belonged to marker Gwm268 with 2 alleles. It should be noted that multiple allelism is very common in SSR markers and they are able to produce different alleles in one locus [46]. This reveals significant differences in allelic diversity among various microsatellite loci.

Many studies have reported remarkable differences in allelic diversity among various microsatellite loci [47, 48, 49, 50]. The average of produced alleles for all loci in this study was 5.4. There are different reports for obtaining different alleles of using SSR markers to study genetic variation among different wheat cultivars and lines. While Fahima *et al*. [51] reported an average of 10 alleles per locus on some wild wheat accessions, Zeb *et al*. [52] reported an average of 5.2 alleles per cultivar. Besides Salem *et al*. [53] reported an average of 3.2 alleles from 7 wheat cultivars. These allelic variations

Table 2: Number of produced alleles with fragment sizes of the alleles in 14 SSR markers for Marker Assisted Selection of 25 wheat genotypes for heat tolerance

Marker	Number of produced allels	Fragment size(bp)
gwm11	9	30-70
Xcfd43	8	80-190
Xgwm356	3	30-180
Xbarc137	8	40-300
Gwm484	6	35-150
Gwm293	9	40-350
gwm291	8	40-200
Gwm325	3	40-100
Xgwm294	5	40-180
Gwm268	2	30-50
Xwmc407	4	50-110
Xcfa2129	3	30-50
Xgwm111.2	3	30-50
wmc527	4	35-50

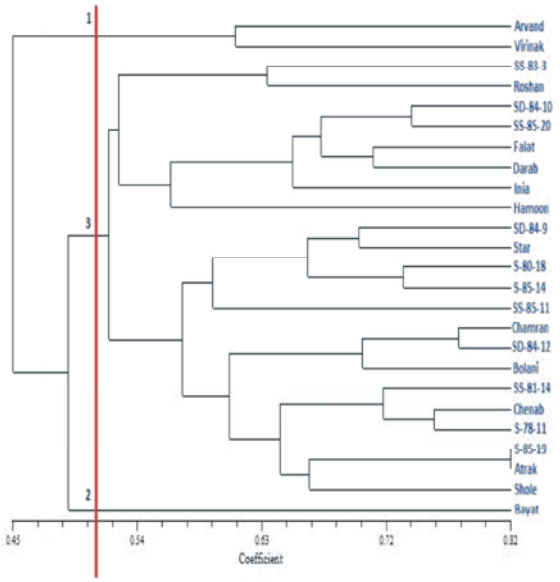

Fig. 5: The dendrogram of the cluster analysis (UPGMA) of 25 bread wheat genotypes using 14 SSR markers for screening to heat tolerance

in different studies is mostly attributed to the kind of wheat genotypes for the mentioned studies. Considerable amount of natural out crossing that occurs in wild wheat accessions and also the landraces which are selected from local germplasm have a wide range of diversity and thus will result in higher alleles [53]. However cultivars which are product of repeated inbreeding, would have lower alleles than both of wild genotypes or landraces. Among 25 genotypes selected for this study, 11 genotypes were lines with repeated inbreeding which resulted in producing lower alleles in comparison with remaining genotypes. The size of the detected alleles produced from using the SSR primer sets ranged from 30 to 350 bp, (Table 2) which reflects not a large difference in the number of repeats between different alleles. While Salem *et al*. [53] obtained an allelic size range between 77 to 266 bp on using 15 microsatellite markers on some wheat genotypes, Moghaieb *et al*. [54] reported an allelic size range between 82 to 1620 bp on using SSR markers associated with salt tolerance in Egyptian wheats. It should be noted that SSR markers not only can show different allelic variations in the same species but also they are able to assess even monoallelic differences in subspecies specifically [46].

The cophenetic correlation coefficient between the dendrogram and the original distance matrix was 0.71 and significant. Based on the formulae $(\sqrt{n/2})$ the SSR based dendrogram grouped the investigated genotypes into three main clusters (Figure 5). Arvand and Virinak were grouped in the first cluster. Bayat was laid in a separated group in the second cluster and the remaining genotypes were grouped in the third cluster. Arvand and Virinak have been proved to be highly tolerant to heat stress from a previous field study [55] and our results were in accord with their results. It should be noted that Arvand and Virinak are 2 cultivars which have been breeded for warm regions of Iran and have been adapted to this climate for a long time. Bayat has also been introduced as a highly tolerant wheat cultivar to heat stress and it was grouped in a separated cluster. Bayat has also passed its breeding period for cultivation in the southern Iran with warm climate. The reason for not grouping Bayat with Arvand and virinak in the same cluster could be that presumably different alleles of thermo-tolerant genes have been produced and these alleles laid Bayat in a different cluster.

Naghavi *et al*. [46] also reported different allelic variations in the same species and even monoallelic differences in subspecies. Among remaining genotypes which have been grouped in another cluster (third cluster) there were Chamran and Atrak which are highly tolerant known wheat cultivars. But surprisingly, they were grouped with some sensitive cultivars and lines in the third cluster. The reason could be that Arvand and Bayat are two late- mature cultivars. Virinak, is an early mature but a late planting cultivar which can tolerate heat stress in the late season with the minimum losing yield. It seems that these three cultivars have the genes with the real

tolerance mechanism (a physiologic heat tolerance due to heat tolerance genes) to heat stress. Hence it is concluded that selected markers have been able to flank to these genes and produced specific alleles which were effective in putting them in different clusters. But Chamran and Atrak are not late mature cultivars. They have no mechanisms for heat tolerance (no physiologic heat tolerance) but have an escape mechanism for heat tolerance. A previous field data on yield of these two cultivars confirms these results [55]. They reported a significant decrease in the yield of these two cultivars when they were cultivated in the late season (2 to 3 months after on time cultivation), in comparison with Arvand, Virinak and Bayat. Among all genotypes which have been clustered in the third cluster, 8 cultivars (Shole, Chenab, Inia, Hamoon, Roshan, Darab, Star, Bolani) have been proved to be highly or partially sensitive to heat tolerance from a previous field study [55]. Besides S-85-19 has been proved to have a considerable adaptation and stability for yield in warm climatic conditions [56]. But the results of this dendrogram have grouped it in to the sensitive cluster. Although this line has a good adaptation and stability for yield in warm conditions but it seems that selected markers were not able to link to genes which are responsible for mentioned traits (Grain-filling duration, HSI Grain-filling duration, HSI_single kernel weight of main spike and HSI kernel weight) for heat tolerance in this line. Besides, some studies have introduced cultivar Falat as a tolerant cultivar and some reports have introduced this cultivar as a sensitive genotype [55]. Our dendrogram has introduced this cultivar as a sensitive cultivar. Accordingly selected markers were not able to produce any specific alleles to put this cultivar to the tolerant cluster.

The reason for these differences is that the stresses and specifically heat stress is a regional problem. In some areas it shocks the plant for just a few hours and in some areas the effect is a lot longer than a shock and the temperature may increase gradually and continuously during the reproductive stage until ripeness. Besides in some areas heat stress comes at the vegetative stage and in some areas it comes at the reproductive stage. Facing the high temperature at the beginning of anthesis or 10 to 15 days after that will also have different effects on yield of a same wheat genotype. Therefore the results of different studies on evaluating of this cultivar for heat resistance in different areas of the world is different. The remaining promising lines including SS-83-3, SD-84-10, SS-85-20, SD-84-12, SS-81-14, S-85-11, S-85-14, S-80-18 S-84-10 and SD-84-9 which have been clustered in sensitive group have not been studied for heat tolerance previously and there were no reliable field data for their tolerance to heat stress.

CONCLUSION

Although there have been numerous QTL mapping studies for a wide range of traits in diverse crop species, relatively few markers have actually been implemented in plant breeding programs [57]. The main reason for this lack of adoption is that the markers used have not been reliable in predicting the desired phenotype. In many cases, this would be attributable to a low accuracy of QTL mapping studies or inadequate validation [17, 57]. However, Young [57] emphasized that scientists must realize the necessity of using larger population sizes, more accurate phenotypic data, different genetic backgrounds and independent verification, in order to develop reliable markers for MAS. Although in this study Selected markers were almost able to make a good screening of selected genotypes but to make sure that the results of marker assisted selection is reliable and validated, a field screening of mentioned genotype for heat tolerance is recommended. Among 25 bread wheat cultivars and lines 15 genotypes had been evaluated previously for heat tolerance in different field experiments. Except for S-85-19 the results of molecular screening grouped remaining 14 genotypes correctly with the lack or having the mechanism of heat tolerance (due to heat tolerance genes and not escape). These results indicate that selected markers have a sufficient efficiency for screening genotypes to heat stress. The remaining 10 genotypes (all were promising lines) had not been evaluated previously for heat tolerance in any field experiments. Hence a field experiment in a compound analysis for more than 2 years is recommended strongly to validate the results of the molecular screening more precisely. Moreover by using more flanking markers from reliable QTL mapping (high-resolution mapping, validation of markers) studies, the results will be more accurate. Since a reduction of grain weight is more important than kernel number for the yield lose in southern Iran (heat stress comes 10 to 15 days after anthesis and continues until ripeness), therefore selected markers are linked to traits mostly related to grain weight. Accordingly, it would be recommended to use these markers in the regions with the same climate to have an acceptable molecular screening for heat tolerance.

REFERENCES

1. IPCC. Climate Change, 2001: The scientific basis. In: Houghton J., Y. Ding, D. Griggs, M. Noguer, P. van der Linden, X. Dai, K. Maskell and C. Johnson, editors. Contributions of working group I to the third assessment report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press, pp: 1-182.
2. Boyer, J.S., 1982. Plant productivity and environment. Science, 218: 443-448.
3. Georgieva, K., 1999. Some mechanisms of damage and acclimation of the photosynthetic apparatus due to high temperature. Bulg. J. Plant Physiol., 25: 89-100.
4. Hassan, I.A., 2006. Effects of water stress and high temperature on gas exchange and chlorophyll fluorescence in *Triticum aestivum* L. Photosynthetica, 44: 312-315.
5. Hays, D.B., J.H. Do, R.E. Mason, G. Morgan and S.A. Finlayson. 2007. Heat stress induced ethylene production in developing wheat grains induces kernel abortion and increased maturation in a susceptible cultivar. Plant Sci., 172: 1113-1123.
6. Macas, B., C. Gomes and A. S. Dias, 1999. Efeito das temperaturas elevadas durante o enchimento do gra˜o em trigo mole e rijo no Sul de Portugal. Melhoramento, 36: 27-45.
7. Macas, B., M.C. Gomes, A.S. Dias and J. Coutinho, 2000. The tolerance of durum wheat to high temperatures during grain filling. In: C. Royo, M.M. Nachit, N. Di Fonzo and J.L. Araus, eds. Options Me´diterrane´ennes. Durum Wheat Improvement in the Mediterranean.
8. Dias, A.S. and F.C. Lidon, 2009. Evaluation of grain filling rate and duration in bread and durum wheat, under heat stress after anthesis. J. Agron. Crop Sci., 195: 137-147.
9. Gibson, L.R. and G.M. Paulsen, 1999. Yield components of wheat grown under high temperature stress during reproductive growth. Crop Science, 39(6): 1841-1846.
10. Wardlaw, I.F., I.A. Dawson, P. Munibi and R. Fewster, 1989. The tolerance of wheat to high temperatures during reproductive growth. I. Survey procedures and general response patterns. Aust. J. Agr. Res., 40: 1-13.
11. Yang, J., R.G. Sears, B.S. Gill and G.M. Paulsen, 2002. Quantitative and molecular characterization of heat tolerance in hexaploid wheat. Euphytica, 126: 275-282.
12. Lande, R. and R. Thompson, 1990. Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics, 124: 743-756.
13. Zhang, W. and C. Smith, 1992. Computer simulation of marker-assisted selection utilizing linkage disequilibrium. Theor. Appl. Genet., 83: 813-82.
14. Edwards, M.D. and N.J. Page, 1994. Evaluation of marker-assisted selection through computer simulation. Theor. Appl. Genet., 88: 376-382.

15a. Gimelfarb, A. and R. Lande, 1994. Simulation of marker assisted selection for nonadditive traits. Genet. Res., 64: 127-136.

15b. Gimelfarb, A. and R. Lande, 1994. Simulation of marker assisted selection in hybrid populations. Genet. Res., 63: 39-47.

16. Distelfeld, A., C. Uauy, T. Fahima and J. Dubcovsky, 2006. Physical map of the wheat high-grain protein content gene Gpc-B1 and development of a high-throughput molecular marker. New Phytol., 169: 753-763.
17. Sharp, P.J., S. Johnston, G. Brown, R.A. McIntosh, M. Pellota, M. Carter, H.S. Bariana, S. Khatkar, E.S. Lagudah, R.P. Singh, M. Kairallah, R. Potter and M.G.K. Jones, 2001. Validation of molecular markers for wheat breeding. Aust. J. Agric. Res., 52: 1357-1366.
18. Giroux, M.J. and C.F. Morris, 1997. A glycine to serine change in puroindoline b is associated with wheat grain hardness and low levels of starch surface friabilin. Theor. Appl. Genet., 95: 857-864.
19. Juhasz, A., M. Gardonyi, L. Tamas and Z. Bedo, 2003. Characterization of the promoter region of Glu-1Bx7 gene from over expressing lines of an old Hungarian wheat variety. In: Pogna, N.E., M. Romano, E.A. Pogna and G. Galterio (eds) Proceedings of the 10th International Wheat Genet Symposium Pasteum, Italy, Instituto Sperimentale per la Cerealicoltura, Rome, 3: 1348-1350.
20. Mclauchlan, A., F.C. Ogbonnaya, B. Hollingsworth, M. Carter, K.R. Gale, R.J. Henry, T.A. Holten, M.K. Morell, L.R. Rampling, P.J. Sharp, M.R. Shariflou, M.G.K. Jones and R. Appels, 2001. Development of PCR-based DNA markers for each homoeo-allele of granule-bound starch synthase and their application in wheat breeding programs. Aust. J. Agric. Res. 52: 1409-1416.

21. Anderson, J.A., R.W. Stack, S. Liu, B.L. Waldron, A.D. Fjeld, C. Coyne, B. Moreno-Sevilla, J.M. Fetch, Q.J. Song, P.B. Cregan and R.C. Frohberg, 2001. DNA markers for fusarium head blight resistance QTLs in two wheat populations. Theor. Appl. Genet., 102: 1164-1168.
22. Lagudah, E.S., H. McFadden, R.P. Singh, J. Huerta-Espino, H.S. Bariana and W. Spielmeyer, 2006. Molecular genetic characterization of the Lr34/Yr18 slow rusting resistance gene region in wheat. Theor. Appl. Genet., 114: 21-30.
23. Mago, R., H.S. Bariana, I.S. Dundas, W. Spielmeyer, G.L. Lawrence, A.J. Prior and J.G. Ellis, 2005. Development of PCR markers for the selection of wheat stem rust resistance genes Sr24 and Sr26 in diverse wheat germplasm. Theor. Appl. Genet., 111: 496-504.
24. Prins, R., J.Z. Groenewald, G.F. Marais, J.W. Snape and R.M.D. Koebner, 2001. AFLP and STS tagging of Lr19, a gene conferring resistance to leaf rust in wheat. Theor Appl. Genet., 103: 618-624.
25. Stoutjesdijk, P., S.J. Kammholz, S. Kleven, S. Matssy, P.M. Banks and P.J. Larkin, 2001. PCR-based molecular marker for the Bdv2 Thinopyrum intermedium source of barley yellow dwarf virus resistance in wheat. Aust. J. Agric. Res., 52: 1383-1388.
26. Collard, B.C.Y., R.A. Grams, W.D. Bovill, C.D. Percy, R. Jolley, A. Lehmensiek, G. Wildermuth and M.W. Sutherland, 2005. Development of molecular. markers for crown rot resistance in wheat: mapping of QTLs for seedling resistance in a '2-49' x 'Janz' population. Plant Breed., 124: 532-537.
27a. Ogbonnaya, F.C., S. Seah, A. Delibes, J. Jahier, I. Lopez-Brana, R.F. Eastwood and E.S. Lagudah, 2001 Molecular-genetic characterization of a new nematode resistance gene in wheat. Theor Appl. Genet., 102: 623-629.
27b. Ogbonnaya, F.C., N.C. Subrahmanyam, O. Moullet, J. De Majnik, H.A. Eagles, J.S. Brown, R.F. Eastwood, J. Kollmorgen, R. Appels and E.S. Lagudah 2001. Diagnostic DNA markers for cereal cyst nematode resistance in bread wheat. Aust J. Agric. Res., 52: 1367-1374.
28. Jefferies, S.P., M.A. Pallotta, J.G. Paull, A. Karakousis, J.M. Kretschmer, S. Manning, A.K.M.R. Islam, P. Langridge and K.J. Chalmers, 2000. Mapping and validation of chromosome regions conferring boron toxicity tolerance in wheat (Triticum aestevum). Theor Appl. Genet., 101: 767-777.
29. Langridge, P., E.S. Lagudah, T.A. Holton, R. Appels, P.J. Sharp and K.J. Chalmers, 2001. Trends in genetic and genome analysis in wheat: a review. Aust J. Agric. Sci., 52: 1043-1077.
30. Hoisington, D., N. Bohorova, S. Fennell, M. Khairallah, A. Pellegrineschi and J.M. Ribaut, 2002. The application of biotechnology to wheat improvement: new tools to improve wheat productivity. In: B.C. Curtis, S. Rajaram and H. Gomez Macpherson (eds) Bread wheat improvement and production. Plant Production and Protection Series No., 30: 175-198.
31. William, H.M., R. Trethowan and E.M. Crosby-Galvan, 2007. Wheat breeding assisted by markers: CIMMYT's experience, Euphytica, 157: 307-319.
32. Dellaporta, S.L., J. Wood and J.B. Hicks, 1983. A plant DNA minipreparation: version II. Plant Mol. Biol. Rep., 1: 19.
33. Mason, R.E., S. Mondal, F.W. Beecher, A. Pacheco, B. Jampala, A.M.H. Ibrahim and D.B. Hays, 2010. QTL associated with heat susceptibility index in wheat (*Triticum aestivum* L.) under short-term reproductive stage heat stress. Euphytica, 174(3): 423-436.
34. Mohammadi, V., M. Modarresi and P. Byrne, 2008. Detection of QTLs for heat tolerance in Wheat measured by grain filling duration. 11th International Wheat Genetic Symposium Proceedings, Brisbane. Australia, Sydney University Press, 3: 1000-1001.
35. Benbouza, H., J.M. Jacquemin, J.P. Baudoin and G. Mergeai, 2006. Optimization of a reliable, fast, cheap and sensitive silver staining method to detect SSR markers in polyacrylamide gels, Biotechnol. Agron. Soc. Environ., 10(2): 77-81.
36. Creste, S.A., N. Tulmann and A. Figueira, 2001. Detection of single sequence repeat polymorphisms in denaturing polyacrylamide sequencing gels by silver staining, Plant Molecular Biology Reporter, 19: 299-306.
37. Sanguinetti, C.J., E. Dias Neto and A.J.G. Simpson, 1994. Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. Biotechniques, 17: 915-919.
38. Rohlf, F.J., 1998. NTSYS-PC. Numerical taxonomy and multivariate analysis system, version 2.00. Exeter Software, Setauket, NY.
39. Nei, M. and W.H. Li, 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA, 76: 5269-5273.

40. Mantel, M., 1967. The detection of disease clustering and a generalized regression approach. Cancer Res., 27: 209-220.
41. Githiri, S.M., S. Watanabe, K. Harada and R. Takahashi, 2006. QTL analysis of flooding tolerance in soybean at an early vegetative growth stage. Plant Breed, 125: 613-618.
42. Kirigwi, F.M., M. Van Ginkel, G. Brown-Guedira, B.S. Gill, G.M. Paulsen and A.K. Fritz, 2007. Markers associated with a QTL for grain yield in wheat under drought. Mol. Breed., 20: 401-413.
43. Peleg, Z., T. Fahima, T. Krugman, S. Abbo, D. Yakir, A.B. Korol and Y. Saranga, 2009. Genomic dissection of drought resistance in durum wheat 9 wild emmer wheat recombinant inbreed line population. Plant Cell Environ., 32: 758-779.
44. Bhullar, S.S. and C.F. Jenner, 1985. Differential responses to high temperature of starch and nitrogen accumulation in the grain of four cultivars of wheat. Aust. J. Plant Physiol., 12: 363-375.
45. Fokar, M., T. Nguyan and A. Blum, 1998. Heat tolerance in spring wheat. II. Grain filling. Euphytica, 104: 9-15.
46. Naghavi, M.R., M. Mardi, S.M. Pirseyedi, M. Kazemi, P. Potki and M.R. Ghafari, 2007. Comparison of genetic variation among accessions of Agilops taushii using AFLP and SSR markers, Genet Resour. Crop. Evol., 54: 237-240.
47. Akagi, H., Y. Yokozeki, A. Inagaki and T. Fujimurati, 1997. Highly polymorphic microsatellites of rice consist of AT repeats and a classification of closely related cultivars with these microsatellite loci. Theor. Appl. Genet., 94: 61-67.
48. McCouch, S.R., S. Temnykh, A. Lukashova and J. Coburn, 2001. Microsatellite Markers in Rice: Abundance, Diversity and Applications. In: Rice Genetics IV. International Rice Research Institute (IRRI), Manila, pp: 117-135.
49. Ravi, M., S. Geethanjali, F. Sameeyafarheen and M. Maheswaran, 2003. Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR Markers. Euphytica, 133: 243-252.
50. Ram, S.G., V. Thiruvengadam and K.K. Vinod, 2007. Genetic diversity among cultivars, landraces and wild relatives of rice as revealed by microsatellite markers. J. Appl. Genet., 48: 337-345.
51. Fahima, T., M. Roder, A. Grama and E. Nevo, 1998. Microsatellite DNA polymorphysim divergence in Triticum dicoccoides accessions highly resistant to yellow rust. Theor. Appl. Genet., 96: 187-195.
52. Zeb, B., I. Ahmad Khan, S. Ali, S. Bacha, S. Mumtaz and Z.A. Swati, 2009. Study on genetic diversity on Pakistani wheat varieties using simple sequence repeat (SSR) markers. Afr. J. Biotechnol., 8(17): 4016-4019.
53. Salem, K.F.M., A.M. El- Zanaty and R.M. Esmail, 2008. Assesing diversity using morphological characters and microsatellite markers. World J. Agric. Sci., 4(5): 538-544.
54. Moghaieb, R.E.A., A.H.A. Abdel-Hadi and N.B. Talaat, 2011. Molecular markers associated with salt tolerance in Egyptian wheats. Afr. J. Biotechnol., 10(79): 18092-18103.
55. Moshatati, A., K. Alami Saeid, S.A. Siadat, A.M. Bakhshandeh and M. R. Jalal Kameli, 2011. Effect of terminal heat stress on yield and yield components of spring bread wheat in Ahvaz. EJCP., 3(4): 195-203.
56. Esmaeilzadeh Moghaddam, M., 2009. The study of some promising bread wheat lines in elite regional uniform yield trial in southern warm zone of Iran. Seed and plant Improvement Institute, App. No: 4-100-120000-11-8305-86057.
57. Young, N.D., 1999. A cautiously optimistic vision for markerassisted breeding. Mol. Breed., 5: 505-510.