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Quantitative and molecular characterization of heat tolerance in hexaploid wheat

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Summary

Understanding the genetic basis of tolerance to high temperature is important for improving the productivity of wheat (*Triticum aestivum* L.) in regions where the stress occurs. The objective of this study was to estimate inheritance of heat tolerance and the minimum number of genes for the trait in bread wheat by combining quantitative genetic estimates and molecular marker analyses. Two cultivars, Ventnor (heat-tolerant) and Karl 92 (heat-susceptible), were crossed to produce F_1, F_2, and F_3 populations, and their grain-filling duration (GFD) at 30/25 °C 16/8 h day/night was determined as a measure of heat tolerance. Distribution of GFD in the F_1 and F_2 populations followed the normal model (χ^2, $p > 0.10$). A minimum of 1.4 genes with both additive and dominance effects, broad-sense heritibility of 80%, and realized heritability of 96% for GFD were determined from F_2 and F_3 populations. Products from 59 primer pairs among 232 simple sequence repeat (SSR) pairs were polymorphic between the parents. Two markers, *Xgwm11* and *Xgwm293*, were linked to GFD by quantitative trait loci (QTL) analysis of the F_2 population. The *Xgwm11*-linked QTL had only additive gene action and contributed 11% to the total phenotypic variation in GFD in the F_2 population, whereas the *Xgwm293*-linked QTL had both additive and dominance action and contributed 12% to the total variation in GFD. The results demonstrated that heat tolerance of common wheat is controlled by multiple genes and suggested that marker-assisted selection with microsatellite primers might be useful for developing improved cultivars.

Abbreviations: GFD – grain filling duration; GLM – general linear model; QTL – quantitative trait loci; PCR – polymerase chain reaction; SSR – simple sequence repeats

Introduction

Terminal or post-anthesis heat stress frequently limits production of wheat (*Triticum asetivum* L.) in many regions (Paulsen, 1994; Reynolds et al., 1994; Wardlaw & Wrigley, 1994). Investigations of the genetic nature of heat tolerance suggested that development of improved cultivars was a practicable solution to the problem (Wardlaw et al., 1989a, 1989b; Moffatt et al., 1990; Saadalla et al., 1990; Ferara et al., 1994; Porter et al., 1995; Fokar et al., 1998; Sun & Xu, 1998). However, improving the trait is complicated by uncertainly regarding its heritability and the number of genes involved.

Most studies found that cytoplasmic effects and nuclear-cytoplasm interactions were involved in heat tolerance, but conclusions about other genetic characteristics varied. Screening F_1 and later generations from crosses between genotypes that differ in heat tolerance showed significant heterosis for the trait (Moffatt et al., 1990; Porter et al., 1995; Fokar et al., 1998; Sun & Xu, 1998). However, Saadalla et al. (1990) observed that F_1 progeny were skewed toward susceptible parents in two crosses. The mean reduction in kernel weight in F_1 progeny from high temperature was intermediate between extreme parents in one cross and close to the susceptible parent in another cross (Wardlaw et al., 1989a). Moffatt et al. (1990) detected

significant genetic additive effects but no dominance effects on heat tolerance in a complete diallel of six cultivars, but Sun & Xu (1998) found both additive and dominance effects in two sets of complete diallels. Several authors concluded that heat tolerance was not simply inherited and more investigation was needed to understand its basis in wheat (Paulsen, 1994, Wardlaw & Wright, 1994; Fokar et al., 1998; Xu & Sun, 1998).

Quantitative trait loci analysis is a powerful tool for qualitative and quantitative genetic analysis of complex traits (Roff, 1997; Liu, 1998; Shah et al., 1999). Simple sequence repeats, also called microsatellites, were interspersed ubiquitously in the DNA of hexaploid wheat (Röder et al., 1998). The PCR-based, sequence-tagged site markers detected a higher level of genetic variation than RFLP and RAPD (Penner et al., 1995; Plaschke et al., 1995; Röder et al., 1995; Korzun et al., 1999) and were useful for genetic analysis of species such as hexaploid wheat that exhibit a narrow genetic base due to their recent origin (Röder et al., 1998; Stephenson et al., 1998). The chromosome-specific feature of the SSR markers was also valuable for localizing linked alleles and detecting the QTL of interest. Wheat microsatellites were used recently for mapping genes in hexaploid wheat (Korzun et al., 1997a,), characterizing the identity of genetic stocks (Korzun et al., 1997b), studying the genetic diversity of hexaploid wheat and related species (Plaschke et al., 1995; Fahima et al., 1998), and identifying QTL that control grain protein content (Prasad et al., 1999) and preharvest sprouting (Roy et al., 1999).

The objective of their study was to estimate the inheritance and minimum number of genes for heat tolerance in bread wheat using a cross between genotypes that were identified previously. The same parents and progeny were utilized to identify SSR markers that are linked with QTL of heat tolerance and to determine the contribution of the mapped QTL to variation in heat tolerance in an F_2 population. The GFD was used as a measure of heat tolerance because the two traits were highly correlated (Reynolds et al., 1994; Stone & Nicolar, 1995), and the GFD was more uniform than the diverse spike forms in the segregating populations. Tolerance of Ventnor, the superior parent, was attributed primarily to its long periods of photosynthesis and grain-filling under stress (Al-Khatib & Paulsen, 1990; Yang et al., 2001).

Materials and methods

Parents and development of F_1, F_2, and F_3 progeny

The parental cultivars were Ventnor (pedigree unknown) (heat-tolerant) and Karl 92 (Plainsman V/3/Kaw/Atlar 50//Parkes*5/Agent) (heat-susceptible) (Al-Khatib & Paulsen, 1990). Five plants of Ventnor were fertilized with the pooled pollen from five plants of Karl 92 in the greenhouse, and the F_1 plants were increased to F_2 and F_3 populations by selfing under the same conditions. Fifty plants of each parent, 50 F_1 plants, and 166 F_2 plants were evaluated for heat tolerance as described below. Nine F_3 seeds were sampled randomly from each of the 10 most tolerant and 10 most susceptible plants in the F_2 population for estimating the heritability of heat tolerance. All 166 F_2 lines were used for QTL analysis.

Plant culture and evaluation of heat tolerance

Seeds of Ventnor (P_1), Karl 92 (P_2), and their F_1, F_2, and F_3 progeny were germinated on moistened filter paper in Petri dishes at 25 °C until the seedling radicals appeared. The seedlings were vernalized at 5 °C for 6 weeks and transplanted into a mixture of soil: sand: peat moss (1:1:1, v:v:v) in 12 × 15-cm pots. The pots, each holding one seedling, were placed randomly in controlled environment chambers (PGW-36, Conviron, Pembina, ND, USA) set at 20/15 °C day/night, 50/70% relative humidity, 16-h photoperiod, and illumination of 420 μmol $m^{-2}s^{-1}$ at the top of the plants. Fertilizer (Peters Professional Plant Food, W.R. Grace & Co., Fogelsville, PA, USA) to supply 100 mg N, 43 mg P, and 87 mg K was applied to each pot at transplanting, jointing, and early anthesis stages of the wheat.

The first three inflorescences that flowered on each plant were labeled when anthers appeared from the florets. At 10 days after anthesis of the second spike, the plants were moved to PGW-36 controlled environment chambers set at 30/25 °C and 16/8 h day/night, with all other conditions the same as before. The plants were watered daily from saucers under the pots to prevent moisture deficiency and were re-randomized weekly to eliminate spatial effects.

Physiological maturity of each spike was noted when the glumes on the center spikes became chlorotic, i.e., lost ca. 90% of their chlorophyll. The GFD of each plant was calculated as the interval between 10 days after anthesis of the second spike and the date of physiological maturity.

Estimation of number of genes and heritability of heat tolerance

Each population was evaluated for fit of its GFD frequency to a normal distribution with one-day intervals by chi-square (χ^2) analysis. The GFD of the P_1, P_2, F_1, and F_2 lines was subjected to an F-test by the GLM procedure (SAS, 1995). Minimum and maximum values and variances were calculated, and generation means were compared by LSD at $\alpha = 0.05$.

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 GFD of Ventnor and Karl 92 plants, V_{F2} was the phenotypic variance of GFD in the F_2 populations, and V_E was the environmental variance. The $V_E = (V_{F1} + V_{p1} + V_{p2})/3$, where V_{F1} was the variance in the F_1 population, and V_{p1} and V_{p2} were variances of P_1 and P_2, respectively (Wright, 1968). The theoretical arithmetic mean GFD of F_1 and F_2 lines under heat stress was estimated as $F_1 = (P_1 - P_2)/2$ and $F_2 = (P_1+2F_1+P_2)/4$, respectively, and the theoretical geometric average GFD of F_2 lines under heat stress was estimated as $F_2 = (\log P_1 + 2\log F_1 + \log P_2)/4$ (Powers & Lyon, 1941). Broad-sense heritability was estimated by two methods: $h^2 = (V_{F2} - V_{F1})/V_{F2}$ (Burton, 1949) and parent-offspring regression of the mean GFD of F_3 families against the GFD of their F_2 parents (Falconer, 1981). Realized heritability of GFD was approximated as $h^2 = (F3_{high} - F3_{low})/(F2_{high} - F2_{low})$ (Falconer, 1981).

Microsatellite marker analyses

Ventnor and Karl 92 were screened for polymorphism of the microsatellite markers, and the 166 F2 plants were used as a mapping population. The distal ends of all leaves were detached when the seedlings reached the 5 to 6-leaf stage and were frozen immediately in liquid nitrogen and held at –80 °C. A sample of 200 to 400 mg of leaf tissue per plant was powdered by grinding in liquid nitrogen and incubated in DNA extraction buffer (100 mM Tris pH 8.0, 50 mM EDTA, 500 mM NaCl, 30 mM NaOH, 1.24% SDS w/v, 3.8% Nabisulfate w/v) at 56 °C for 30 min. The extract was washed with chloroform/isoamyl alcohol (24: 1, v/v), and the emulsion was centrifuged at 12000 g for 12 min. The supernatant was extracted with phenol: chloroform (1:1, v/v), and DNA was precipitated with 2.5 volumes of 95% ethanol, washed with 70% ethanol, and dissolved in Tris-EDTA. The DNA concentration was estimated by comparing its intensity to standard amounts of DNA after the samples were separated on ethidium bromide-stained agarose gels.

Table 1. Statistical estimates of grain-filling duration (GFD) of Ventnor and Karl 92 wheat cultivars and their F_1 and F_2 progenies at 30/25 °C day/night

Statistic	Ventnor	Karl 92	F1	F2
χ^2-test*	P>0.10	P >0.10	P >0.10	P > 0.10
Mean	21.98	12.96	17.31	15.74
Min	17.00	9.00	13.00	8.00
Max	27.00	18.00	20.00	24.00
Variance	4.35	4.73	3.30	11.56
Number	50.00	50.00	50.00	166.00

* Chi-square test for the goodness-of-fit to a standard normal curve.

Microsatellite primers developed by Röder et al. (1995, 1998) were used. The PCR was performed in a volume of 25 μl containing 50 ng DNA, 1 × reaction buffer, 1.5 mM $MgCl_2$, 1 μl Taq polymerase (Bioline, Kenilworth, NJ, USA), 250 μM of dNTP (Promega, Madison, WI, USA), and 0.5 μm of primer DNA. The reaction mixture was run on a thermocycler (Perkin Elmer, GeneAmp PCR System 9700, Foster City, CA, USA) by the procedure of Röder et al. (1998) with minor modifications. 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. Amplification products were resolved by electrophoresis of 25 μl of reaction mixture on 2.3% Metaphor agarose gels (FMC BioProducts, Rockland, ME, USA) at 70 V for 6h, visualized by ethidium bromide staining, and recorded for analysis by photography.

Single-marker QTL analysis was performed by the method of Liu (1998). Significant association of a tested marker with a QTL for heat tolerance was detected by single-factor ANOVA. Contribution of a marker-linked QTL to total variation in heat tolerance in F_2 lines was estimated by linear regression. Additive and dominance action of the QTL was determined by significance of the slopes (β_1 and β_2) in the linear model $Y_j = \beta_0 + \beta_1 X_{1j} + \beta_2 X_{2j} + \epsilon_j$ (Liu, 1998). The homozygous susceptible marker allele was coded 0, the heterozygous allele was coded 1, and the homozygous tolerant allele was coded 2. All statistical procedures were performed with SAS (1995).

Table 2. Observed and calculated theoretical mean grain filling duration (GFD) of F_1 and F_2 progeny of Ventnor × Karl 92 wheat cultivars assuming arithmetic and geometric gene action at 30/25 °C day/night

Cross	F_1 population mean		F_2 population mean			
	Observed	Theoretical arithmetic	Arithmetic		Geometric	
			Observed	Theoretical	Observed	Theoretical
Ventnor ♀ × Karl 92 ♂	17.30	17.37	15.74	17.25	1.23	1.20

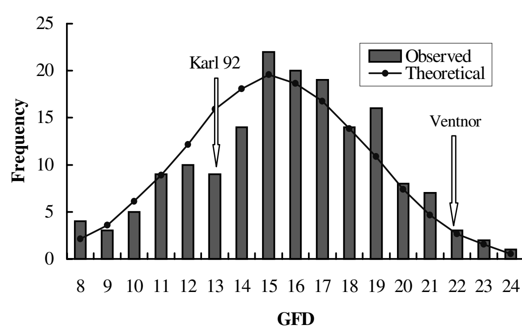

Figure 1. Observed and theoretical distribution of the grain filling duration (GFD) of F_2 progeny of Ventnor × Karl 92 wheat cultivars at 30/25 °C day/night.

Table 3. ANOVA of regression of mean grain filling duration (GFD) of F_3 progeny against GFD of F_2 progeny of Ventnor × Karl 92 wheat cultivars at 30/25 °C day/night

	DF	SS	MS	F	P
Regression	1	550.452	550.452	57.640	< 0.001
Residual	18	171.896	9.550		
Total	19	722.348			

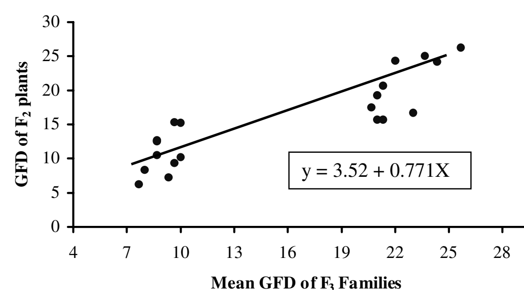

Figure 2. Regression of the mean grain filling duration (GFD) of selected F_3 progeny against F_2 progeny of Ventnor × Karl 92 wheat cultivars at 30/25 °C day/night.

Results

Quantitative nature of heat tolerance

The χ^2-test indicated that the frequency distribution of GFD of the F_1 and F_2 lines and their parents, Ventnor and Karl 92, was expressed by a normal model ($P > 0.10$, Table 1). The observed frequency of GFD of the F_2 lines closely followed the theoretical frequency (Figure 1). The difference between the mean GFD of the two parents under heat stress, 2.3 standard deviations of the F_2 population, was highly significant ($P < 0.01$). The mean GFD of the F_1 lines, which was midway between Ventnor and Karl 92, differed significantly ($P < 0.01$) from the two parents. However, the mean GFD of the F_2lines did not differ significantly from the F_1 lines.

The minimum number of genes or factors controlling heat tolerance was estimated as 1.4 by the equation of Wright (1968). The pattern of gene action involved in inheritance of heat tolerance could be estimated roughly by comparing the observed and theoretical means of the F_1 and F_2 lines (Powers & Lyon, 1949). The difference between observed and theoretical arithmetic means of the F_2 lines was uncertain due to a lack of suitable statistics. However, consistency among the observed and theoretical arithmetical means of F_1 lines and the geometric means of F_2 lines suggested the possibility of both additive and dominance gene actions associated with the GFD under heat stress (Table 2).

The mean GFD of F_3 families regressed significantly with the GFD of the corresponding F_2 plants (Table 3). The regression coefficient of $\beta = 0.771$ between F2 and F3 lines for estimating broad-sense heritability (Falconer, 1981) was close to the estimate of $h^2 = 0.802$ by the method of Burton (1951) (Figure 2). The realized heritability from the F_2 to F_3 lines was 0.965.

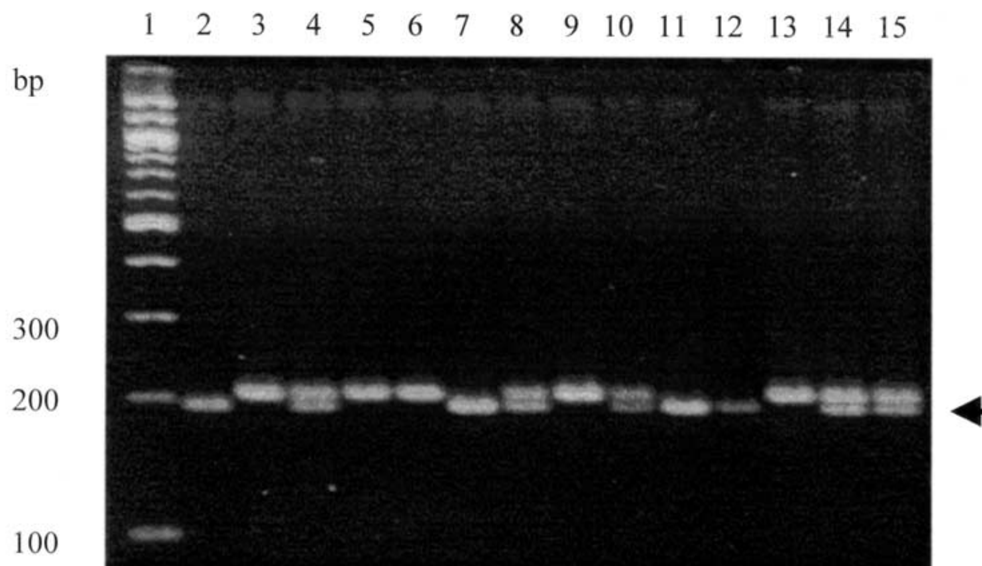

Figure 3. Selective genotyping of F_2 progeny of Ventnor × Karl 92 wheat cultivars with the *Xgwm11* marker for heat tolerance. Ladder 1 is 100-bp DNA marker, Ladder 2 is Karl 92 (susceptible), Ladder 3 is Ventnor (tolerant), Ladders 4–9 are the six most tolerant F_2 lines, and Ladders 10–15 are the six most susceptible F_2 lines. Arrow points to polymorphic bands of the *Xwgm11* marker.

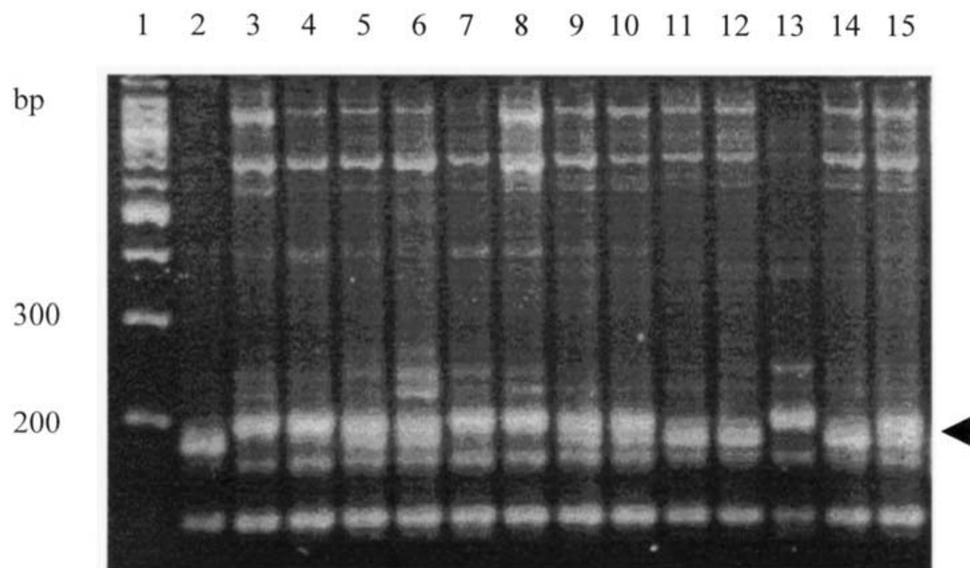

Figure 4. Selective genotyping of F_2 progeny of Ventnor × Karl 92 wheat cultivars with the *Xgwm293* marker for heat tolerance. Ladder 1 is 100-bp DNA marker, Ladder 2 is Karl 92 (susceptible), Ladder 3 is Ventnor (tolerant), Ladders 4–9 are the six most tolerant F_2 lines, and Ladders 10–15 are the six most susceptible F_2 lines. Arrow points to polymorphic bands of the *Xwgm293* marker.

Table 4. ANOVA of grain-filling duration (GFD) of F_2 progeny of Ventnor × Karl 92 wheat cultivars on *Xgwm11* and *Xgwm293* markers

	DF	SS	MS	F	P
GFD on *Xgwm11*					
Model	2	131.89	65.94	11.39	0.0001
Error	152	880.33	5.79		
Total	154	1012.23			
GFD on *Xgwm293*					
Model	2	133.48	66.74	10.68	0.0001
Error	156	974.61	6.24		
Total	158	1108.09			

SSR markers associated with heat tolerance

Among 232 SSR primer pairs, 198 gave scorable amplification products in both Ventnor and Karl 92, and 59 of the scorable primers detected polymorphism between the parents. The ratio of polymorphic markers to scorable markers of nearly 30% was consistent with recent results by Roy et al. (1999) and Prasad et al. (1999). When the 59 primers were used to screen the 166 lines in the F_2 population, 57 of the pairs were not significantly associated with heat tolerance as estimated by single-marker ANOVA ($P = 0.05$) (Liu, 1998). Two markers, *Xgwm11* and *Xgwm293,* were significantly linked ($P < 0.001$) to heat tolerance (Table 4). The amplification profiles of the two primer pairs were characterized by the six most tolerant individuals and the six most susceptible individuals in the F_2 progeny and their parents (Figures 3 and 4). Among the six most tolerant F_2 lines, three had profiles of Ventnor, one of Karl 92, and two were heterozygotes. Among the six most susceptible lines, one had a profile of Ventnor, and three were heterozygotes (Figure 3). Figure 4 shows similar results. Three of the most tolerant lines had profiles of Ventnor and three were heterozygotes. Three of the most susceptible individuals had profiles of Karl 92, two were heterzygotes, and one resembled Ventnor. The *Xgwm11* and *Xgwm293* markers were assigned to short arms of chromosomes 1B and 5A following Röder et al.(1998).

Regressions of GFD under heat stress on both markers were highly significant (Table 5). The adjusted R^2 values of 0.12 and 0.11 suggested that the *Xgwm11*-linked QTL and *Xgwm293*-linked QTL accounted for 11% and 12% of the total phenotypic variation in heat tolerance in the F_2 population. The regression coefficients $\beta_1 = 1.19$ ($P = 0.0001$) and $\beta_2 = -0.79$ ($P = 0.049$) for *Xgwm293* indicated both additive and dominance actions of the gene linked with the marker. In contrast, the gene linked with *Xgwm11* showed only additive action ($\beta_1 = 1.30$, $P = 0.0001$) and no dominance ($\beta_2 = 0.07$, $P = 0.85$).

Discussion

Understanding inheritance of heat tolerance of plants from their overall response to high temperature was difficult. Precision of estimates was low due to effects of plant developmental status and interactions of environmental factors with genes for the trait (Ottaviano et al., 1991; Shah et al., 1999). In this study, extraneous effects were minimized by growing the plants in controlled environment chambers and exposing them to the temperature treatments at exact stages of grain development. The procedure probably enabled the close fit of the observed frequency distributions of the grain filling duration under high temperature in the F_1 and F_2 populations and their parents with the theoretical prediction from binomial expansion $(X + Y)^n$. The normal distribution was typical of traits controlled by multiple genes (Roff, 1997; Prasad et al., 1999) and supported the hypothesis of Blum (1988) that heat tolerance is quantitatively inherited.

The small total contribution of 23% for the two loci associated with *Xgwm11* and *Xgwm293* to the variation in heat tolerance in the F_2 population was within the range of 8.9 to 38.2% for seversl agronomic traits (Shah et al., 1999). The low value suggested that the markers were either tightly linked to QTL that had a small effect or were loosely linked to QTL that had a large effect (Melchinger, 1998). Our evidence supported the former hypothesis. The ratio of 30% of polymorphic markers to scorable markers was higher than the average of less than 10% from RFLP (Röder et al., 1998) and less than 3.3% from RAPD (Penner et al., 1995) in hexaploid wheat. However, fewer than three ($59/21 < 3$) markers on average could be allocated to each chromosome pair of hexaploid wheat. The density of the marker was too low to cover the whole genome of wheat. Morever, the 59 scorable markers were not evenly distributed among the chromosome complement. Six SSR markers, for example, were assigned to the long arm of chromosome 1B, whereas none was assigned to the long arms of chromosomes 6A and 6D according to Röder et al. (1998). Some

Table 5. Parameters from regression analyses of grain filling duration (GFD) of F_2 progeny of Ventnor × Karl 92 wheat cultivars on *Xgwm293* and *Xgwm11* markers*

Marker	β_0	β_1	β_2	Adjusted R^2
Xgwm11	14.49	1.30	0.07	0.12
	(P = 0.0001)	(P = 0.0001)	(P = 0.8541)	
Xgwm293	15.09	1.19	–0.79	0.11
	(P = 0.0001)	(P = 0.0001)	(P = 0.0492)	

* Linear model $Y_j = \beta_0 + \beta_1 x_{1j} + \beta_2 \times {}_{2j} + \epsilon_j$ (Liu, 1998).

QTLs were undetected by the screening because of the low density of the markers.

Homoeologous groups of chromosomes 5 and 6 of wheat contain a number of genes that are important for resistance to abiotic stress (Dubcovsky et al., 1995). Discovery of the *Xgwm293*-linked QTL was probably attributable to the four scorable markers on the short arm of chromosome 5A. Due to the lack of available markers, QTL on homoeologous group 6, if any, were not assayed.

Tight linkage of the markers to QTL that had small effects on heat tolerance was also indicated by a simulation model showing that the QTL number increased and effect per QTL decreased roughly as sample size increased (Roff, 1997). The simulation predicted that only those QTL having large or modest effects ($>5\%$) were detectable in a sample of 200 individuals. The identification in this study of two QTL with effects little more than 10% in a sample of 166 individuals was consistent with the prediction by the simulation. However, the result implied that more QTL with small effects would have been detected if the sample size were larger.

The estimate of only 1.4 genes or factors affecting GFD of the parents under heat stress was contingent on several assumptions (Wright, 1968; Lande, 1981). The assumption of a large number of loci with both epistatic and dominance effects and their possible linkages that can be ignored was the most important (Roff, 1997). The exact number of loci controlling heat tolerance and epistatic effects could not be estimated in the study, but the assumption that dominance effects could be ignored probably was not warranted because of the significant dominance action of the *Xgwm293*-linked QTL and the inability to separate it from epistasis in the model. As a result, the method likely underestimated the number of genes that were involved (Lande, 1981; Roff, 1997).

Additive effects of the two QTL that were identified might be important for accumulating tolerant alleles in breeding programs. The absence of significant transgression in the F_1 and F_2 populations and the presence of the two beneficial alleles in the heat-tolerant genotype suggested that Karl 92 was unlikely to provide favorable alleles. The positive additive effect (β_1 = 1.30) and negative dominance effect (β_2 = –0.79) of the allele linked with marker *Xgwm293* indicated that selection for long GFD under high temperature is likely to be successful in early generations.

The broad-sense heritibility of about 80% and realized heritability of over 96% for GFD under heat stress that was estimated in this study were similar to the value of 89% for heat tolerance determined by Fokar et al. (1998). The high heritibilty, together with the 23% variation from the two SSR marker-linked QTL, would facilitate breeding heat-tolerant cultivars. However, our estimates and those of Fokar et al. (1998) were based on data obtained in controlled environments, which usually underestimate variation under field conditions (Roff, 1997). Nonetheless, the 23% of true-breeding genetic variability that the two QTL contributed suggested a promising application of molecular-assisted techniques for improving heat tolerance of new wheat cultivars.

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