Australian Journal of Crop Science AJCS
AJCS 5(2):104-110 (2011) ISSN:1835-2707

Identification of new microsatellite marker linked to the grain filling rate as indicator for heat tolerance genes in F_2 wheat population

Mohamed Najeb Barakat[1]*, Abdullah Abdlulaziz Al-Doss[1,2], Adel Ahmed Elshafei[1], Khaled Ahmed Moustafa[2]

[1]Plant Genetic Manipulation and Genomic Breeding Group, Center of Excellence in Biotechnology Research, [2]Plant Production Department, College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia

***Corresponding author: mnrbarakat@yahoo.com** (On leave from Biotechnology Lab., Crop Science Department, Faculty of Agriculture, Alexandria University, Egypt)

Abstract

The objective of this study was to estimate inheritance of the grain filling rate as indicator for heat tolerant genes. The minimum number of genes for the trait in bread wheat was also assessed by combining quantitative genetic estimates and SSR marker analyses. Two cultivars, Debra (heat-tolerant) and Yecora Rojo (heat-sensitive) crossed and F_1 and F_2 populations generated. The parents, F_1 and 162 F_2 plants were planted in winter season 2009 to evaluate heat tolerance during the grain-filling period. The sowing date in the present investigation represents the heat stress conditions in Saudi Arabia. The minimum number of genes or factors controlling heat tolerance was estimated (1.5) and the broad sense heritability was estimated as 47.7 %. The results revealed that three SSR markers; *Xgwm132*, *Xgwm577* and *Xgwm617* were linked to grain filling rate (GFR) by quantitative trait loci (QTL) analysis of the F_2 population. The results showed that regression analysis for the relationship between the three markers (*Xgwm132*, *Xgwm577* and *Xgwm617*) and the phenotypes of F_2 plants were highly significant and the coefficients of determination (R^2) were 0.07, 0.25 and 0.03, respectively. This indicates that these three markers were associated with the grain filling rate as indicator for heat tolerant genes. The adjusted R^2 values suggested that the *Xgwm132*, *Xgwm577* and *Xgwm617* accounted for 7%, 25% and 3% of the total phenotypic variation of heat tolerance in the F_2 population, respectively. The results demonstrated that SSR markers, combined with bulked segregant analysis, could be used to identify molecular markers linked to the grain filling rate as indicator for heat tolerance in wheat.

Keywords: Grain filling rate, QTL analysis, SSR marker, Wheat.
Abbreviations: BSA- bulked segregant analysis; GFR - grain filling rate; QTL- quantitative trait loci; SSR – simple sequence repeats.

Introduction

Heat stress due to increased temperature is an agricultural problem in many areas in the world (Wahid et al., 2007). High temperature stress during post-anthesis is a major cause of wheat yield reduction in some regions in Saudi Arabia as well as in many wheat-growing regions of the world. Heat stress during grain filling is commonly occurred in wheat. High temperatures, typically above 34°C, affect final grain weight by reducing the duration of grain filling due to suppression of current photosynthesis (El-Khatib and Paulsen, 1984), and by direct inhibition of starch biosynthesis in the endosperm (Jenner, 1994; Keeling et al., 1993). The grain filling rate (GFR) plays a significant role in the final yield of wheat (Beiquan and Kronstad, 1994). In cereal crops, the final yield depends on carbohydrates derived from two different sources: leaf photosynthetic assimilates during grain filling and accumulated non-structural carbohydrates in culms and leaf sheaths (Takai et al., 2005). For these two sources of carbohydrate, the grain-filling rate and duration are critical and dynamic processes that determine the final grain yield (Takai et al., 2005). The GFR is positively associated with final grain weight (Wiegand and Cuellar, 1981). Direct selection for heat stress tolerance under field conditions is generally difficult because uncontrollable environmental factors adversely affect the precision and repeatability of trials. Often, no consistent high-temperature conditions can be guaranteed in field nurseries, as heat stress may or may not occur in the field. Furthermore, stress tolerance is a developmentally regulated, stage-specific phenomenon; tolerance at one stage of plant development may not be correlated with tolerance at other developmental stages. Consequently, the genetic dissection of the quantitative traits controlling the adaptive response of crops to abiotic stress is a prerequisite to allow cost-effective applications of genomics-based approaches to breeding programs aimed at improving the sustainability and stability of yield under adverse conditions (Collins et al., 2008). Recent genetic studies and efforts to improve heat tolerance of crop

Table 1. Statistical estimates and heritability of grain filling duration, grain yield per plant and grain filling rate of Debra (heat-tolerant) and Yecora Rojo (heat- sensitive) wheat cultivars and their F_1 and F_2 progenies under heat stress

Traits	Genotype	Mean	Range	Standard deviation	h^2 (%)
Grain filling duration					93
(GFD day)	Debra	28.3	24--32	3.12	
	Yecora Rojo	33.5	33--35	1.00	
	F_1	33.8	33--35	1.10	
	F_2	30.4	17--42	4.17	
					33
Grain Yield/plant	Debra	4.42	2.25-10.11	2.90	
(GY/P Gram)	Yecora Rojo	2.20	0.53-7.07	3.25	
	F_1	3.35	1.90-7.07	2.14	
	F_2	2.49	0.12-17.11	2.61	
					48
Grain filling rate	Debra	154	81--326	92	
(GFR mg/day)	Yecora Rojo	63	16--202	92	
	F_1	98	58--202	60	
	F_2	80	3.5--518	83	

plants using traditional protocols have largely determined that plant heat-tolerance is a polygenic trait and different components of tolerance, controlled by different sets of genes, are critical for heat tolerance at different stages of development or in different tissues (Howarth, 2005; Bohnert et al., 2006). Thus, the use of correlation and co-segregation analysis, and molecular marker techniques in genetic stocks with different degrees of heat tolerance are promising approaches to dissect the genetic basis of thermo-tolerance (Maestri et al., 2002). Because of the general complexity of abiotic stress tolerance and the difficulty in phenotypic selection for tolerance, MAS has been considered as an effective approach to improve plant stress tolerance (Foolad, 2005). The use of this approach, however, requires identification of genetic markers that are associated with genes or QTLs affecting whole plant stress tolerance or individual contributing components. Quantitative and molecular characterization of heat tolerance in hexaploid wheat has been reported yet (Yang et al., 2002). Recently, QTL associated with heat susceptibility index in wheat (*Triticum aestivum* L.) under short-term reproductive stage heat stress have been reported (Mason et al., 2010). However, up to now, QTL for heat tolerance at grain filling rate (GFR) in wheat have not been reported, whereas only QTL for GFR under drought stress have been reported (Kirigwi et al., 2007; Wang et al., 2009). The objective of this study is to estimate inheritance of the grain filling rate, as indicator for heat tolerance, and detection of minimum number of genes for the trait in bread wheat by combining quantitative genetic estimates and SSR marker analyses

Materials and methods

Plant materials and population development

The wheat genotypes used in this study were Debra, a soft white wheat obtained from Sudan, and Yecora Rojo, the adapted cultivar in Saudi Arabia. The pedigree of Debra is unknown but it is a heat tolerant. The Yecora Rojo is a cultivar produced in USA and recommended for environment of Saudi Arabia since 1981. Yecora Rojo is a high yield, 2-gene dwarf cultivar but is very sensitive to environmental factors such as temperature, especially during the critical period of grain filling towards the end of the growing season (Gandoura, 1989). Two wheat genotypes that had contrasting response to heat stress were crossed to generate a F_1 seeds during winter season 2008 at the College of Food and Agriculture Sciences, Experimental Research Station, at Dierab near Riyadh (24ºN, 46ºE), King Saud University. F_1 seeds population derived from the cross (Debra × Yecora Rojo) were obtained. The F_2 seeds were obtained by selfing in the summer season in 2008 under green house conditions

Evaluation of heat tolerance

162 F_2 plants and their F_1, as well as parents, were planted on 20th January in winter season 2009 at Experimental Research Station, King Saud University, to evaluate the heat tolerance during the grain-filling period. The sowing date in the present investigation represents the heat stress conditions in Saudi Arabia. The cultural practices were carried out according to the recommended practices followed in Riyadh area. The agronomic traits such as grain filling duration, grain yield per plant and grain filling rate (GFR) were determined. GFR is the rate at which assimilates are transported from the source to the sink. It was estimated as the ratio between grain yield per plant and grain filling duration.

Estimation of number of genes and heritability of heat tolerance

Minimum and maximum values and variances were calculated. An estimate of the minimum number of genes or factors controlling heat tolerance in the two parents was calculated as $n = (P_1–P_2)^2/8(V_{F2}–V_E)$, where P_1 and P_2 were mean GFR of the tolerant parent and the sensitive parent in each population, respectively, V_{F2} was the phenotypic variance of GFR in the F_2 populations, and V_E was the environmental variance. The $V_E = (V_{F1}+Vp_1+V_{P2})$ /3, where V_{F1} was the variance in the F_1 population, and V_{P1} and Vp_2 were variances of P_1 and P_2 population, respectively (Wright, 1968).

Table 2. Characteristics of the five screened microsatellite markers

Marker	Primer sequences	Ann. Tem. (ºC)	Product size (bp)
Xgwm-368-4B	CCA TTT CAC CTA ATG CCT GCAAT AAA ACC AT AGC TCA CTT GC	60	259-271
Xgwm-617-6A	GAT CTT GGC GCT GAG AGA GA CTC CGA TGG ATT ACT CGC AC	60	133
Xgwm-132-6B	TAC CAA ATC GAA ACA CAT CAG G CAT ATC AAG GTC TCC TTC CCC	60	116-118
Xgwm-577-7B	ATG GCA TAA TTT GGT GAA ATT G TGT TTC AAG CCC AAC TTC TAT T	55	155-164
Xgwm-428-7D	AGC GTT CTT GGG AAT TAG AGA CCA ATC AGC CTG CAA CAA C	60	133-137

Table 3. Genetic characteristics of QTL related to the grain filling rate as indicator for heat tolerance genes in the 162 F_2 plants population of Debra X Yecora Rojo

Marker	QTL(cM)	LOD	R^2 (%)
Xgwm132-6A	36.6	6.3	7.0
Xgwm577-6B	6.1	50.4	25.0
Xgwm617-7D	17.1	23.9	3.3

Table 4. ANOVA of grain filling rate (GFR) of F_2 progeny of Debra X Yecora Rojo wheat cultivars on *Xgwm-617, Xgwm-132* and *Xgwm-577* markers

Marker	Source	d.f.	Sum of squares	Mean of squares	F-value	P-value
Xgwm-617-6A	Model	2	40381.9	20190.95	2.99	0.05
	Error	159	1073368.8	6750.75		
	Total	161	1113749.17			
Xgwm-132-6B	Model	1	72471.73	72471.75	11.13	0.0001
	Error	160	1041277.44	6507.98		
	Total	161	1113749.17			
Xgwm-577-7B	Model	1	275707.7	275707.7	52.64	0.0001
	Error	160	838041.4	5237.76		
	Total	161	1113749.17			

Broad- sense heritability for grain filling duration, grain yield per plant and grain filling rate were estimated by Burton method: $h^2 = (V_{F2} – V_{F1}) / V_{F2}$ (Burton,1951).

DNA extraction

Frozen young leaves (500 mg) of 162 F_2 plants and their parents were individually ground to a powder in a mortar with liquid nitrogen. The DNA extraction was done using CTAB method (Sagahi-Maroof et al., 1984).

PCR amplification

A set of 46 microsatellite primers developed by several investigators (Roder et al., 1998) were used in this study. The PCR reaction mixture consisted of 20-50 ng genomic DNA, 1×PCR buffer, 2.0 mM $MgCl_2$, 100 µM of each dNTP, 0.1 µM primer and 1U *Taq* polymerase in a 25 µL volume. The PCR cycle included an initial denaturation at 94 ◦C for 3 min followed by 35 cycles of denaturation at 94 ◦C for 1 min; annealing at 50, 55 or 60 ◦C (depending on the individual microsatellite primer) for 1 min; and extension at 72 ◦C for 2 min followed by a 17-min final extension at 72 ◦C. The amplification products have been electrophoresed in 2-3% agarose gels

Bulked segregant analysis

Bulked – segregant analyses (BSA) was used in conjunction with SSR analysis (Michelmore et al., 1991) to find markers linked to genes of heat tolerance. Tolerant and sensitive bulks were prepared from F_2 individuals by pooling aliquots, containing equivalent amounts of total DNA, approximately, 50

ng /µl from each of ten sensitive and ten tolerant F_2 plants selected, based on phenotypic assessments. SSR primers were,

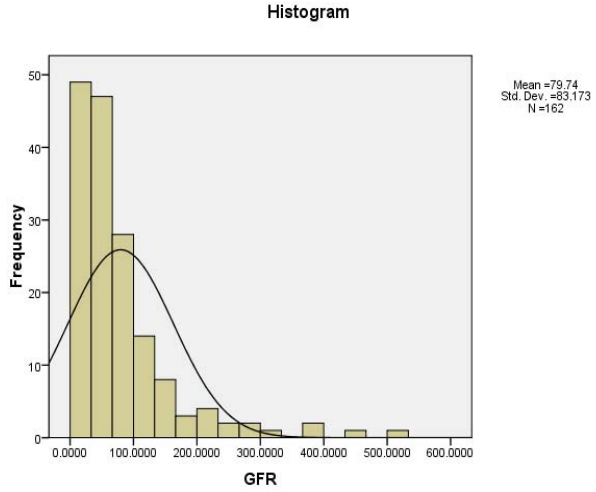

Fig 1. Frequency distribution of grain filling rate in F_2 population under heat stress

then, screened on the parents and the two bulk DNA samples, from which some primer combinations revealed bands that were polymorphic, not only among parental genotypes, but also between the pair of the bulk DNA. Based on the evaluations of DNA bulks, individual F_2 plants were analyzed with co-segregating primers to confirm SSR markers linkage to the grain filling rate as indicator for heat tolerance genes.

Data and Linkage analysis

The association between molecular markers (SSR) and the grain filling rate as indicator for heat tolerance genes was assessed with simple regression analysis, using PROC REG of SAS version 9.1 software packages (SAS Institute, Cary, NC, 2007). Magnitude of the marker associated with phenotypic effect was described by the coefficient of determination (R^2) which represented the fraction of variance explained by the polymorphism of the marker. Single-marker analysis to detect main effect of QTL was performed by the method of Liu (1998). Significant association of a tested marker with a QTL for grain filling rate was detected by single-factor ANOVA. Map manager QTX Version 0.22 (Meer et al., 2001) was used to analyze the linkage relationship of SSR markers detected from bulked segregate analysis. Linkage was detected when a log of the likelihood ratio (LOD) threshold of 3.0 and maximum distance was 50 cM. The Kosambi's mapping function was used.

Results and discussion

Field data analysis

The difference between the mean GFD, GY/P and GFR of the two parents under heat stress were significant ($P<0.05$). The mean of the F_2 lines for the GFD, GY/P and GFR, which were midway between Debra and Yecora Rojo, significantly varied only for the GY/P and GFR from the parent Debra. On the other hand, the mean of the F_2 lines for the GFD, only, was significantly different from the Yecora Rojo. However, the mean GY/P and GFR of the F_2 lines did not differ from the F_1 lines but was high significant for GFD (Table 1). The broad-sense heritability of GFD, GY/P and GFR were 93%, 33% and 48%, respectively, (Table 1). Previously, the broad-sense heritability of 80%, and realized heritability of 96% for GFD were determined from F_2 and F_3 populations (Yang et al., 2002). Similar value of high heritability (89 %) for heat tolerance was determined by Fokar et al. (1998). Their estimates were based on data obtained in controlled environments, which usually underestimate variation under field conditions (Roff, 1997). The minimum number of genes or factors controlling heat tolerance was estimated as 1.5 by the equation of Wright (1968). The distribution of GFR of F_2 lines was somewhat skewed with too many heat sensitive observations (Fig. 1), indicating that the GFR controlled by multigenes, and a locus might have a strong influence on GFR in these population.

SSR markers analysis

Out of 46 SSR markers used in this study, only five primer pairs generated polymorphism between the parents (Table 2). Each of these markers was used to screen DNA bulks of the ten tolerant and the ten sensitive F_2 plants. SSR markers *Xgwm132*, *Xgwm577* and *Xgwm617* were, only, amplified polymorphic bands. The amplification profiles of the three primer pairs were characterized by the ten most tolerant individuals and the ten most sensitive individuals in the F_2 progeny and their parents (Fig. 2). The SSR primers *Xgwm132* and *Xgwm577*, generated one polymorphic fragments at 160bp, and 130bp, respectively, which were present only in the tolerant bulk and Debra (tolerant parent) and were missing in sensitive bulk and Yecora Rojo (sensitive parent). A typical amplification pattern generated by *Xgwm617-6A* was shown in Fig.2. Among the ten most tolerant F_2 lines, three had profiles of Debra, three of Yecora Rojo, and four were heterozygotes. Among the ten most sensitive F_2 lines, two had profiles of Debra, three of Yecora Rojo, and five were heterozygotes (Fig. 2). The *Xgwm617* allele from the tolerant parent was smaller than from the sensitive parent. This locus was inherited in a Mendelian co-dominant manner. There were clear co-segregations between the amplification of the smaller *Xgwm617* allele and the F_2 plants showing the tolerant phenotypes. In the homozygous sensitive F_2 plants, only the large *Xgwm617* allele was amplified. In a proportion of tolerant F_2 plants, both the larger and the smaller alleles were amplified, these plants were presumably heterozygous. The co-dominant microsatellite marker *Xgwm617* was able to identify the heterozygotes, and would serve as an important tool to rapidly transfer the heat tolerance genes into other wheat cultivars. The *Xgwm132*, *Xgwm577* and *Xgwm617* were assigned to chromosomes 6B, 7B and 6A, respectively, following Röder et al. (1998). Homoeologous groups of chromosome 6 of wheat contain a number of genes that are important for tolerance to abiotic stress (Dubcovsky et al., 1995). To check for potential co-segregantion of DNA fragments and heat tolerant phenotypes, simple regression analysis was carried out in order to confirm an association between the *Xgwm132*, *Xgwm577* and *Xgwm617* markers and the grain filling rate as indicator for heat tolerance genes in all 162 F_2 progenies. The results showed that the regression analysis for the relationship between the three markers (*Xgwm132, Xgwm577 and Xgwm617*) and the phenotypes of F_2 individuals were highly significant. The coefficient of determination (R^2) were recorded 0.07, 0.25 and

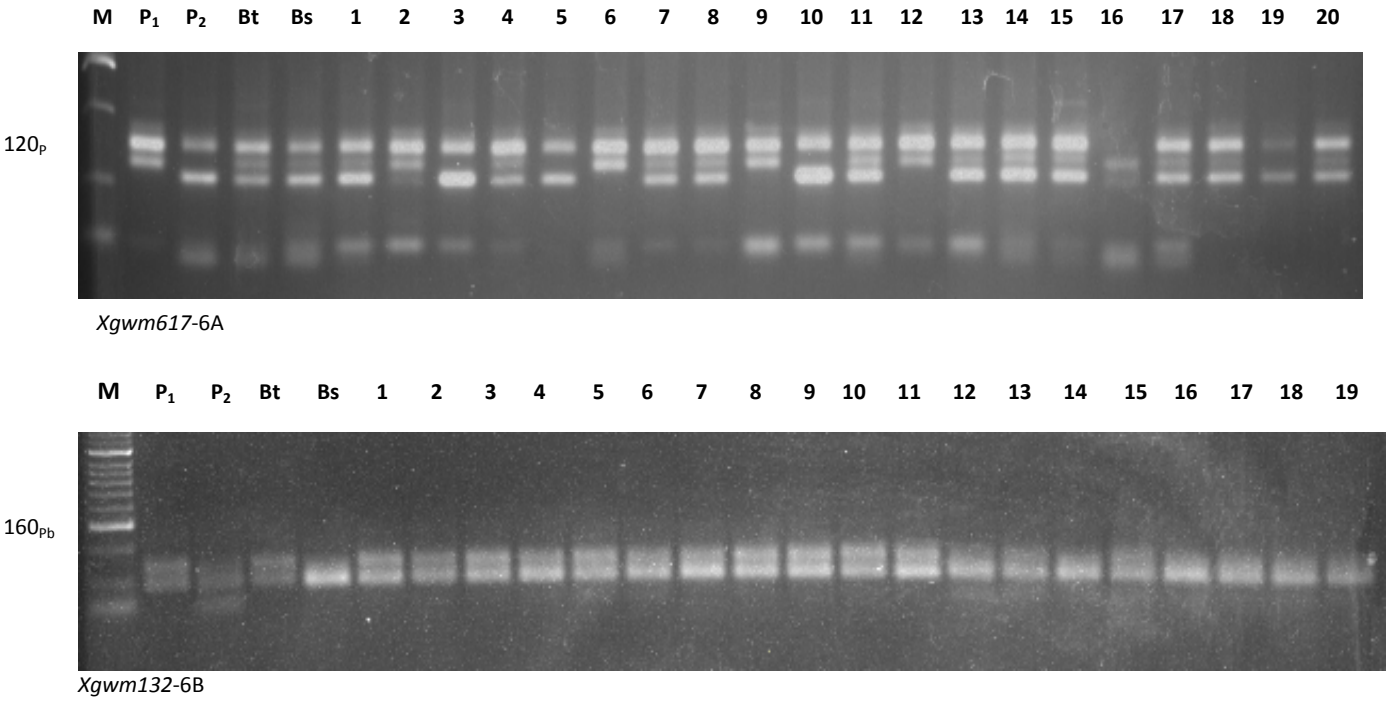

Fig 2. Selective genotyping of F_2 progeny of Debra × Yecora Rojo wheat cultivars with the *Xgwm617*-6A and *Xgwm132*-6B markers for heat tolerance. M: Molecular weight, followed by P_1 and P_2 parents Debra and Yecora Rojo, respectively. Ladders 1–10 are the ten most tolerant F_2 lines, and Ladders 11–20 are the ten most sensitive lines. Arrow points to polymorphic bands of the SSR markers.

0.03, respectively (Table 3). This indicates that the three markers were associated with the grain filling rate as indicator for heat tolerance genes. The adjusted R^2 values suggested that the *Xgwm132*-linked QTL, *Xgwm577*-linked QTL and *Xgwm617*-linked QTL accounted for 7%, 25% and 3% of the total phenotypic variation, respectively, in heat tolerance in the F_2 population.

QTL analysis

The linkage relationship between the SSR markers (*Xgwm132*, *Xgwm577* and *Xgwm617*) and the grain filling rate as indicator for heat tolerance genes were estimated, using F_2 population, derived from the cross, Debra × Yecora Rojo. The genetic distance between SSR markers (*Xgwm132*, *Xgwm577* and *Xgwm617*) and heat tolerance genes were determined as 36.6, 6.1 and 17.1 cM, respectively (with LOD scores of 6.3, 50.4 and 23.9, respectively) (Table 3 and Fig 3). Therefore, SSR markers (*Xgwm132*, *Xgwm577* and *Xgwm617*) were linked to the quantitative trait loci (QTL) for the grain filling rate as indicator for heat tolerance genes. One-way ANOVA was carried out using marker genotypes as groups. The ANOVA on SSR markers (*Xgwm132*, *Xgwm577* and *Xgwm617*) and genotypes as groups for the grain filling rate established high significant association between SSR marker and phenotype (Table 4). The single marker ANOVA analysis revealed that the *Xgwm132*-linked QTL, *Xgwm577*-linked QTL and *Xgwm617*-linked QTL accounted for 7%, 25% and 3% of the total phenotypic variation, respectively, in heat tolerance in the F_2 population. Recently, some markers tightly linked to genes were found by using bulked segregant analysis (BSA) (Xu et al., 1995; Mackay and Caligari, 2000; Zheng et al., 2002; Altinkue et al., 2003; Podlich et al., 2004; Govindaraj et al., 2005, Barakat et al., 2010). BSA was firstly reported by Michelmore et al., (1991) to identify RAPD markers tightly linked to genes for resistance to lettuce downy mildew. Using a method inspired by BSA, we are able to identify three SSR markers (*Xgwm132, Xgwm577* and *Xgwm617*) associated with the grain filling rate in wheat under heat-stressed. These markers should be useful for marker-assisted selection. The present results support the idea that BSA can provide fast detection of molecular markers linked to genes of interest. The quantitative and molecular characterization of heat tolerance in hexaploid wheat have previously been investigated (Yang et al., 2002). They reported that two markers, *Xgwm11* and *Xgwm293*, were linked to the grain filling duration (GFD) by quantitative trait loci (QTL) analysis of the F_2 population. Recently, bulked segregant analysis to detect QTL related to heat tolerance in rice (*Oryza sativa* L.) using SSR markers have been reported (Zhang et al., 2009). The results of SMA revealed that SSR markers, RM3735 on chromosome 4 and RM3586 on chromosome 3 showed significant association with heat tolerance, respectively, accounted for 17 and 3% of the total variation, respectively. In this study, regressions of GFR under heat stress on the three SSR markers were highly significant. The adjusted R^2 values of 0.07, 0.25, and 0.03 suggested that

the *Xgwm132*-linked QTL, *Xgwm577*-linked QTL and *Xgwm617*-linked QTL accounted for 7%, 25% and 3% of the

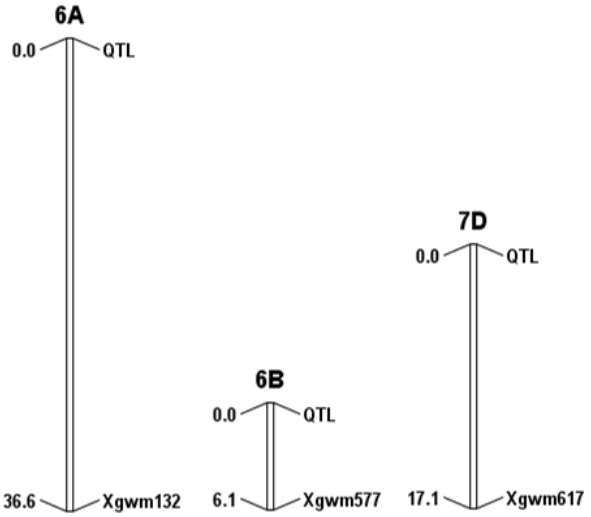

Fig 3. SSR markers (*Xgwm132, Xgwm577* and *Xgwm617*) and QTL for the grain filling rate as indicator for heat tolerance genes were located through the MAPMAKER-QTL analysis. All distances are given in centi-Morgan, using Kosambi's mapping function.

total phenotypic variation, respectively, in heat tolerance in the F_2 population. The present study indicated that SSR markers, combined with bulked segregant analysis, could be used to identify molecular markers linked to the grain filling rate as indicator for heat tolerance genes in wheat and suggested that marker-assisted selection with microsatellite primers might be useful for developing improved cultivars.

Acknowledgements

The investigators acknowledges the Center of Excellence in Biotechnology Research (grant CEBR2 - 08), King Saud University and Ministry of Higher Education, Saudi Arabia for this generous financial support.

References

Altinkut A, Gozukirmizi N (2003) Search for microsatellite markers associated with water-stress tolerance in wheat through bulked segregant analysis. Mol Biot 23:97-106.

Barakat MN, El-Shafei AA, Al-Doss AA (2010) Molecular mapping of QTLs for resistance to northern corn leaf blight in maize. J of Food, Agriculture & Environment 8:547-552.

Beiquan M., Kronstad WE (1994). Duration and rate of grain filling in selected winter wheat population. I. Inheritance. Crop Sci. 34:833-837.

Bohnert HJ, Gong Q, Li P, Ma S (2006) Unraveling abiotic stress tolerance mechanisms—getting genomics going. Curt. Opin. Plant Biol. 9:180–188.

Burton GW (1951) Quantitative inheritance in pearl millet (*Pennistum glaucum*). Agron J 9:409–417.

Collins NC, Tardieu F, Tuberosa R (2008) Quantitative Trait Loci and Crop Performance under Abiotic Stress: Where Do We Stand? Plant Physiol 147:469–486.

Dubcovsky J, Luo MC, Dvorák J (1995) Linkage relationships among stress-induced genes in wheat. Theor Appl Genet 91:795–801.

El-Khatib K, Paulsen GM (1984) Mode of high temperature injury of wheat during grain development. Plant Physiol. 61:363-368.

Fokar M, Nguyen HT, Blum A (1998) Heat tolerance in spring wheat. I. Estimating cellular thermotolerance and its heritability. Euphytica 104:1–8.

Foolad MR (2005) Breeding for abiotic stress tolerances in tomato. In: Ashraf, M, Harris, P.J.C. (Eds.), Abiotic Stresses: Plant Resistance Through Breeding and Molecular Approaches. The Haworth Press Inc., New York, USA, pp. 613–684.

Gandourah MO (1989) Grain yield and its stability of some selected wheat varieties in Saudi Arabia. J King Saud Univ.1:75-85.

Govindaraj P, Arumugachamy S, Maheswaran M (2005) Bulked segregant analysis to detect main effect QTL associated with grain quality parameters in Basmati 370/ASD16 cross in rice (*Oryza sativa* L.) using SSR markers. Euphytica 144:61-68.

Howarth CJ (2005) Genetic improvements of tolerance to high temperature. In: Ashraf, M, Harris, P.J.C. (Eds.), Abiotic Stresses: Plant Resistance Through Breeding and Molecular Approaches. Howarth Press Inc., New York.

Jenner CF (1994) Starch synthesis in kernels of wheat under high temperature conditions. Aust J Plant Physiol 21:791-806.

Keeling PL, Bacon PJ, Holt DC (1993) Elevated temperature reduces starch deposition in wheat endosperm by reducing the activity of soluble starch synthase. Planta 191:342-348.

Kirigwi FM, Ginkel MV, Guedira GB, Gill BS, Paulsen GM, Fritz AK (2007) Markers associated with a QTL for grain yield in wheat under drought. Mol Breed 20:401–413.

Liu BH (1998) Statistical Genomics. CRC Press, Boca Raton, FL. Pp.:387-416.

Mackay IJ, Caligari PDS (2000) Efficiencies in F_2 and backcross generations for bulked segregant analysis using dominant markers. Crop Sci 40:626-630.

Maestri E, Klueva N, Perrotta C, Gulli M, Nguyen HT, Marmiroli N (2002) Molecular genetics of heat tolerance and heat shock proteins in cereals. Plant Mol. Biol. 48:667-681.

Mason RE, Pacheco A, Hays DB, Mondal S, Jampala B, Beecher FW, Ibrahim AM (2010) QTL associated with heat susceptibility index in wheat (*Triticum aestivum L.*) under short-term reproductive stage heat stress. Euphytica. DOI 10.1007/s10681-010-0151-x

Meer J, Robert HC, Kenneth FM (2002) Map manager version 0.22. http://manager.roswellpark.org/mmQTX.html

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease- resistance genes by bulked segregant anaylsis: A rapid method to detect markers in specific genomics regions by using segregating populations. Proc Natl Acad Sci USA. 88:9828-9852.

Podlich DW, Winkler CR, Cooper M (2004) Mapping as you go: An effective approach for marker-assisted selection of complex traits. Crop Sci 44:1560-1571.

Röder MS, Korzun V, Gill BS, Ganal MW (1998) The physical mapping of microsatellite markers in wheat. Genome 41:278-283.

Roff DA (1997) Evolutionary Quantitative Genetics, pp. 1–72. Chapman & Hall, New York, NY.

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci. 81:8014-8018.

SAS (2007) SAS/STAT. Guide for personal computers. Version9 edn.SAS end. SAS institute, Cary. N.C, U. S. A.

Takai T, Fukuta Y, Shiraiwa T (2005) Time-related mapping of quantitative trait loci controlling grain-filling in rice (*Oryza sativa* L.). J Exp Bot 56:2107–2118.

Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: An overview Envoi and Exp Botany 61:199–223.

Wang RX, Hai L, Zhang XY, You GX, Yan CS, Xiao SH (2009) QTL mapping for grain filling rate and yield-related traits in RILs of the Chinese winter wheat population Heshangmai 3 Yu8679. Theor Appl Genet 118:313–325

Wiegand CL, Cuellar JA (1981) Duration of grain filling and kernal weight as affected by temperature. Crop Sci 21:95–101.

Wright S (1968) Evolution and the genetics of populations. Vol. 1, Genetic and Biometric Foundations. Univ. of Chicago, Chicago, IL.

Xu BY, Shen ZT, Chen Y (1995) Molecular mapping for quantitative trait loci controlling rice yield. Acta Genetica Sinica 22:46-52.

Yang J, Sears RG, Gill BS, Paulsen GM (2002) Quantitative and molecular characterization of heat tolerance in hexaploid wheat. Euphytica 126:275–282.

Zhang G, Chen L, Xiaq G, Xiaq Y, Chen X, Zhang S (2009) Bulked segregant analysis to detect QTL related to heat tolerance in rice (*Oryza sativa* L.) using SSR markers. Agricultural Sciences in China 8:482-487.

Zheng XY, Wang YJ, Song SH, Li L, Yu SC (2002) Identification of heat tolerance linked molecular markers of Chinese cabbage *Brassica campestris* ssp. *pekinensis*. Agricultural Sciences in China 1:309-313.