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Full Length Research Paper

Bulked segregant analysis to detect quantitative trait loci (QTL) related to heat tolerance at grain filling rate in wheat using simple sequence repeat (SSR) markers

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The grain-filling rate (GFR) plays an important role in determining grain yield. An F_2 population of wheat was developed from a cross between the 2 wheat cultivars, Ksu106 (heat-tolerant) and Yecora Rojo (heat-sensitive). The parents and 205 F_2 plants were planted on the 20th of January during the winter season of 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. Bulked – segregant analyses (BSA) was used in conjunction with simple sequence repeats (SSR) analysis to find markers linked to genes of heat tolerance. Composite interval mapping was used for mapping quantitative trait loci (QTL). The results reveal that 12 SSR markers: *Wmc24, Wmc168, Wmc326, Xgwm30, Xgwm456, Wmc25*, *Wmc44*, *Wmc94*, *Wmc161*, *Wmc273*, *Wmc327* and *Xgwm566* were linked to GFR by QTLs analysis of the F_2 population. The results show that regression analysis for the relationship between the 12 markers and the phenotypes of F_2 individuals were highly significant. The results demonstrate that SSR markers combined with bulked segregant analysis could be used to identify molecular markers linked to the grain filling rate as an indicator for heat tolerance in wheat.

Key words: Grain filling rate, QTL analysis, SSR marker, wheat.

INTRODUCTION

Due to the global change in climatic conditions that results from increasing atmospheric concentrations of carbon dioxide and other greenhouse gases, high temperature stress have become one of the major factors exerting serious influence on wheat production (Maestri et al., 2002). The heat stress especially occurring during flowering stage in wheat growth area results in a reduction in both individual kernel weight and kernel number (Plaut et al., 2004; Hays et al., 2007). So the research on the genetic mechanism of heat tolerance is getting more and more important for the utilization of heat tolerant genes and the development of new wheat varieties with heat tolerance. Grain filling is a crucial and dynamic process of wheat growth. Its duration and rate determine the individual grain size, grain weight and as a result, the economic yield of the crop (Li and Pan, 2005). Grain filling duration is much influenced by temperature, particularly in the presence of stress (Wiegand and Cuellar, 1981; Knott and Gebeyehou, 1987), while grain-filling rate (GFR) appears to be largely under genetic control (Van Sanford, 1985).

Moreover, since wheat is harvested at the beginning of

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Abbreviations: BSA, Bulked segregant analysis; **CIM,** composite interval mapping; **GFR,** grain filling rate; **QTL,** quantitative trait loci; **SSR,** simple sequence repeats.

summer and some other crops are planted in succession in wheat growth area, the selection of genotypes with high GFR appears to be a successful strategy for increasing overall grain yield. The physiological mechanisms by which crops regulate GFR have been widely explored (Egli et al., 1989), but little is known until now about the genetics and loci affecting this complex trait. Availability of molecular maps helps to dissect complex quantitative traits into component loci and study their relative effects on a specific trait in a segregating population by quantitative trait loci (QTL) analysis (Roder et al., 1998). In recent years, with the development of molecular marker technique and widespread application, there were reports on genetic research of heat tolerance in wheat by utilizing molecular marker (Yang et al., 2002; Mason et al., 2010). Previous research on the genetic mechanism of heat tolerance in wheat was focused on the grain failing duration (Yang et al., 2002) and the heat susceptibility index (Mason et al., 2010) under high temperature as a measure of heat tolerance.

Also, QTLs for GFR under drought stress have been reported (Kirigwi et al., 2007; Wang et al., 2009). Recently, identification of new microsatellite markers linked to the grain filling rate as an indicator for heat tolerance genes in an F_2 wheat population have been reported (Barakat et al., 2011). However, in the present investigation, composite interval mapping (CIM) has been used for mapping QTLs which is considered statistically more powerful compared to single-point analysis (Lander and Botstein, 1989; Liu, 1998). In the present study, we present the mapping of 12 QTLs associated with heat tolerance using pooled bulks from the F_2 population of Ksu106 (heat tolerant) / Yecora Rojo (heat-sensitive) cross. This strategy is expected to contribute towards a deeper understanding of the genetic mechanism of wheat heat tolerance as well as the cloning of heat tolerance QTLs and marker assisted selection breeding.

MATERIALS AND METHODS

Population development

The wheat genotypes used in this study were Ksu106; advanced lines (F_8) selected from the wheat breeding program at the Plant Production Department, College of Food and Agricultural Sciences, King Saud University, Saudi Arabia, and Yecora Rojo; the adapted cultivar in Saudi Arabia. The pedigree of Ksu106 is Barouk /R1474-75-3-53-3-3 and it is heat tolerant (Al-Doss et al., 2009). The Yecora Rojo is a USA cultivar and the elite recommended cultivar adapted to Saudi Arabia environment since 1981. Yecora Rojo is a 2-gene dwarf high yielding cultivar but is very sensitive to any change in the environmental factors such as temperature, especially during the critical period of grain filling towards the end of the growing season. The increase in temperature during this period causes the plant to speed up its life cycle and shorten the time for grain filling and, thus, the kernel weight is reduced and consequently so is grain yield (Gandourah, 1989). Two wheat genotypes that had contrasting response to heat stress were crossed to generate F_1 seeds during the winter season 2008 at the college of food and agricultural sciences, experimental research station, at Dierab near Riyadh, King Saud University. F_1 seeds population derived from the cross (Ksu106 × 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

205 F_2 plants and the parents were planted on 20th January in the winter season 2009 at the 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 region. The agronomic traits such as grain filling duration, grain yield per plant and 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.

DNA extraction

Frozen young leaves (500 mg) of 205 F_2 plants and their parents were individually ground to a powder in a mortar with liquid nitrogen. The DNA extraction was done using the CTAB method (Sagahi-Maroof et al., 1984).

PCR amplification

A set of 100 microsatellite primers developed by several investigators (Roder et al., 1998; Gupta et al., 2002) were used in this study. The PCR reaction mixture consisted of 20 to 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 to 3% agarose gels.

Bulked segregant analysis

Bulked – segregant analyses (BSA) was used in conjunction with simple sequence repeats (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, 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 an indicator for heat tolerance genes.

Data and linkage analysis

The computer program Map Manager QTX Version 0.22 (Meer et al., 2002) was used to perform composite interval mapping (CIM)

(Zeng, 1994) to evaluate marker intervals putatively associated with trait phenotypes. 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. Genetic loci with the most significant effect for each QTL were assembled into multiple regression models, using PROC REG of SAS version 9.1 software packages (SAS Institute, Cary, NC 2007), to determine the total amount of the phenotypic variation explained (Nelson, 1997).

RESULTS

SSR markers analysis

Out of the 100 SSR markers used in this study, only 25 primer pairs generated polymorphisms between the parents. Each of these markers was used to screen DNA bulks of the 10 tolerant and the 10 sensitive F_2 plants. Twenty SSR markers (*Wmc24, Wmc25, Wmc44, Wmc47, Wmc48, Wmc52, Wmc94, Wmc149, Wmc154, Wmc161, Wmc168, Wmc215, Wmc219, Wmc261, Wmc273, Wmc326, Wmc327, Xgwm30, Xgwm456*, and *Xgwm56*) only amplified polymorphic bands (Table 1). The amplification profiles of the twenty primer pairs were characterized by the F_2 progeny and their parents. The SSR primers *Wmc24*, *Wmc47*, *Wmc48*, *Wmc52*, *Wmc149*, *Wmc154*, *Wmc168*, *Wmc219*, *Wmc261*, *Wmc326*, *Xgwm30* and Xgwm456 generated one polymorphic fragment at 152, 160, 130, 170, 230, 180, 310, 180, 120, 225, 160 and 140 bp, respectively which was present only in the tolerant bulk and Ksu106 (tolerant parent) and were missing in the sensitive bulk and Yecora Rojo (sensitive parent) (Figure 1).

The SSR primers *Wmc25*, *Wmc44*, *Wmc94*, *Wmc161*, *Wmc215*, *Wmc273*, *Wmc327*, and *Xgwm566* generated one polymorphic fragment at 200, 260, 120, 200, 207, 180, 200 and 140 bp, respectively, which was present only in the tolerant bulk and Ksu106 (tolerant parent) and another polymorphic fragments at 220, 290, 105, 190, 195, 170, 180, and 150 bp, respectively which were present only in the sensitive bulk and Yecora Rojo (sensitive parent) (Figure 1). These co-dominant microsatellite markers were able to identify the heterozygotes plants. A typical amplification pattern generated by *Wmc25* and *Wmc327* is shown in Figure 1. The *Wmc25* of the tolerant parent was smaller than the sensitive parent. This locus was inherited in a Mendelian co-dominant manner. There was clear co-segregation between the amplification of the smaller *Wmc25* and the F_2 plants showing tolerant phenotypes. In the homozygous sensitive F_2 plants, only the large *Wmc25* was amplified.

In a proportion of tolerant F_2 plants, both the larger and the smaller were amplified; these plants were presumably heterozygous. In contrast, the *Wmc327* of the tolerant parent was larger than the sensitive parent. This locus was also inherited in a Mendelian co-dominant manner. Again there was clear co-segregation between the amplification of the larger *Wmc327* and the F_2 plants showing tolerant phenotypes. In the homozygous tolerant F_2 plants, only the large *Wmc327* was amplified. In a proportion of tolerant F_2 plants, both the larger and the smaller alleles were amplified, so these plants were presumably heterozygous. The co-dominant microsatellite markers *Wmc25*, *Wmc44*, *Wmc94*, *Wmc161*, *Wmc215*, *Wmc273*, *Wmc327*, and *Xgwm566* were able to identify the heterozygotes, and would serve as an important tool to rapidly transfer the heat tolerance genes into other wheat cultivars.

To check for potential co-segregantion of DNA fragments and heat tolerant phenotypes, multiple regression analysis was carried out in order to confirm an association between the twenty SSR markers and the grain filling rate as an indicator for heat tolerance genes in all 205 F_2 progenies. The results show that the regression analysis for the relationship between the dominant markers (*Wmc47*, *Wmc48*, *Wmc52*, *Wmc149*, *Wmc154*, *Wmc219* and *Wmc261***)** and the phenotypes of F_2 individuals were non-significant. However, the dominant markers *Wmc24, Wmc168, Wmc326, Xgwm30* and *Xgwm456* were significantly ($P < 0.05$) associated with the grain filling rate and explained 31, 27, 22, 29, and 33% of the variation. Also, the co-dominant microsatellite markers *Wmc25*, *Wmc44*, *Wmc94*, *Wmc161*, *Wmc273*, *Wmc327*, and *Xgwm566* were significantly ($P < 0.05$) associated with the grain filling rate and explained 45, 30, 39, 43, 48, 32 and 64% of the variation, respectively (Table 2). This indicates that the 12 markers were associated with the grain filling rate as an indicator for heat tolerance genes. The R^2 values suggested that *Wmc24*-linked QTL**,** *Wmc168*-linked QTL**,** *Wmc326*-linked QTL**,** *Xgwm30*-linked QTL**,** *Xgwm456*-linked QTL**,** *Wmc25*-linked QTL**,** *Wmc44*-linked QTL**,** *Wmc94*-linked QTL**,** *Wmc161*-linked QTL**,** *Wmc273*-linked QTL, *Wmc327*-linked QTL, and *Xgwm566*-linked QTL account 0.31, 0.27, 0.22, 0.29, 0.33, 0.45, 0.30, 0.39, 0.43, 0.48, 0.32 and 0.64 of the total phenotypic variation, respectively, in heat tolerance in the F_2 population.

QTL analysis

The linkage relationship between the 12 SSR markers (*Wmc24, Wmc168, Wmc326, Xgwm30, Xgwm456, Wmc25*, *Wmc44*, *Wmc94*, *Wmc161*, *Wmc273*, *Wmc327*, and *Xgwm566*) and the grain filling rate as an indicator for heat tolerance genes were estimated using the F_2 population, deriving from the cross, Ksu106 × Yecora Rojo. The genetic distance between the 12 SSR markers and heat tolerance genes were determined as 9.9, 9.0, 9.0, 7.6, 8.9, 7.8, 9.7, 8.4, 7.3, 7.0, 8.6, and 5.7cM, respectively, with LOD scores of (29.8, 31.7, 31.2, 34.0, 29.9, 56.6, 52.2, 56.0, 59.4, 60.5, 53.5, and 66.4, respectively) (Table 2). Therefore, these SSR markers

Table 1. Characteristics of the twenty SSR markers generated polymorphism between the parents.

Marker	Primer sequences	Annealing temperature (°C)	Product size (bp)
Wmc24-2A	GTGAGCAATTTTGATTATACTG TACCCTGATGCTGTAATATGTG	51	152
Wmc25-2AS,2BS,2DS	TCTGGCCAGGATCAATATTACT TAAGATACATAGATCCAACACC	51	166
Wmc44-1B	GGTCTTCTGGGCTTTGATCCTG TGTTGCTAGGGACCCGTAGTGG	61	232 to242
Wmc47-4B,5A	GAAACAGGGTTAACCATGCCAA ATGGTGCTGCCAACAACATACA7A	61	127 to141
Wmc48-4A,4B,4D	GAGGGTTCTGAAATGTTTTGCC ACGTGCTAGGGAGGTATCTTGC	61	139 to 190
Wmc52-1B,4D	TCCAATCAATCAGGGAGGAGT AGAACGCATCAAGGCATGAAGTA	61	192
Wmc94-7D	TTCTAAAATGTTTGAAACGCTC GCATTTCGATATGTTGAAGTAA	51	107
Wmc149-3B,2A,2B,5B	ACAGACTTGGTTGGTGCCGAGC ATGGGCGGGGGTGTAGAGTTTG	61	180 to230
Wmc154-2B	ATGCTCGTCAGTGTCATGTTTG AAACGGAACCTACCTCACTCTT	61	147 to 149
Wmc161-4A,5D	ACCTTCTTTGGGATGGAAGTAA GTACTGAACCACTTGTAACGCA	61	200
Wmc168-7A	AACACAAAAGATCCAACGACAC CAGTATAGAAGGATTTTGAGAG	51	319
Wmc215-5D,3A,5A	CATGCATGGTTGCAAGCAAAAG CATCCCGGTGCAACATCTGAAA	61	207 to 211
Wmc219-4A	TGCTAGTTTGTCATCCGGGCGA CAATCCCGTTCTACAAGTTCCA	61	204
Wmc261-2A,1D,2B,3B,7B	GATGTGCATGTGAATCTCAAAAGT AAAAGAGGGTCACAGAATAACCTAAA	61	110
Wmc273-6A,7B,7D	AGTTATGTATTCTCTCGAGCCTG GGTAACCACTAGAGTATGTCCTT	51	182-235`
Wmc326-3B,3A,5B	GGAGCATCGCAGGACAGA GGACGAGGACGCCTGAAT	61	186
Wmc327-5A	TGCGGTACAGGCAAGGCT TAGAACGCCCTCGTCGGA	61	183
Xgwm30-2D	ATCTTAGCATAGAAGGGAGTGGG TTCTGCACCCTGGGTGAT	60	156
Xgwm456-1D	TCTGAACATTACACAACCCTGA TGCTCTCTCTGAACCTGAAGC	55	138-165
Xgwm566-3B	TCTGTCTACCCATGGGATTTG CTGGCTTCGAGGTAAGCAAC	60	122-131

were linked to the quantitative trait loci (QTL) for the grain filling rate as an indicator for heat tolerance genes. All of the QTLs had a positive additive effect indicating contribution of alleles increasing GFR by the tolerant

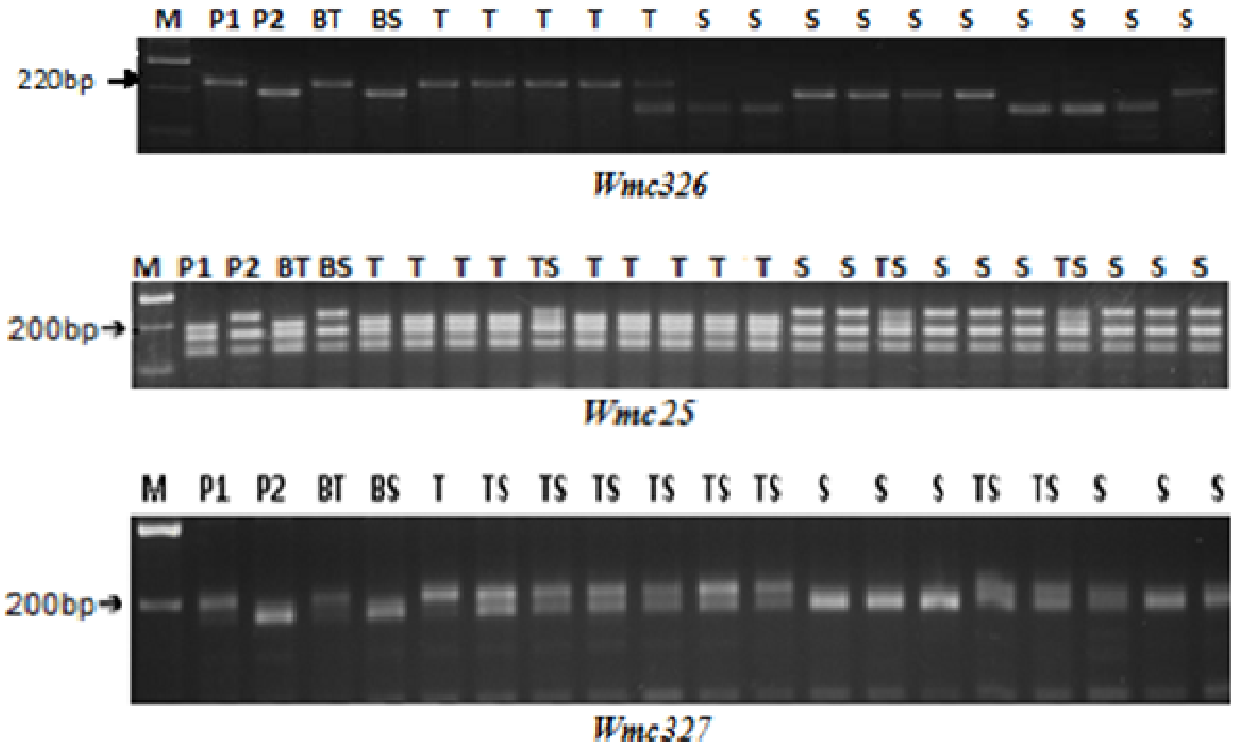

Figure 1. Selective genotyping of F_2 progeny of Ksu 106 X Yecora Rojo wheat cultivars with the *Wmc326-3B, 34A, 5B*; *Wmc25-2AS, 2BS,2DS* and *Wmc327-5A* markers for heat tolerance. Lane M: Molecular weight followed by P_1 and P_2 parents Ksu106 and Yecora Rojo, respectively. T=F_2 Tolerant plant; S=F_2 Sensitive plant; TS = F_2 Heterozygous plant. Arrow points to polymorphic bands of the SSR markers.

Table 2. Genetic characteristics of QTL related to the grain filling rate as indicator for heat tolerance genes in the 205 F_2 plants population of Ksu106 X Yecora Rojo.

Marker	Chromosome arm	Type of marker	QTL (cm)	LOD	R^2 (%)	P value	Additive effect
Wmc24	1AS	Dominant	9.9	29.8	31	0.05	122.48
Wmc25	2AS,2BS,2DS	Co-dominant	7.8	56.6	45	0.01	135.89
Wmc44	*1B*L	Co-dominant	9.7	52.2	30.00	0.05	103.99
Wmc47	*4B,5A*	Dominant	10.9	29.4	15.00	0.21	52.64
Wmc48	*4A,4BS,4D*S	Dominant	44.9	2	-	0.16	-
Wmc52	*1B,4DS*	Dominant	32.9	6.9	8.00	0.29	68.71
Wmc94	7DL	Co-dominant	8.4	56	39	0.05	121.61
Wmc149	*3B,2A,2B,5B*S	Dominant	20.7	14.5	8.00	0.22	77.98
Wmc154	*2Bs*	Dominant	26.8	10.1	8.00	0.55	22.9
Wmc161	4A,5DL	Co-dominant	7.3	59.4	43	0.05	131.06
Wmc168	7AS	Dominant	9.0	31.7	27	0.05	87.76
Wmc215	*5D,3A,5A*L	Co-dominant	16.4	35.4	28.00	0.37	99.24
Wmc219	*4A*L	Dominant	16.3	20.8	16.00	0.38	23.85
Wmc261	*2AL,1D,2B,3B,7B*	Dominant	17.2	19.8	17.00	0.70	23.39
Wmc273	*7AL,7B,7D*	Co-dominant	7.0	60.5	48.00	0.01	148.23
Wmc326	3B,3A,5B	Dominant	9.0	31.2	22	0.05	141.2
Wmc327	5AL	Co-dominant	8.6	53.5	32	0.05	112.95
Xgwm30	2D	Dominant	7.6	34.0	29	0.01	117.54
Xgwm456	1D	Dominant	8.9	29.9	33	0.05	61.11
Xgwm566	3B	Co-dominant	5.7	66.4	64	0.05	158.6

parent 'Ksu106' (Table 2). Positive additive effect of the QTL on chromosomes 1A, 2A, 3A, 4A, 5A, 7A, 1B, 2B, 3B, 5B, 7B, 1D, 2D, 5D and 7D indicates contribution of QTL alleles in these loci from the tolerant parent, 'Ksu106'. In all of the detected QTLs, additive effects were positive. This indicates the relative importance of additive gene effects in controlling GFR as an indicator for heat tolerance in 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 an indicator for heat tolerance genes in wheat and suggested that marker-assisted selection with microsatellite primers might be useful for developing improved cultivars.

DISCUSSION

Breeding for heat tolerance is still in its infancy and warrants more attention than it has been given in the past. It is unfortunate that the literature contains relatively little information on breeding for heat tolerance in different crop species. However, despite all the complexity of heat tolerance and difficulties encountered during transfer of tolerance, some heat-tolerant inbred lines and hybrid cultivars with commercial acceptability have been developed and released, at least in a few crop species such as tomato (Scott et al., 1986, 1995). Composite interval mapping (CIM) has been used in the present investigation for mapping QTLs associated with heat tolerance using pooled bulks from the F_2 population of Ksu106 (heat tolerant)/Yecora Rojo (heat-sensitive) cross. This method combines interval mapping with linear regression and includes additional genetic markers in the statistical model in addition to an adjacent pair of linked markers for interval mapping (Jansen, 1993; Jansen and Stam, 1994; Zeng, 1994). The main advantage of CIM is that it is more precise and effective at mapping QTLs compared to single-point analysis and interval mapping.

Microsatellites are highly popular genetic markers because of their co-dominant inheritance, high abundance, enormous extent of allelic diversity, and the ease of assessing SSR size variation by PCR with pairs of flanking primers. The reproducibility of microsatellites is such that they can be used efficiently by different research laboratories to produce consistent data (Saghai-Maroof et al., 1984). In the present study, the markers *Wmc24, Wmc168, Wmc326, Xgwm30, Xgwm456, Wmc25*, *Wmc44*, *Wmc94*, *Wmc161*, *Wmc273*, *Wmc327*, and *Xgwm566* were assigned to chromosomes 1A, 7A, (3B, 3A, 5B), 2D, 1D, (2A, 2B, 2D), 1B, 7D, (4A, 5D),(7A, 7B, 7D), 5A, and 3B, respectively following several investigators (Roder et al., 1998; Somers et al., 2004; Golabadi et al., 2011). Homoeologous groups of chromosomes 2, 3, 5 and 7 of wheat contain a number of genes that are important for tolerance to abiotic stress (Dubcovsky et al., 1995; Somers et al., 2004; Golabadi et al., 2011).

Some markers tightly linked to genes were found by using bulked segregant analysis (BSA) (Xu et al., 1995; Mackay and Caligari, 2000; Altinkuet and Gozukirmizi, 2003; Podlich et al., 2004; Govindaraj et al., 2005; Zhang et al., 2009; Barakat et al., 2010; Milad et al., 2011). BSA was firstly reported by Michelmore et al. (1991) to identify random amplified polymorphic DNA (RAPD) markers tightly linked to genes for resistance to lettuce downy mildew. Using a method inspired by BSA, we were able to identify the aforementioned 12 SSR markers associated with the grain filling rate in wheat under heat-stress. 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 has 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 an F_2 population. Recently, a bulked segregant analysis to detect QTLs related to heat tolerance in rice (*Oryza sativa* L.) using SSR markers has been reported (Zhang et al., 2009). In this study, multiple regressions of GFR under heat stress on the 5 dominant SSR markers and 7 co-dominant SSR markers were significant. The R^2 values suggested that *Wmc24*-linked QTL**,** *Wmc168*-linked QTL**,** *Wmc326*-linked QTL**,** *Xgwm30*-linked QTL**,** *Xgwm456*-linked QTL**,** *Wmc25*-linked QTL**,** *Wmc44*-linked QTL**,** *Wmc94*-linked QTL**,** *Wmc161*-linked QTL**,** *Wmc273*-linked QTL**,** *Wmc327*-linked QTL**,** and *Xgwm566*-linked QTL account 31, 27, 22, 29, 33, 45, 30, 39, 43, 48, 32, and 64% of the total phenotypic variation, respectively, in heat tolerance in the F_2 population.

Conclusion

The aforementioned results, although preliminary, because of the low number of SSR primers and SSR markers associated with the grain filling rate in wheat under heat-stress, are nevertheless encouraging. Moreover, it is suggested that using more SSR primers to construct a linkage map from $F_{4.5}$ families in future studies in our breeding program will help to detect more closely linked markers associated with the grain filling rate in wheat under heat-stress.

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