QUANTITATIVE TRAIT LOCI (QTL) ANALYSIS OF YIELD COMPONENTS AND HEAT TOLERANCE IN WHEAT (*Triticum aestivum*)

A Dissertation

by

JUNG HWA DO

Submitted to the Office of Graduate Studies of
Texas A &M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2007

Major Subject: Molecular and Environmental Plant Sciences

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ABSTRACT

Quantitative Trait Loci (QTL) Analysis of Yield Components and Heat Tolerance in Wheat (*Triticum aestivum*). (December 2007)

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This study was conducted to identify and map QTLs for yield components and heat tolerance of wheat in response to two kinds of heat treatment (short term-and long term-heat treatment) during seed formation in a set of 62 RILs derived from a cross of '7C' (heat resistant variety) and 'Seri M82' (heat susceptible variety) in environmentally controlled growth rooms and field. Phenotypic variations of yield components (kernel number, kernel weight, spike number and grain filling duration) were evaluated as indicators of heat tolerance / susceptibility. Most of the phenotypic variations of yield components exhibited a normally distributed pattern in response to heat stress treatments. This suggests that the yield component responses to high temperature stress are likely quantitatively inherited. A transgressive segregation pattern compared to the two parents was observed in several yield traits. This suggests that genetic variation from optimal recombination from the two parents have occurred in the progeny population. The Pearson correlation coefficients revealed significant correlations between yield components. This suggests the probability of co-segregation of genes controlling each yield components. The ANOVA also revealed a significant genotype x environment

effect on individual yield components in response to reproductive stage high temperature stress. The heritability of the individual yield components was low (0.42 to 25%, 0.1~ 2% for heat tolerance). One hundred two polymorphic SSRs markers among 323 SSRs markers tested were used to construct a linkage coverage and average interval distance of 1860.2 cM and 18.2 cM/marker, respectively. Eighty-one QTLs for yield components and 68 QTLs for heat tolerance were detected with high LOD values (2.50~8.35 for yield components, 2.51~ 9.37 for heat tolerance) and that explained significant phenotypic variations (7~40% for individual QTL for yield components, 2~40 % for individual heat tolerance QTLs) from seven individual environments and the four individual heat stress environments, respectively. Specifically the regions between wmc48 and wmc89, and between wmc622 and wmc332 on the chromosome 4A and 6A, respectively possessed QTLs for both yield components and heat tolerance from various environments.

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ABBREVIATIONS

Ker. no. / ms –	Kernel Number per Main Spike
Ker. wt / ms –	Kernel Weight per Main Spike
Sin. Ker. wt. / ms –	Single Kernel Weight per Main Spike
Ker. no. / sp –	Kernel Number per Spike
Ker. no. / pl –	Kernel Number per Plant
Sp. no. / pl –	Spike Number per Plant
GFD. / ms –	Grain Filling Duration per Main Spike
QS_Kno_ms_con –	QTL Short-term Kernel Number per main Spike Control
QL_Kwt_pl_heat –	QTL Long -term Kernel Number per Plant Heat
QF_Kno_ pl_con –	QTL Field Kernel Number per Plant Control
QF26_Kno_ heat –	QTL Field 26~31˚C Kernel Number Heat
QF32_Skwt_heat –	QTL Field 32~31˚C Single Kernel Weight Number Heat
QS_red_Kno_heat –	QTL Short-term % Reduction Kernel Number Heat
QS_ss_Kno_heat –	QTL Short- term Simple Score Kernel Number Heat

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CHAPTER I

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important grain crop due to considerable utility for bread making, starch-gluten manufacture, cake and biscuit production, and a wide range of manufactured foods and animal feeds. Wheat is grown in various environmental regions which affect yield and end-use quality based on interaction between varying genotypes with the environment (Atak et al., 2002). The yield of wheat varieties are often affected by unfavorable stressful environments rather than their own genetic potential (Gusta & Chen 1987; Assad & Paulsen, 2002). Wheat prefers an optimal growing temperature in the range of 15 -20˚C throughout the whole life cycle, especially during reproductive development (Chowdhury & Wardlaw, 1978: Yang et al., 2002a).

It has been shown that most current wheat cultivars are heat susceptible and lose approximately 50% of their yield potential due to exposure to one to two days of heat stress during reproductive development (Wardlaw *et al*., 1989a). High temperatures during reproductive development are common in many wheat growing regions worldwide including the U.S. Southern Great Plains as well as many wheat-growing regions in China, India, the Middle East, Africa and many European countries. Recent research has focused on the physiological and quantitative results of agronomically important traits such as dwarfing, the vernalization response (Korzun et al., 1997), leaf

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This dissertation follows the style and format of Crop Science.

rust resistance (Feuillet et al., 1995, 1997; Naik et al., 1998), kernel hardness (Sourdille et al., 1997), pre-harvest sprouting tolerance (Roy et al., 1998), high protein content (Prasad et al., 1999; Mesfin et al., 1999), powdery mildew resistance (Qi et al., 1996), kernel traits (Campbell et al., 1999) and flour viscosity (Udall et al., 1999). Few studies however have focused on the relationship between QTL for yield components and QTLs for reproductive stage heat tolerance in wheat. This represents an important hole in our knowledge in terms of developing cultivars with optimal yields and yield stability for high temperature environments.

The specific objectives of this project are as follows:

Chapter II. Define quantitative yield traits (e.g. kernel weight, kernel number per main spike, grain filling duration) which are indicators of reproductive stage heat tolerance.

Chapter III. Identify QTL for yield and its components in 7 unique environments.

Chapter IV. Identify QTL for reproductive stage heat tolerance.

Chapter V. Determine the relationship between QTLs for yield components with QTLs for yield heat tolerance.

CHAPTER II

ANALYSIS OF QUANTITATIVE TRAITS OF RIL OF WHEAT AFTER SHORT-TERM AND LONG-TERM PERIODS OF HEAT STRESS

1. INTRODUCTION

Quantitative traits such as yield, plant height, grain filling duration and *etc*., vary continuously among hybrid lines and inbred line populations derived from crosses of two contrasting parents. The phenotypic variation observed within a segregating population is due in large part to the genotypic differences between the parents. The variation of a particular quantitative trait can be affected by the number of genes involved, the type of gene action (additive or dominant), as well as the degree of interaction between genes and environments. Yield components, including grain weight, size and number, are all examples of quantitative traits which are major contributors to the production of cereal crops.

Crop plants have been shown to have a more rapid and sensitive response to heat stress than any other adverse environmental factors (Poehlman & Sleper, 1995). The enzymatic dependent mechanism of photosynthesis and respiration are damaged within a few minutes of exposure to high temperature. Heat stress is known to be a major factor contributing to the reduction of grain weight, size and number each of which can result from damage to source leaves or the developing grain itself. Therefore, the performance of yield components in response to heat stress can be used as a measure of heat tolerance in crop plants.

In the heat susceptible wheat genotypes, heat stress during vegetative and reproductive development reduces photosynthesis, promotes premature senescence, and leads to reduced yields by inducing pollen sterility and seed abortion. Heat stress on the also reduces tests weights, flour yields, and dough quality through an early initiation of programmed seed desiccation that reduces the grain-filling duration. It has been reported that susceptible wheat genotypes exhibited a 3 % reduction in yield for every temperature degree rise above 15°C. In addition, it has been shown that most current wheat cultivars are heat susceptible and lose approximately 50% of their yield potential due to exposure to one to two days of heat stress at 38°C during reproductive development (Wardlaw et al., 1989a, b). These levels of high temperature stress have been reported to be common in wheat growing regions worldwide. Injury from heat stress is particularly critical during flowering, pollen growth, stigma receptivity and seed formation (Poehlman and Sleper, 1995). In contrast, wheat genotypes with greater tolerance to high temperature have been identified and are defined by maintenances of photosynthesis, chlorophyll content, stomatal conductance, and yield through higher seed set, grain weight, and an extended grain filling duration even at elevated temperatures. However, the genetic and molecular mechanism of heat tolerance in crop plants is poorly understood, yet is critical to the development of heat-tolerant cultivars.

Kernel size and flowering date in wheat have been shown to be traits which have relatively high heritability during high temperatures (Poehlman & Sleper, 1995). This indicates that cultivars breed for heat tolerance will exhibit a greater maintenance of grain weight, size and number during heat stress than heat susceptible cultivars due to

the inheritance of heat tolerance from their parents. In the recent decades, heat stress accompanying drought stress during seedling and reproductive development have been primary constraints to wheat production and profitability in the U. S. Southern Great Plains and many wheat-growing regions in China, India, the Middle East, Africa and many European countries. Additional yield reductions from the predicted rise in global temperature will add further strain to food security and economic viability of farming and related farm based businesses. In an international consultancy, leaders of national wheat programs identified the improvement of heat tolerance as one of their major priorities (International Center for Maize and Wheat Improvement-CIMMYT, 1995).

The objective of this chapter is to define the quantitative yield traits (e.g. kernel weight, kernel number per main spike, and grain filling duration) which are indicators of sensitivity to heat stress. Treatments included a short-term heat stress at 38°C for 2 days beginning at 10 days after pollination (DAP) and a long-term heat stresses of 30°C beginning at 10 DAP until grain maturity in an environmentally controlled growth room and a long-term heat stress conducted in the field (field data were provided by Cimmyt in 2004). This was used to determine which components of yield most highly correlated with yield maintenance during the two periods of heat stresses, and for use as measures of heat tolerance within the RIL population.

1-1. Yield and yield components of wheat during heat stress

Yield components such as kernel number, kernel weight and kernel size are thought to be inherited quantitatively (Benmoussa et al., 2005). Kernel weight is the major contributing factor to wheat grain yield, with kernel number a following factor

(Fisher, 1993). These individual yield components are affected by high temperature stress during kernel development, and ripening. Gibson and Paulsen (1999) reported that grain yields were reduced by 78% through the reduction of kernel weight and kernel number by 29% and 63%, respectively when exposed to a 35/20 °C (day/night) high temperature stress at 10 days after anthesis until ripeness. When exposed to a 36/31°C (day/night) from 7 days after anthesis, an 80% reduction in kernel weight was observed. However, exposure of wheat grain to high temperature in the later stages of seed development at 15 and 20 days after anthesis, showed less yield loss. Stone and Nicolas (1994) reported that the largest reduction of kernel weight occurred when the wheat was grown at 40/15°C (day/night) for 3 days beginning at 30 days after anthesis. Therefore, timing, duration, and degree of temperature difference between day and night are important factors influencing yield (Tashiro and Wardlaw, 1989). Wheat yield also decreases as a result of a shortened grain filling duration that results in reduced grain weight (Tashiro and Wardlaw, 1990a; Stone and Nicolas, 1994). Grain filling in cereals is supported by current photosynthesis in leaves, stems, and ears (Johnson and Moss 1976, Araus et al., 1993) and by reallocation of carbon stored in stems and leaves during grain filling (Gebber and Schnyder 1999, Yang et al., 2001). During grain filling, when plants are subjected to drought and high temperature, the amount of starch deposited per endosperm cell is reduced, leading to a reduction in the amount of starch per granule and hence reduced grain weight (Jenner, 1991; Chojeck et al., 1986). This study also showed grain filling duration to be highly correlated with grain weight. Under normal growth and development, leaf photosynthesis declines during grain filling as leaves begin to

senescence. Senescence can also occur early in response to stress. High temperature stress is one such stress. High temperatures are often associated with high radiation, with their combination having a strong impact on photosynthesis (Paulsen, 1994). Elevated temperatures enhance photo-inhibition (Fuse et al., 1993) and photorespiration (Leegood and Edwards 1996) and can have deleterious effects on thylakoid function (Pastenes and Horton 1996). Photosystem II in particular is highly heat sensitive. Significant correlations between increased photosynthesis and stomatal conductance with yields have been reported between wheat varieties (Waddington et al., 1987; Gutierrez et al., 2000; Yang et al., 2002) and RILs of crosses between heat tolerant and susceptible varieties (Reynolds et al., 2002). In this way, heat stress alters optimal photosynthesis, respiration and vegetative growth as source of photo-assimilates for seed development. As a result, disruption of these processes leads to reductions in yield through reduction of weight, size, and number of grains.

1-2. Genetic and molecular characteristics of heat

Heat tolerance, like yield components is believed to be inherited quantitatively (Maestri et al., 2002). Using QTL analysis to dissect heat tolerance in maize, 6 QTL accounting for 53% of the genetic variability for cellular membrane thermostability (CMS) were detected in a RIL population. Using the same population, 5 QTL were detected controlling pollen germination during heat stress and thermo-tolerance (Frova and Sari-Gola, 1993; Frova, 1996). Using grain filling duration as a measure of heat tolerance in wheat, a minimum of 1.4 genes, with additive and dominant effects, were estimated to control the trait (Yang et al., 2002b).These estimates were similar to the

estimates using co-segregation analysis in RILs of wheat which showed a significant correlation between cellular membrane thermo stability (CMS) and yield when grown in hot summer conditions (Blum et al, 2001). Ristic *et al*., (1998) and Malik et al., (1999) reported in their studies of maize and carrot callus culture that heat shock proteins (45kDa HSP and 17.7 kDa HSP) correlated with thermotolerance. Similar evidence was obtained in a study of 9 genotypes of tomatoes which showed a significant correlation between chloroplast-localized HSPs and heat tolerance (Preczewski et al., 2000; Maestri et al., 2002). However, Singla et al., (1998) found significant constitutive levels of HSP101 in developing and mature grains of wheat in the absence of heat stress. In addition, HSP70, a chloroplast-localized HSP26 and low-molecular weight HSPs of both class I and class II were observed at high levels in grains of 20 diverse wheat cultivars grown in temperate conditions (Maestri et al., 2002).

2. MATERIALS AND METHODS

2-1. Plant growth condition

A set of 62 recombinant inbred lines (RILs) derived from a cross between a parent exhibiting heat tolerfance '7C' (Mexico, spring wheat) and another parent exhibiting heat susceptibility 'Seri M82' (Mexico, spring wheat) and these parents were provided by Cimmyt in Mexico. Each RIL and parent was seeded at 3 plants per pot (1 : 3 ratio of Metro Mix 200 (Sun Gro Horticulture, Bellevue, WA) potting mix plus sandy loam soil in 12 x 15 cm pots) replicated 6 times and grown in an environmentally controlled growth room. Nutrition was provided 3 times with Peters Professional Fertilizer, 20 (N): 20 (P): 20 (K) (W.R. Grace and Co., Fogelsville, PA) to supply a total

of 100mg N, 43mg P, and 83 mg K per pot between young seedling stage to anthesis. Initially all RILs and their parents were grown under optimal management in a growth room in a 20°C/18 °C at day/night cycle with 12 hours of photoperiod under halogen lamps to obtain a 500 μmol m^{-2} s^{-1} photosynthetic photon flux. Inflorescence from each plant was scored on the day of flowering. At 10 DAP, two identical sets of RILs and their parents were split between two identical growth chambers. Plants in both chambers were watered twice daily to eliminate water stress as a component of heat stress. Plants in the controlled chamber were grown for two days as described above, while plants in the heat stress chamber were grown for two days at 38/20°C day / night cycle under fluorescent lamps to obtain a 600 μmol m^{-2} s^{-1} photosynthetic photon flux. Following the two days all plants were returned to identical growth rooms set a 20/18°C day / night. The relative humidity of chambers and growth rooms was 60%. For long- term heat stress, plants in the control room were grown as described above while plants in the heat stress room were transferred to a second identical growth room at 10 DAP set at a 30/20°C day/night cycle. Experiments in chambers and growth rooms were designed in a complete randomized design with 3 replicates (9 plants per genotype) in both of the short- term heat treatment, the long-term heat treatment population, and control treatment. The three growth condition in the field were a temperate climate during the whole life cycle for the RIL growth period (1996-1997 year),while for the heat stress treatment, the RILs were grown in the range of 26~31°C in 2001, and 32~35°C in 2002 during the reproductive stages. This data was provided by Cimmyt in Mexico. Plants

were harvested by hand and kernels were threshed with a single-spike thresher (Lincoln Manufacturing Co., Lincoln, NE) after counting spikes.

2-2. Quantification of changes in yield and its components after heat

Kernel weight and number per main spike, per total spikes, and per plant were scored in the 62 RILs and their parents 'Seri M82' and '7C'. The % reduction between control group and heat treated group was calculated as indicators of sensitivity to heat stress and as a primary indicator for heat tolerance among yield components. Single kernel weight was measured by ratio of kernel weight to kernel number from main spike and total plant kernel number respectively. The grain filling duration (GFD), one contributor to wheat yield and heat tolerance, was estimated as the interval between 10 days after pollination of the main spike and date at which the glumes on the main spike became 90% becoming chlorotic

2-3. Statistical analysis

Correlations between phenotypic traits were determined using PROC CORR procedure in SAS. A univariate procedure in SAS was performed to determine the normality of the distribution of each phenotypic trait. The estimation for genetic and environment effect on each phenotypic trait and on the heat tolerance estimated by the % reduction of the phenotypic traits of 62 RILs population was made using PROC GLM procedure in SAS (SAS, 1996) on the basis of the analysis of variance (ANOVA) using genotype as fixed effects and environments as random effects.

The linear model for phenotypic traits analysis based on yield components such as kernel number and kernel weight of the main spike and the plant, single kernel weight of the main spike and the plant using a complete randomized experimental design is:

$Y_{ijk} = u + G_i + r_{j(k)} + E_k + (G*E)_{ik} + e_{ijk}$.

Where Y_{ijk}= phenotypic value for the ith genotype in the jth replication at the kth environment.

u = population mean

G_i = effect of the i^{th} genotype

$r_{j(k)}$= effect of the j^{th} replication in the k^{th} environment.

E_k = effect of the k^{th} environment.

$(G*E)_{ik}$ = effect of the i^{th} genotype at the k^{th} environment.

e_{ijk} = residual error of the i^{th} genotype in the j^{th} replication at the k^{th} environment.

For each trait, narrow sense heritability was estimated from the source of variance from ANOVA and calculated as follows:

$$h^2 = \frac{\sigma^2_a}{\sigma^2_e + \sigma^2_{GE} + \sigma^2_G} \quad \text{with} \quad \sigma^2_e = MS_e, \quad \sigma^2_a = \sigma^2_G / 2$$

$$\sigma^2_G = \frac{MS_G - MS_{GE}}{re}$$

$$\sigma^2_{GE} = \frac{MS_{GE} - MS_e}{r}$$

where MSG, MSGE and MSe are the mean square of genotype, genotype x environment interaction and residual error, respectively.

3. RESULTS AND DISCUSSION

Short-term heat stress was more deleterious to grain development than long – term heat stress for both parental lines, 'Seri M82' and '7C' based on photographs of their kernels. Kernels of 'Seri M82' grown in a short-heat stress at 38℃ for 2 days were smaller in size and more shriveled than those of '7C'(Fig.1a). In contrast, in the long-term heat stress imposed at 30℃ from 10DAP until grain maturity, the kernel development of 'Seri M82' and '7C' were not significantly affected (Fig. 1b).

3-1. Grain yield and its components of two parental lines 'Seri M82' and '7C'

The suitable selection of parents is very important for the development of varieties that assemble together many desirable genes. Hybridization of two highly contrasting inbred lines with different genetic backgrounds is one strategy for obtaining superior progeny through optimal combination of desirable alleles for traits of interest (Mohan et al., 1997).

In this study, using the relative % reduction of yield components such as kernel number, kernel weight, single kernel weight per main spike and per plant, and the grain filling duration per main spike after long-term and short-term heat stress was analyzed for the two parental lines 'Seri M82' (moderate heat susceptible variety) and '7C'

(moderate heat tolerant variety). Significantly different % reduction for yield (α=0.01) and its components was revealed (Table I). Following long-term heat stress the single kernel weight per plant were significantly reduced at α= 0.05 (Table I).

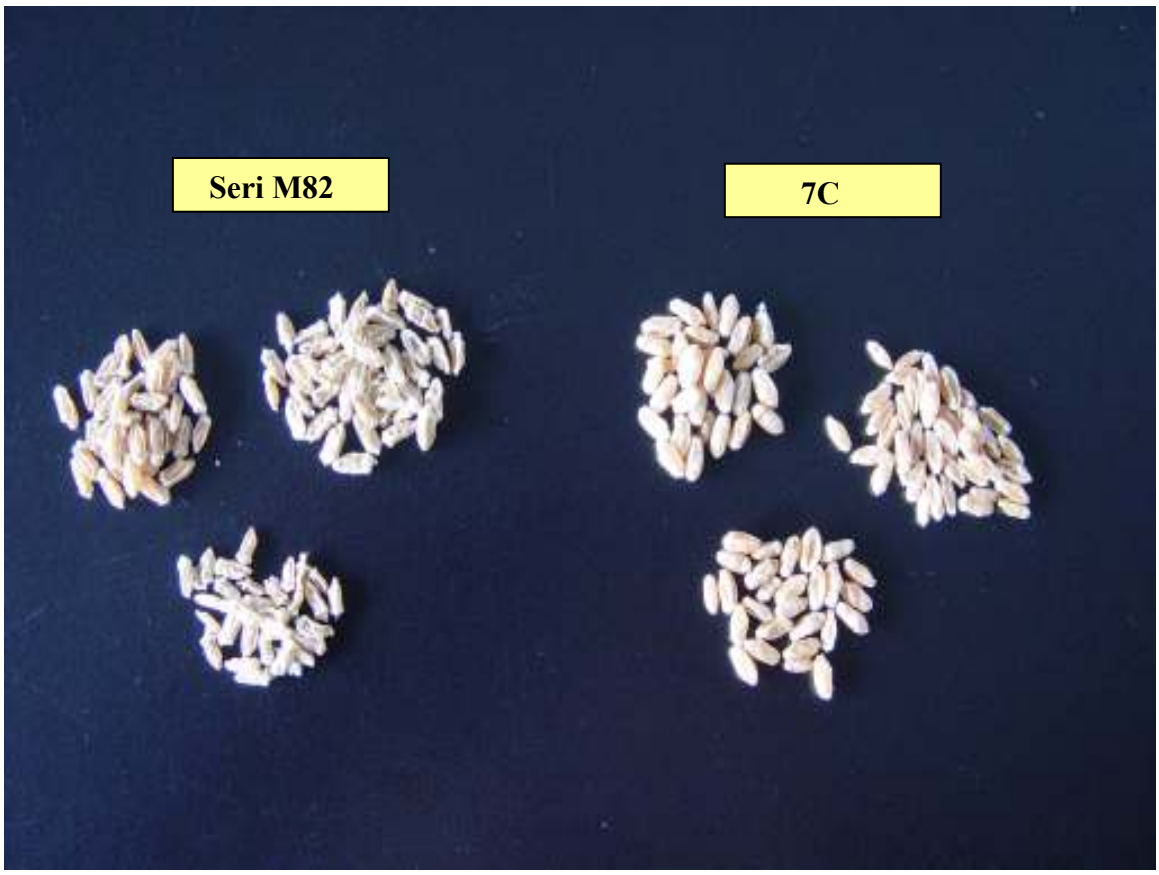


Fig. 1. (a) Kernels of 'Seri M82' and '7C' exposed to a short-term heat stress of a 38/20℃ day/night cycle for two days beginning at 10 DAP in environmentally controlled growth chambers.

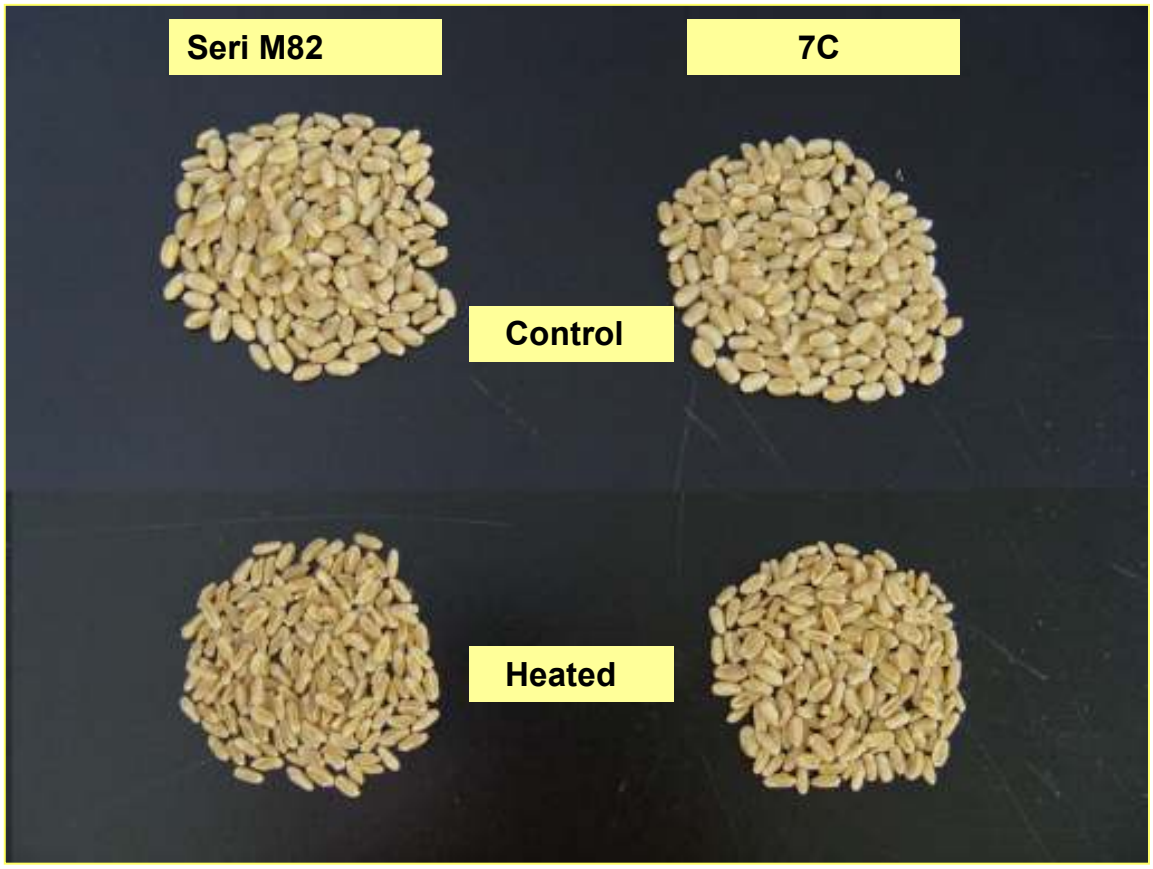


Fig. 1 (continued) (b) Kernels of 'Seri M82' and '7C' exposed to a long-term heat stress of a 30/18℃ day/night cycle beginning at 10 DAP until grain maturity in environmentally controlled growth rooms.

In '7C', the kernel number per main spike and per plant increased after the two day short-term heat stress, but not following long-term heat stress (Table 1). Kernel weight however was reduced in both of parental lines after both long and short term heat stress. This result demonstrated that kernel weight is more sensitive to heat stress than kernel number in these two genotypes. Previous reports have drawn the same

Table I. Statistical analysis of mean, standard deviation and paired samples T-test for the relative % reduction of phenotypic traits for the two parental lines 'Seri M82' and '7C' grown under temperate temperature, short-term heat stress(at 38°C for 2 days) and long- term heat stress (at 30°C) beginning at 10DAP until grain maturity.

Relative % Reduction for traits	Short- Term at 38˚C Relative % reduction Mean	Long-Term at 30˚C Mean
Kernel No. per Main Spike		
Seri M82	31.0 ± 2.0**	5.2 ± .21**
7C	-23.0 ± 2.1	1.3 ± 0.12
Kernel No. per plant		
Seri M82	38.0 ± 1.0**	9.3 ± 2.0**
7C	-10.0 ± 0.1	-6.3 ± 0.1
Kernel Wt. per Main Spike		
Seri M82	66.0 ± 0.3**	60.4 ± 0.2*
7C	24.0 ± 0.2	42.4 ± 0.1
Kernel Wt. per Plant		
Seri M82	66.0 ±2.0**	63.0± 2.0**
7C	25.0 ± 0.6	51.0 ± 1.0
Single Kernel Wt. per Main Spike		
Seri M82	52.0 ± 2.0**	58.0± 2.0*
7C	35.0 ± 1.0	42.4 ± 0.1
Single Kernel Wt. per Plant		
Seri M82	45.0 ± 2.0*	61.0±1.0*
7C	35.0 ± 1.7	53.0 ± 1.5
GFD per Main Spike		
Seri M82	24.0 ± 2.0**	58.0± 2.0*
7C	1.3 ± 0.6	49.0 ± 3.0

± indicates Standard Deviation
*, ** indicate pair was significantly different from each other at the levels of $p < 0.05$ and $P< 0.01$, respectively.

conclusion that the main contributor to wheat yield reduction is attributed to lower kernel weight rather than kernel number (Sofield et al., 1977, Chowdhury & Wardlaw, 1978). The difference in the % reduction of yield and its components was more significant for both parental lines after short-term heat stress. Heat- shock stress during a short-term treatment at 38°C may not provide enough time for plants to respond to high temperature stress through the expression for self-protective transcripts. The present selection of the two parental lines appears to be a suitable choice for development of RILs based on the results obtained. Yang et al. (2002) reported, however, that grain yields of 'Seri M82' and '7C' had 1.361, 1.223 g/spike at 20/15°C, respectively and 1.019, 0.942 per g/ spike at 30/25 °C, respectively. Their heat susceptibility index (HSI) calculated by their grain yield was 0.679 and 0.829, respectively. In their study based on the results of HSI, 'Seri M82' was regarded as a slightly heat tolerant variety than '7C'. However, based on the relative % reduction in g/spike, which was calculated to be 25.1% and 22.9% for 'Seri M82' and '7C', respectively, '7C' appears to be the slightly more heat tolerant variety.

3-2. Grain yield and its components in the 62 RILs population in response to heat stress

The mean grain yield and mean yield components of the 62 RILs were used to estimate the effects of heat stress after short-term and long-term heat stress in environmental control growth room treatments and long-term heat stress in the field. Significant differences for mean yield and mean yield components were observed between the control and heat treated RILs. Most individual lines were significantly

reduced after heat stress (Table II Table III). The mean kernel number per main spike and per plant reduced significantly (α=0.01) in response to short-term heat stress but not to long-term heat stress in the environmentally controlled growth room (Table II). The mean kernel weight and mean single kernel weight per main spike and per plant also reduced significantly (α=0.01) in response to both heat stress treatments, but more significantly to long term heat stress (Table II). The mean kernel weight was also more sensitive than mean kernel number for both environmentally controlled heat stress treatments. Similar results have been reported with kernel weight being more sensitive to stress than kernel number in growth chamber studies (Sofield et al., 1977; Chowdhury & Wardlaw 1978; Wardlaw et al., 1989 a, b; Tashiro & Wardlaw, 1990a, b). The mean grain filling duration per main spike was also significantly (α=0.01) different for both heat stress treatments showing a greater decrease in response to long-term heat stress (Table II). A shortened grain filling duration is known to cause an early senescence of most of organs in plants leading to a reduction of kernel weight (Tashiro & Wardlaw, 1990a, b; Stone & Nicolas, 1994). Thus the present results that the mean kernel weight per main spike and per plant were more sensitive to long-term heat stress than short-term is in agreement with previous reports (Tashiro & Wardlaw, 1990a, b; Stone & Nicolas, 1994).

In the field, both mean kernel number, mean kernel weight, and mean single kernel weight per plant exhibited significant reductions when grown under long-term reproductive stage heat stress of 26~31°C (in 2002) and 32~35°C (in 2003) (Table III). Similar to data obtained from the environmentally controlled experiments in the field,

the mean kernel weight and mean single kernel weight per plant reduced to a greater degree than mean kernel number (Table III). The yield of wheat which was subjected to high temperature in field environment was four-fold less than the yield in the favorable environment (Midmore et al., 1984, Shipler & Blum, 1986; Zhong-hu and Rajaram 1994). In other studies, the degree of yield decline due to high temperature was much lower in controlled condition compared to field conditions (Chowdhury and Wardlaw, 1978; Nicolas et al., 1984; Tashiro and Wardlaw, 1989; Wardlaw et al., 1989a). In this study, the degree of decrease of yield including kernel number per plant and kernel weight per plant was much higher in the field than in the environmentally controlled growth room on the basis of relative % reduction of yields. In field, the kernel number, reduced by 37% and 11% and the kernel weight reduced by 93% and 90% at 26~31˚C and 32~35˚C, respectively versus temperate field conditions. In contrast, the kernel number reduced by 21% and 5.4% and kernel weight reduced by 43% and 59 % in response to short-term and long-term heat stress, respectively in the controlled condition (Table II, Table III). These results may be expected due to more complicated environmental effects and the higher light conditions found in the field compared to environmentally controlled growth rooms.

Table II. Statistical analysis of mean, standard deviation and paired samples T-test of phenotypic traits for the 62 recombinant inbred lines grown under temperate temperature, short-term heat stress (at 38°C) for 2 days, long-term heat stress (at 30°C) beginning 10DAP until grain maturity in environmentally controlled growth room.

Phenotypic Traits of 62 RILs	Short- Term at 38˚C Mean	Long-Term at 30˚C Mean
Kernel No. per Main Spike		
Contol	33.0 ± 7.7**	64.0 ± 14.8
Heat	30.0 ± 5.9	61.0 ± 6.4
Kernel No. per plant		
Control	52 ± 19**	222 ± 53
Heat	41 ± 13	210 ± 26
Kernel Wt. per Main Spike		
Control	0.66 ± 0.3**	2.84 ± 0.8*
Heat	0.41 ± 0.2	1.33 ± 0.3
Kernel Wt. per plant		
Control	0.93 ± 0.54**	8.82 ± 2.9**
Heat	0.53 ± 0.28	3.85 ± 0.86
Single Kernel Wt. per Main Spike		
Control	0.02± 0.01**	0.045 ± 0.01*
Heat	0.013± 0.003	0.022 ± 0.003
Single Kernel Wt. per plant		
Control	0.017± 0.01**	0.04 ± 0.01* *
Heat	0.012± 0.004	0.02 ± 0.003
GFD per Main Spike		
Control	33.0 ± 3.0**	62.0 ± 8.0* *
Heat	29.0 ± 0.6	29.0 ± 3.0

± indicates Standard Deviation
*, ** indicate pair was significantly different from each other at the levels of $p < 0.05$ and $P< 0.01$, respectively.

Table III. Statistical analysis of mean, standard deviation and paired samples T-test of phenotypic traits for the 62 recombinant inbred lines grown under temperate temperature, long-term heat stress at 26~31°C and 32~ 35°C during reproductive development stage until grain maturity.

Phenotypic Traits of 62 RILs	Long-Term at 26~31°C	Long- term at 32~35 °C
	Mean	Mean
Kernel No. per plants / m^2		
Control	15081.0 ± 2297.0**	15081.0 ± 2297.0**
Heat	9474.0 ± 1418.0	13401.0 ± 2217.0
Kernel Wt. per Plants / m^2		
Control	5095.4 ± 625.5**	5095.4± 625.51**
Heat	351.79 ± 52.1	485.7 ± 78.3
Single Kernel Wt. per Plants		
Control	0.341 ± 0.027**	0.341 ± 0.027**
Heat	0.037 ± 0.002	0.036 ± 0.002

± Standard deviation

*, ** indicate pair was statistically different from each other at the levels of $p<0.05$, $p<0.01$

3-3. Distribution of yield and each trait of yield components

The population of 62 RILs from the cross of the heat tolerant variety '7C' and heat susceptible variety 'Seri M82' exhibit broad segregation and significant trangressive segregation compared to the two parents for most the yield components. This suggests that superior progenies resulted from optimal recombination of desirable alleles. These traits may be differently regulated by additive and dominance effects (Zhuang et al., 1997). The distribution of kernel number per main spike in the 62 RILs exhibited a broader distribution, yet less phenotypic variation than kernel number per

plant in both response to control and short-term heat stress treatments (Fig. 2). A similar pattern was found in the long-term heat stress in the environmentally controlled growth room (Fig. 3a, b) except that a narrower distribution occurred for main spike versus whole plant yield components. Specifically, the kernel number per main spike response to the short-term heat stress and kernel number per plant response to long- term heat stress exhibited a continuous variation with small difference between classes (Fig. 2c, Fig. 3d). The variation in kernel number per m^2 was greater when grown in a temperate (control field) temperature, followed by 32~35°C and 26~32°C temperatures (Fig.4a, b, c).

The distribution of kernel weight per main spike showed less variation than those per plant for both control and heat-treated RILs in both short-term and long-term heat stress, yet still had the same number of classes that fell into a normal distribution (Fig. 5, Fig.6). In the field, kernel weight per m^2 had the greatest variation and classes of traits in the temperate environments followed by the 32~35°C (in 2003) and 26~31°C (in 2002) long-term heat stress experiments (Fig. 7). Similar variations were found for both control and heat treated populations for single kernel weight for both short-term and long-term heat stress treatments (Fig. 8, Fig. 9). In the field, when the RIL population grown in temperate condition, a continuous and greater variation in distribution was observed (Fig. 10). The distribution of spike number per plant showed similar variation when the population was grown under control and heat controlled environment conditions (Fig. 11). Grain filling duration per main spike was more broadly distributed when the RIL population was grown under heated stress versus control controlled environment conditions (Fig. 12).

The distribution of the relative % reduction of yield components was similar to the yield components in that a broad segregation and significant level of transgressive segregation over their two parental lines occurred. The relative % reduction of kernel number per main spike and plant had similar variation in both short-term, long-term and field imposed heat stresses (Fig. 13a, b, Fig 14a, b, Fig 15a, b). The relative % reduction of kernel weight per main spike and per plant also had similar variation in agreement with the results of kernel number (Fig 13c, d Fig. 14c, d, Fig15c, d). The distribution of relative % reduction on single kernel weight per main spike and plant were similar in the short-term (Fig. 16a, b), in the long-term controlled environment heat stress treatments (Fig. 16c, d) and the two field heat stress treatments (Fig. 17a, b).The variation of relative % reduction in the grain filling duration per main spike in the short-term heat stress was greater and had more classes than those in the long- term heat stress treatments (Fig.18a, b).

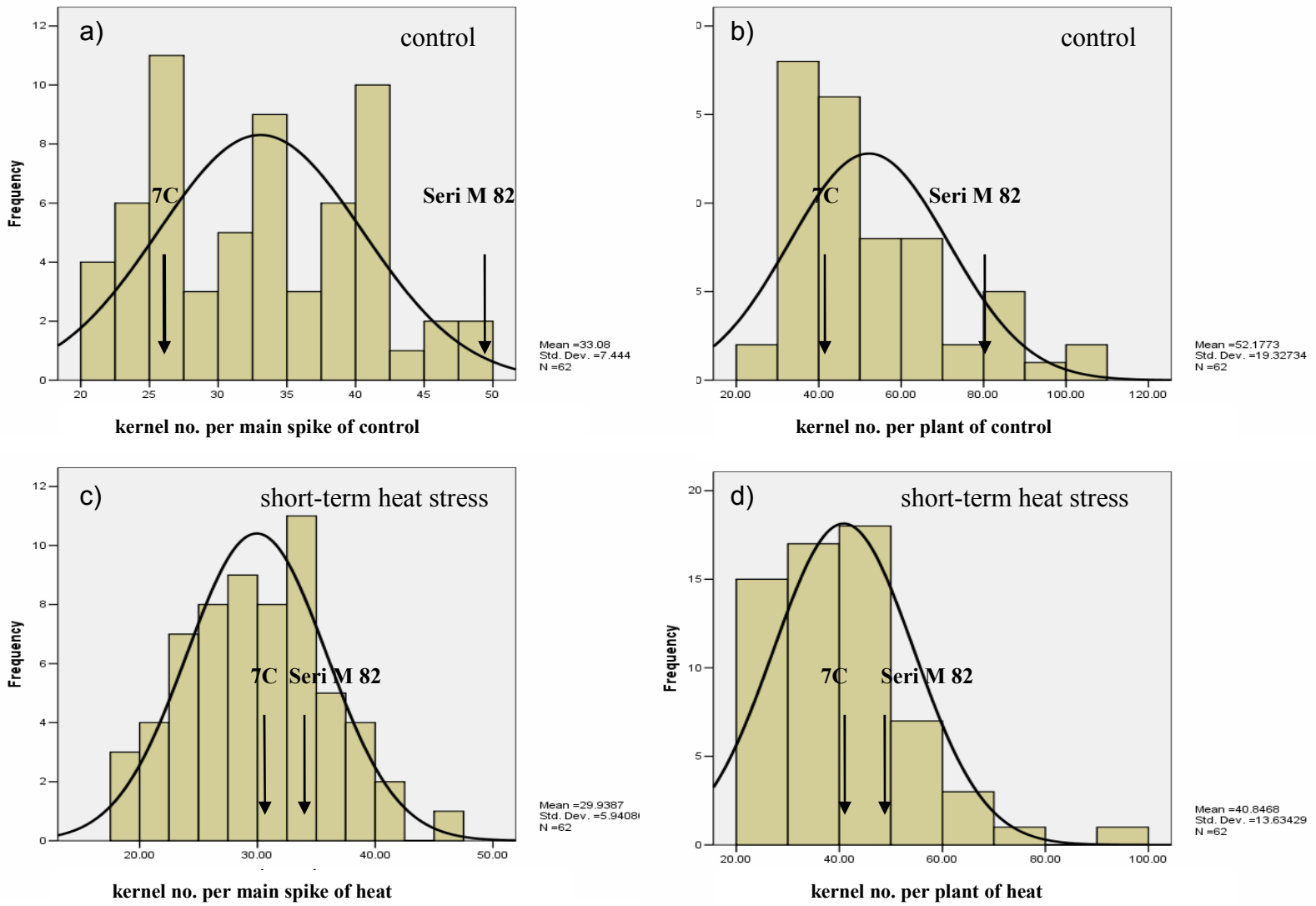


Fig. 2. Histogram of the distribution of the kernel number (no.) per main spike and per plant of control (a, b) and heat-treated (c, d) RILs after short-term heat stress of 38℃ 2 days beginning at 10 DAP for in an environmentally controlled growth rooms.

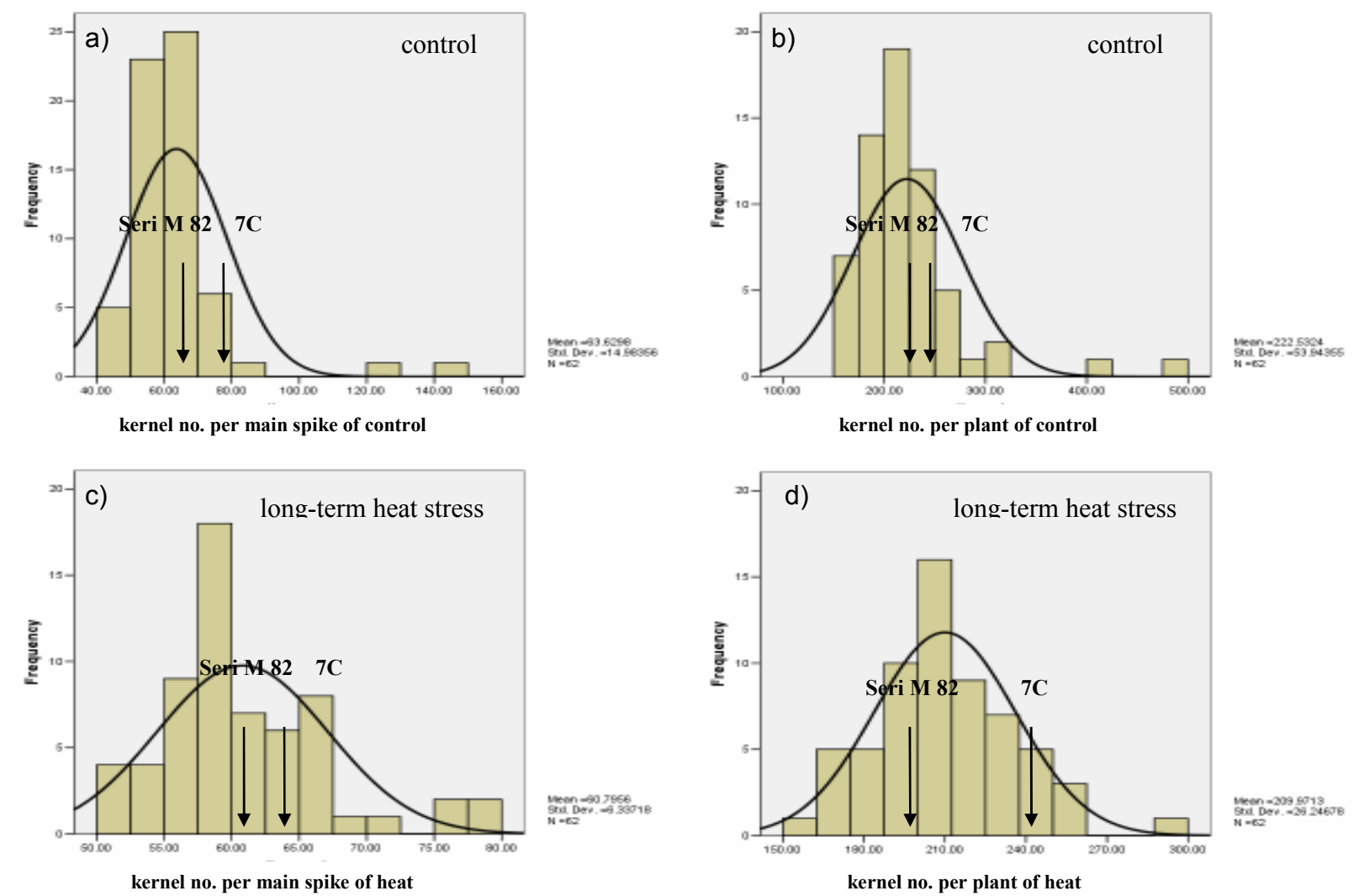


Fig. 3. Histogram of the distribution of the kernel number (no.) per main spike and per plant of control (a, b) and heat-treated (c, d) RILs after long-term heat stress of 30˚C beginning at 10 DAP until grain maturity in environmentally controlled growth rooms.

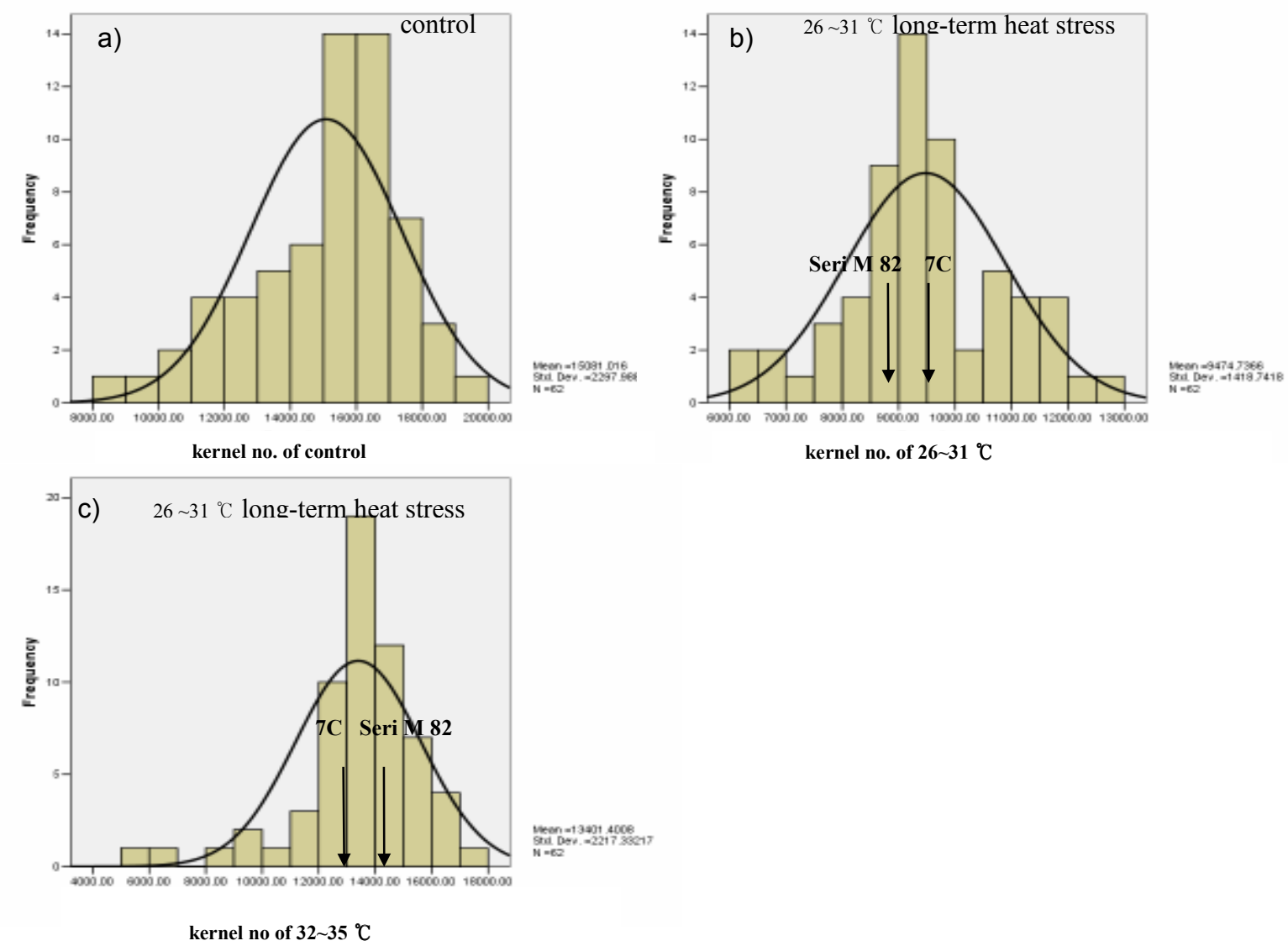


Fig. 4. Histogram of the distribution of the kernel number (no.)/m^2 (a, b) for the 62 RILs after temperate (a), and long-term heat stress of 26~31℃ (b) and 32~35℃ (c) in the field. Data provided by CIMMYT (1996-1997 for control, 2002-2003 for heat-treated RILs).

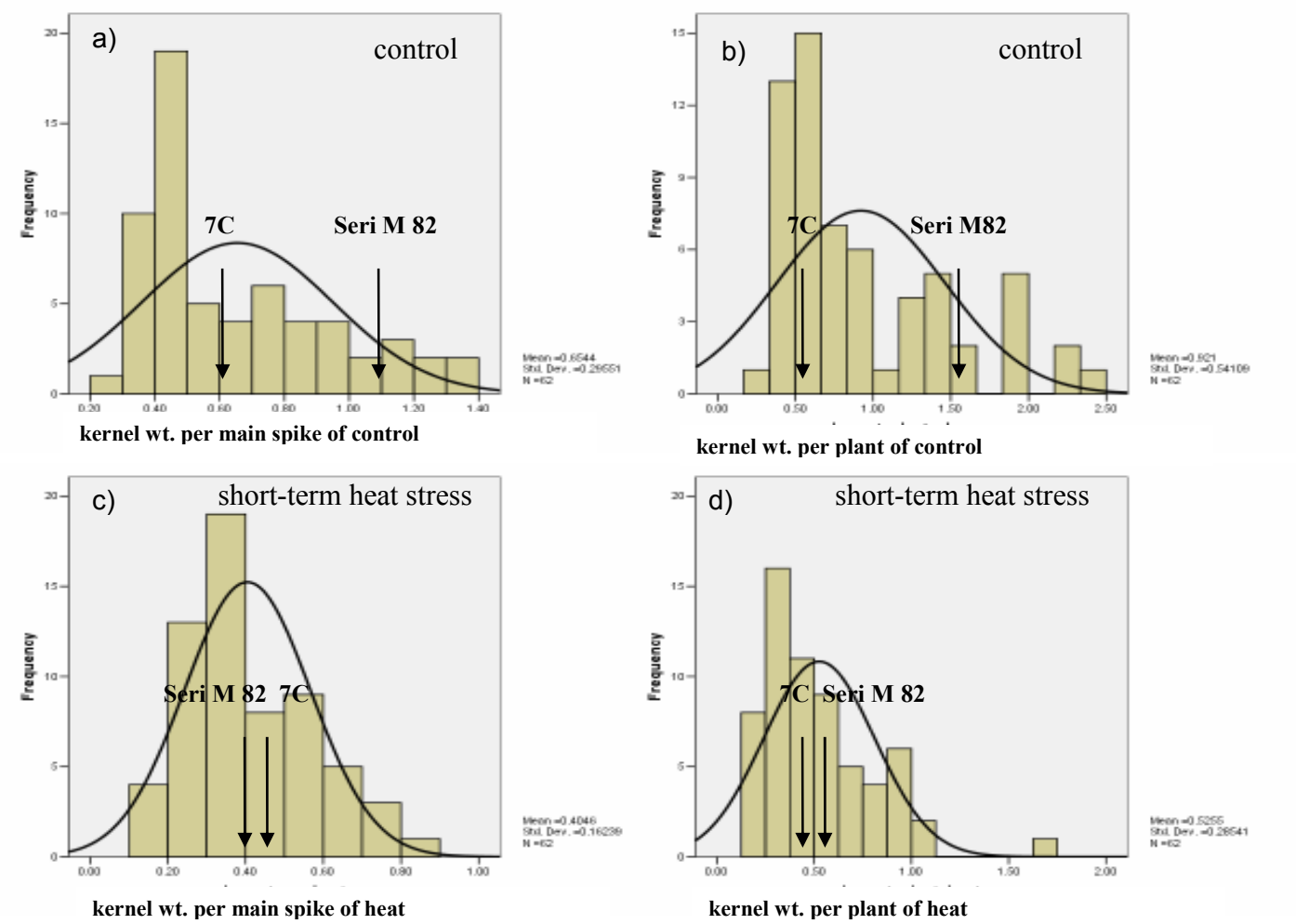


Fig. 5. Histogram of the distribution of the kernel weight (wt.) per main spike and per plant from control (a,b) and heat-treated group (c,d) RILs after a short-term heat stress of 38℃ for 2 days beginning at 10 DAP in an environmentally controlled growth chambers.

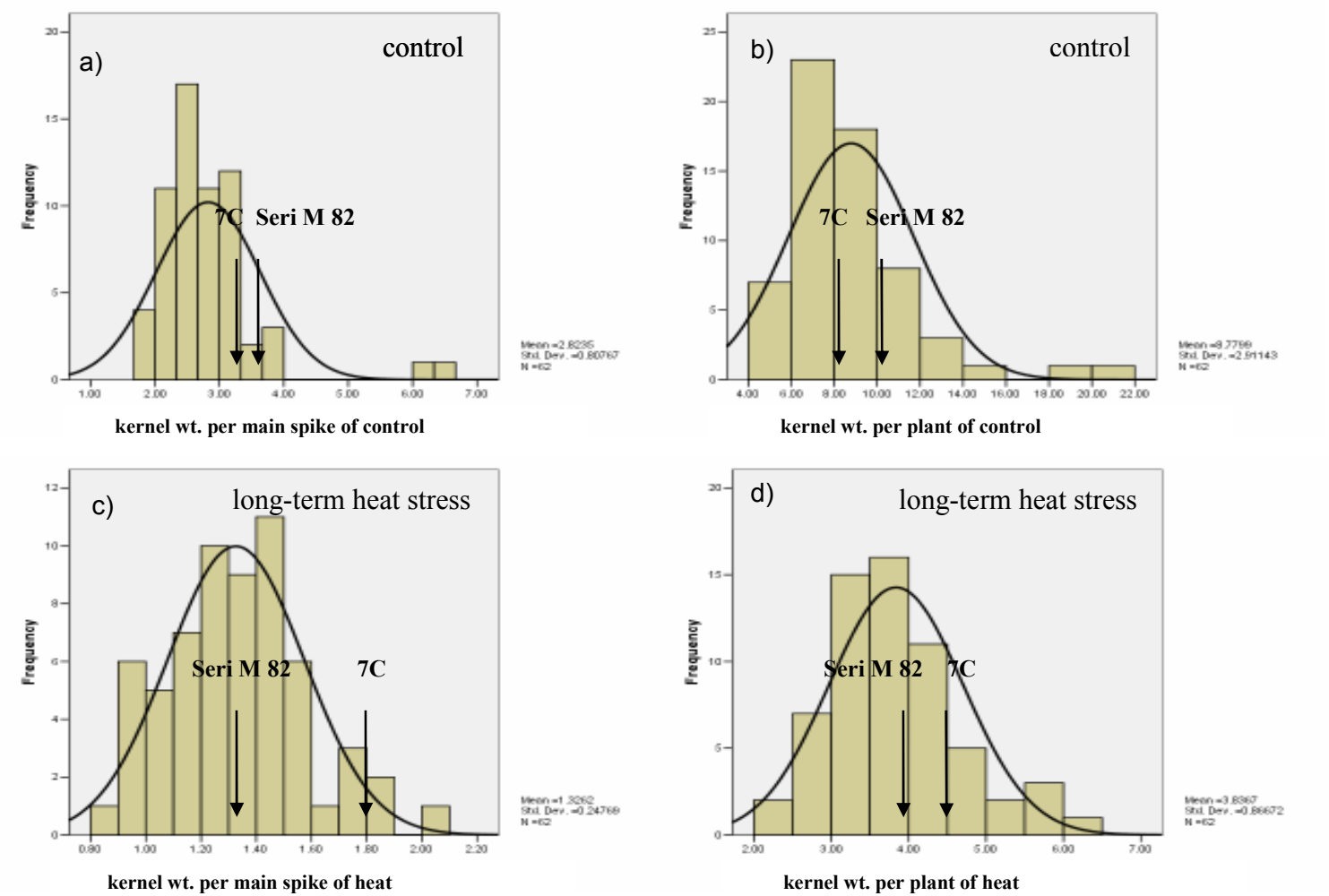


Fig. 6. Histogram of the distribution of the kernel number (no.) per main spike and per plant of control (a,b) and heat-treated (c,d) RILs after a long-term heat stress of 30℃ beginning at 10 DAP until grain maturity in environmentally controlled growth rooms.

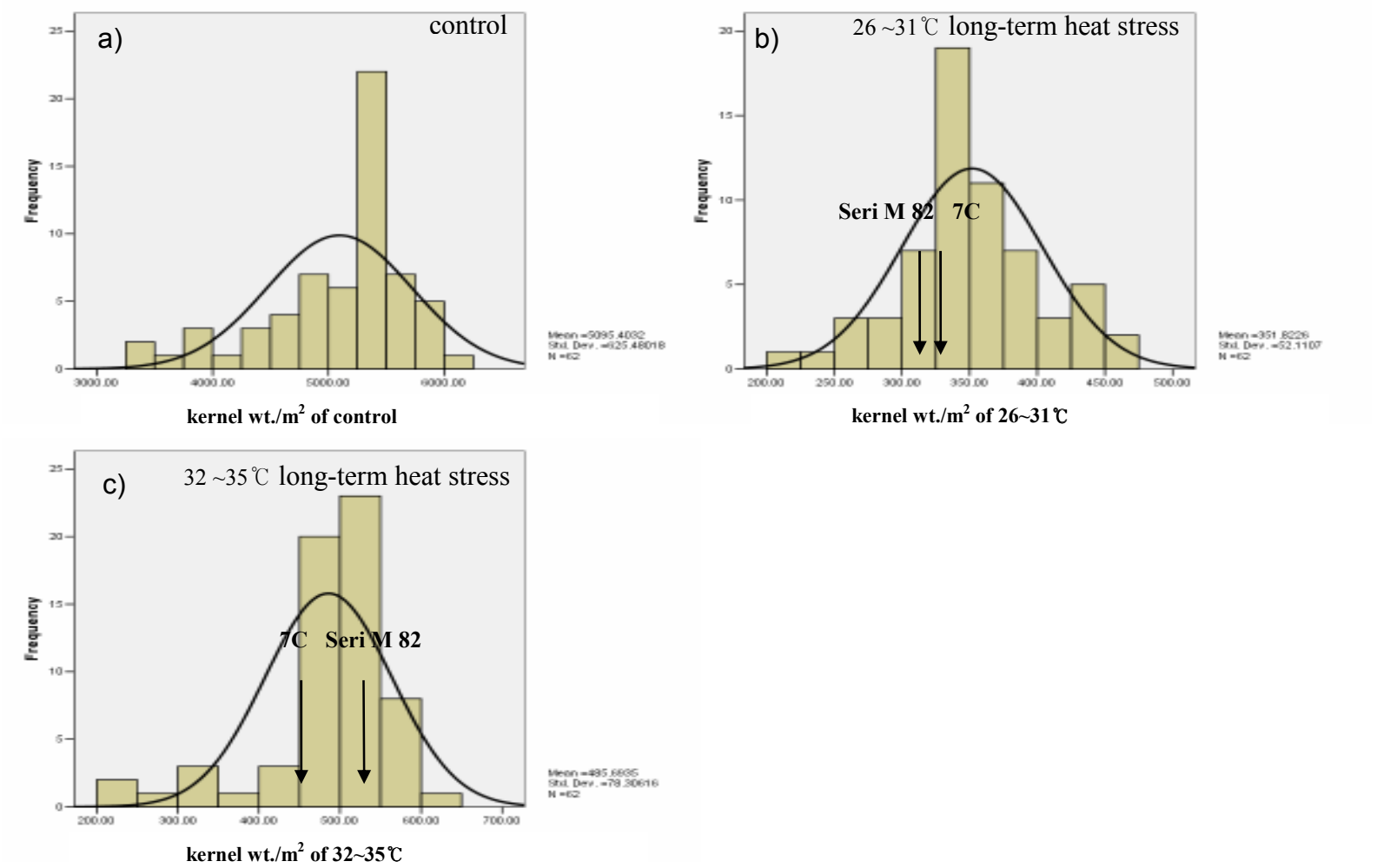


Fig. 7. Histogram of the distribution of the kernel weight (wt.)/m^2 for the 62 RILs after temperate(a) and long-term heat stress of 26~31℃(b) and 32~35℃(c) in the field. Data provided by CIMMYT (1996-1997 for control, 2002-2003 for heat-treated group).

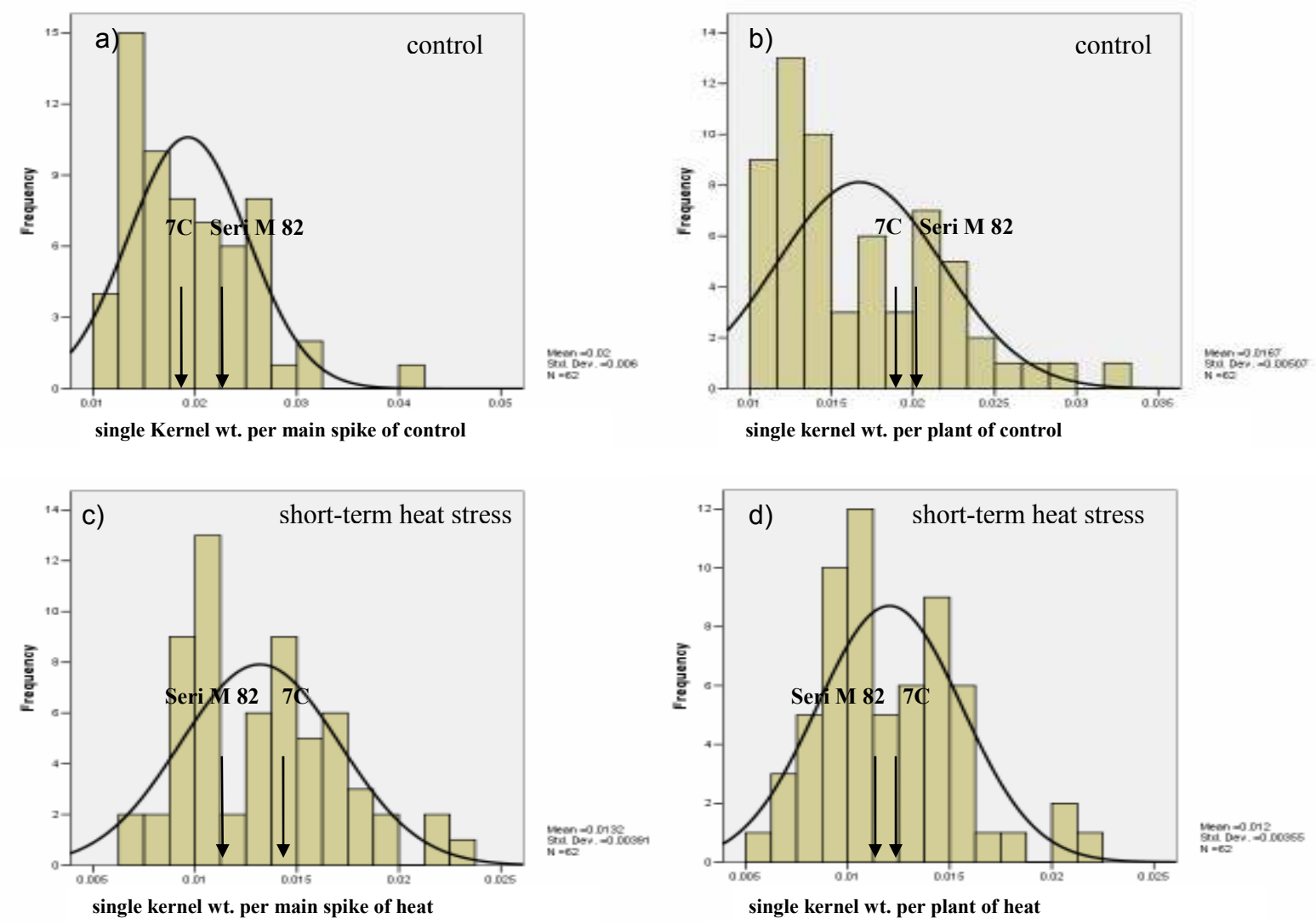


Fig. 8. Histogram of the distribution of the single kernel weight (wt.) per main spike and per plant of control (a,b) and heat-treated (c,d) RILs after a short-term heat stress of 38℃ for 2 days beginning at10 DAP in environmentally controlled growth rooms.

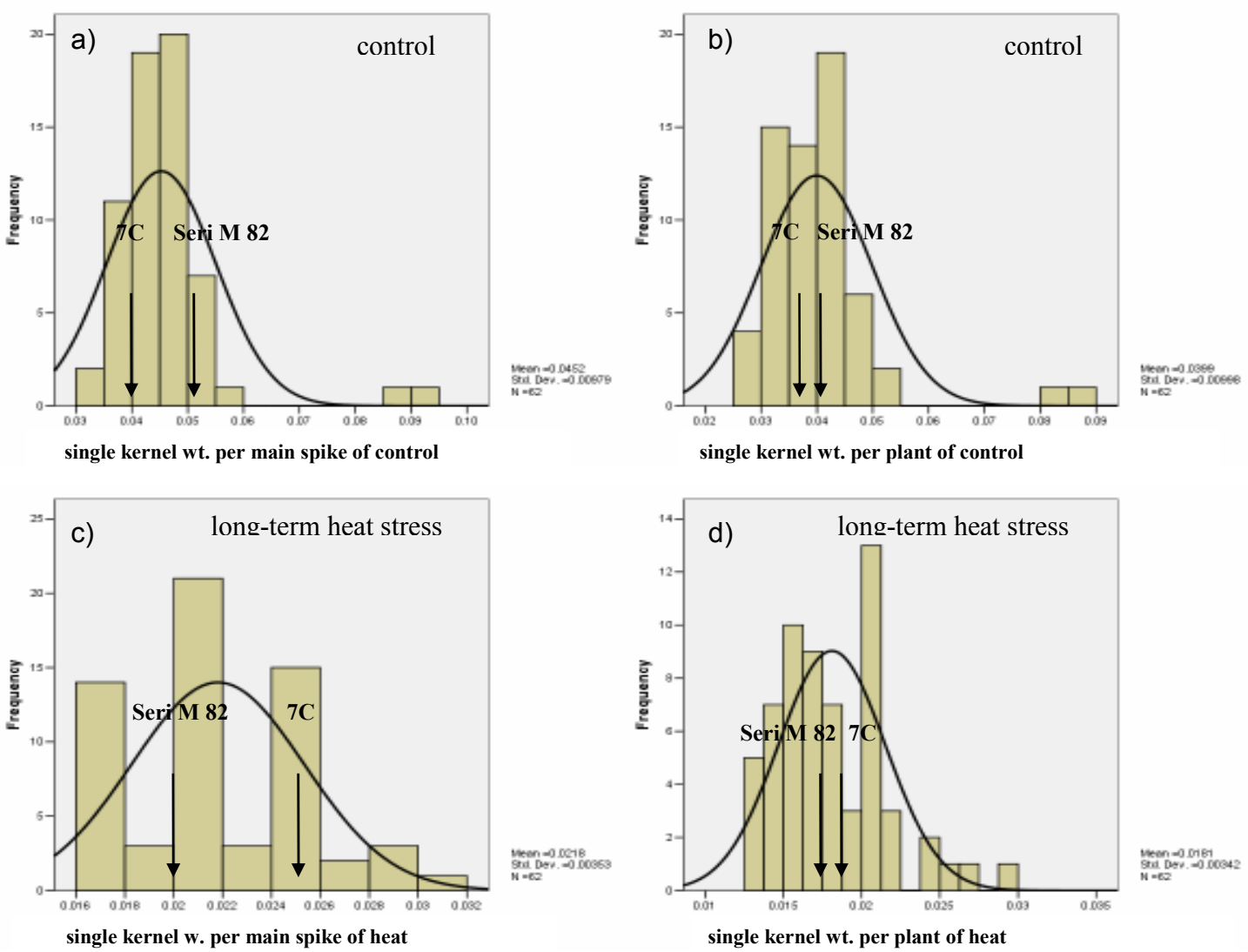


Fig. 9. Histogram of the distribution of the single kernel weight (wt.) per main spike and per plant of control (a, b) and heat treated (c, d) RILs after a long-term heat stress of 30℃ beginning at10 DAP until grain maturity in environmentally controlled growth rooms.

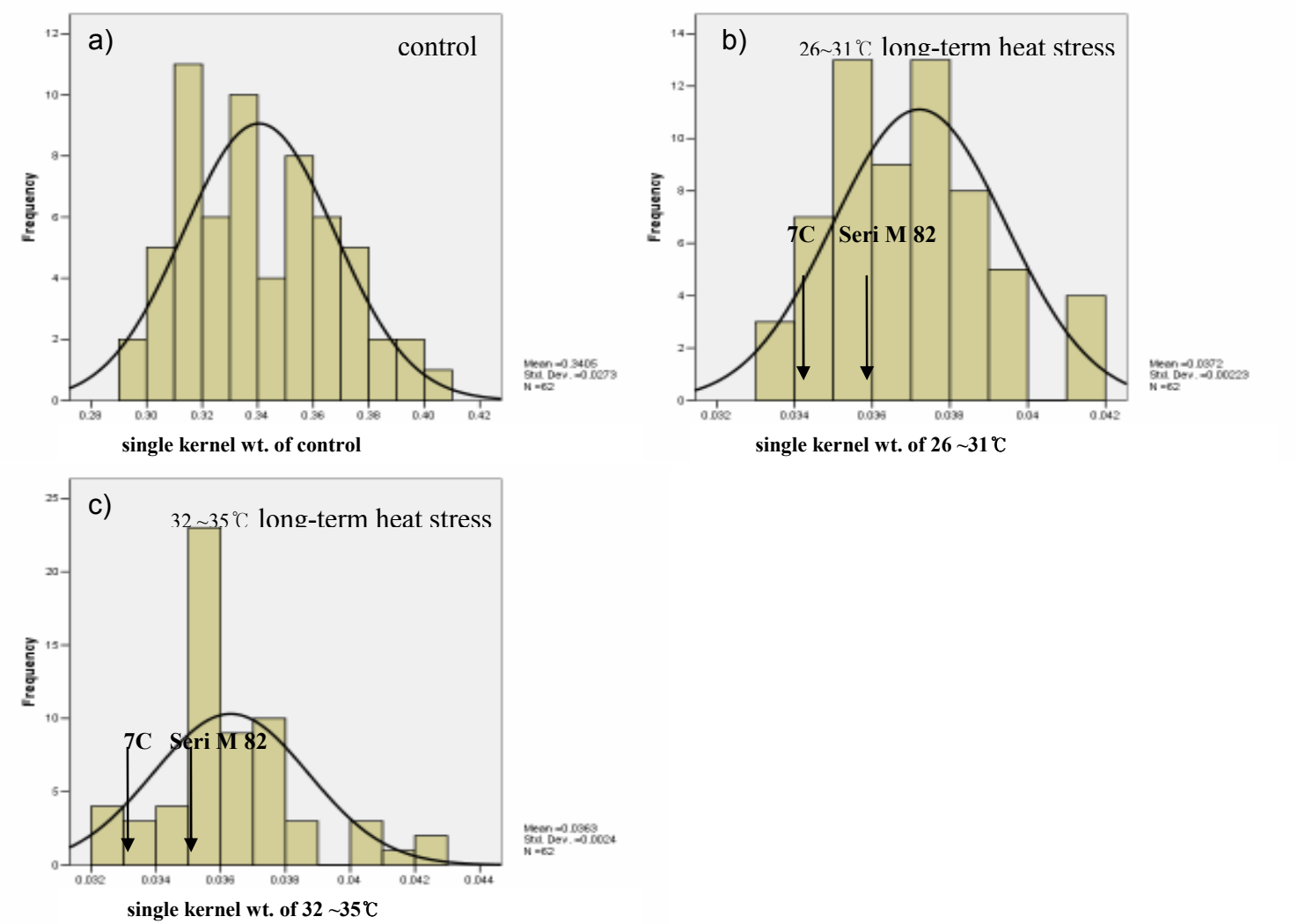


Fig. 10. Histogram of the distribution of the single kernel weight(wt.) for the 62 RILs after temperate(a) and long- term heat stress of 26~31℃(b) and 32~35℃(c) in the field. Data provided by CIMMYT (1996-1997 for control, 2002-2003 for heat-treated group).

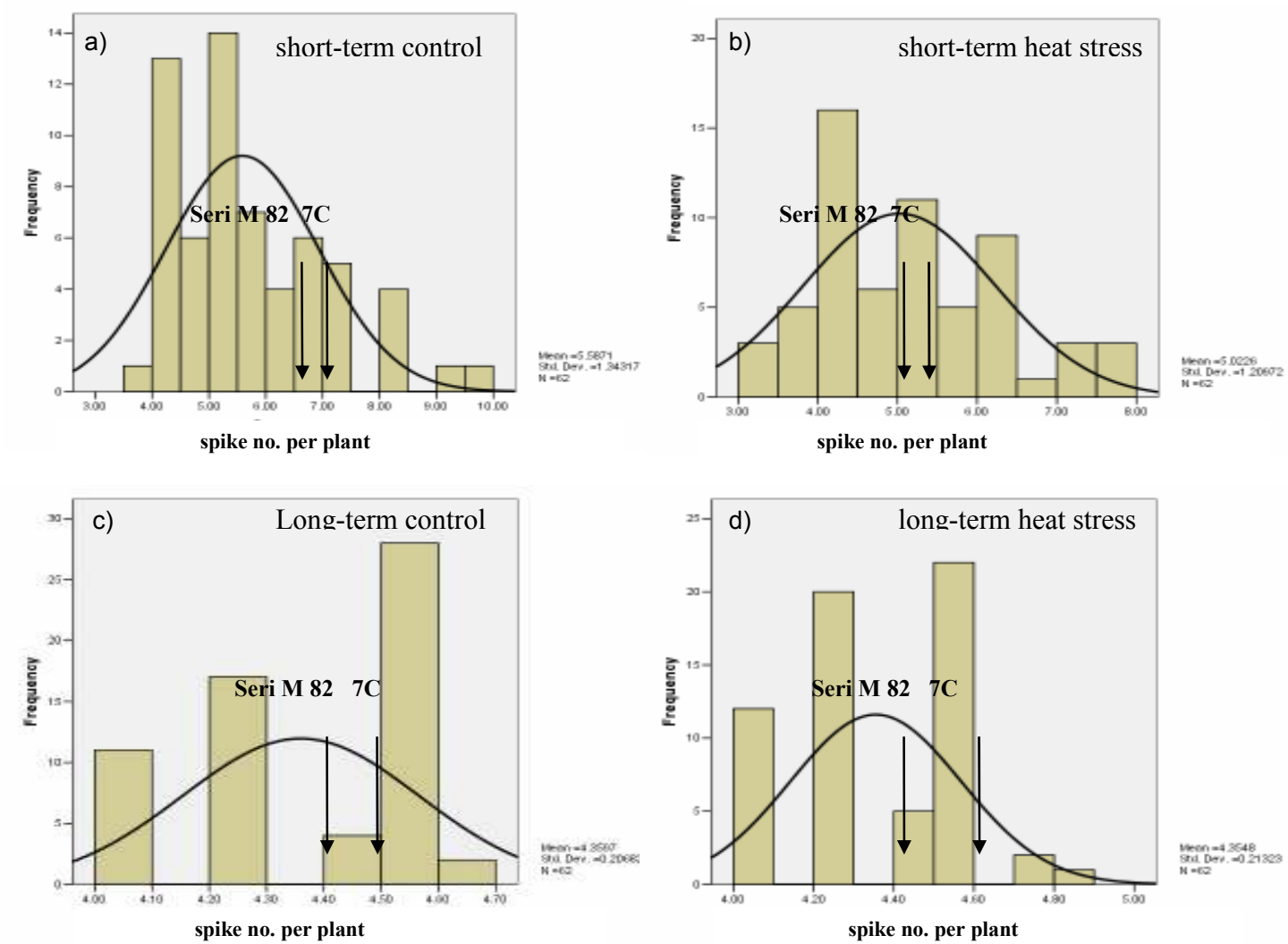


Fig. 11. Histogram of the distribution of the spike number (no.) per plant for the 62 RILs grown in a ideal control condition (a, c) and after a short-term heat stress of 38℃ for 2 days (b) beginning at 10 DAP in growth chambers and long-term heat stress of 30℃ (c, d) beginning at 10 DAP until grain maturity in environmentally controlled growth rooms.

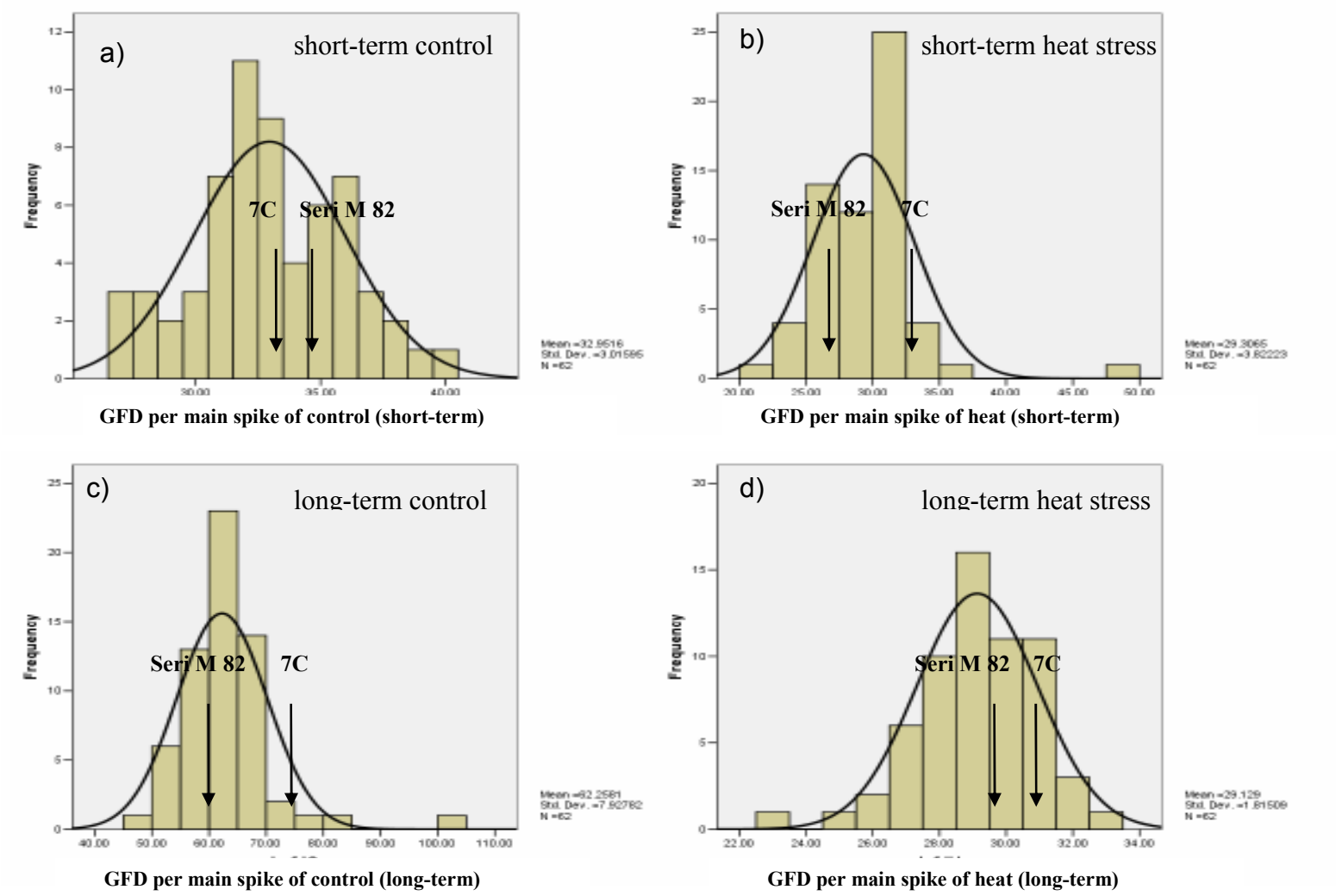


Fig. 12. Histogram of the distribution of the grain filling duration(GFD) per main spike for the 62 RILs after ideal conditions (a, c) or after a short-term heat stress of 38℃(a) for 2 days (b) or a long-term heat stress of 30℃ (d) beginning at 10 DAP until grain maturity in environmentally controlled growth rooms.

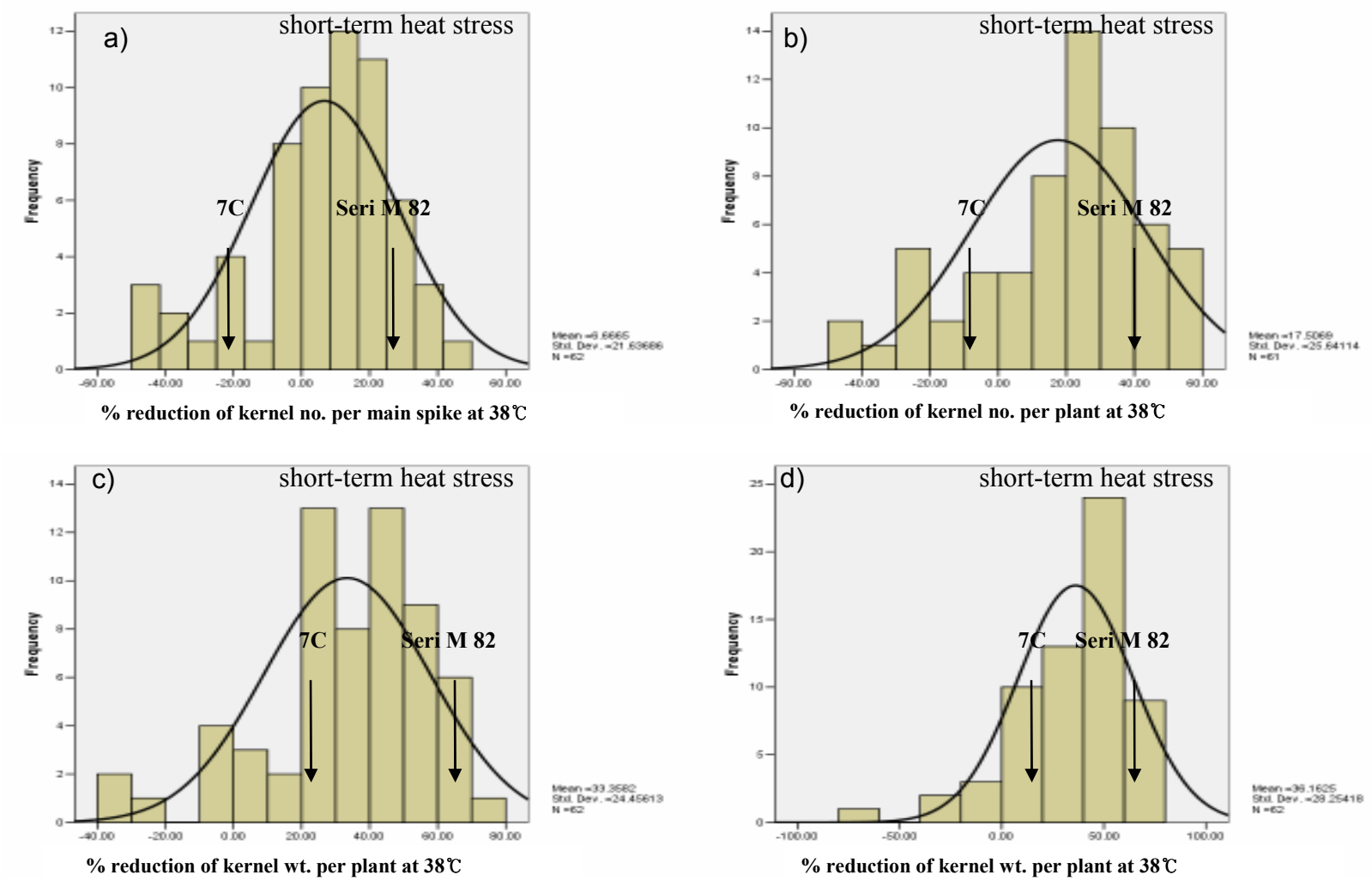


Fig. 13. Histogram of the distribution of the % reduction of kernel number (no.) (a, b) and kernel weight (wt) (c, d) per main spike and per plant for the 62 RILs after a short-term heat stress of 38℃ for 2 days beginning at 10 DAP in environmentally controlled growth chambers.

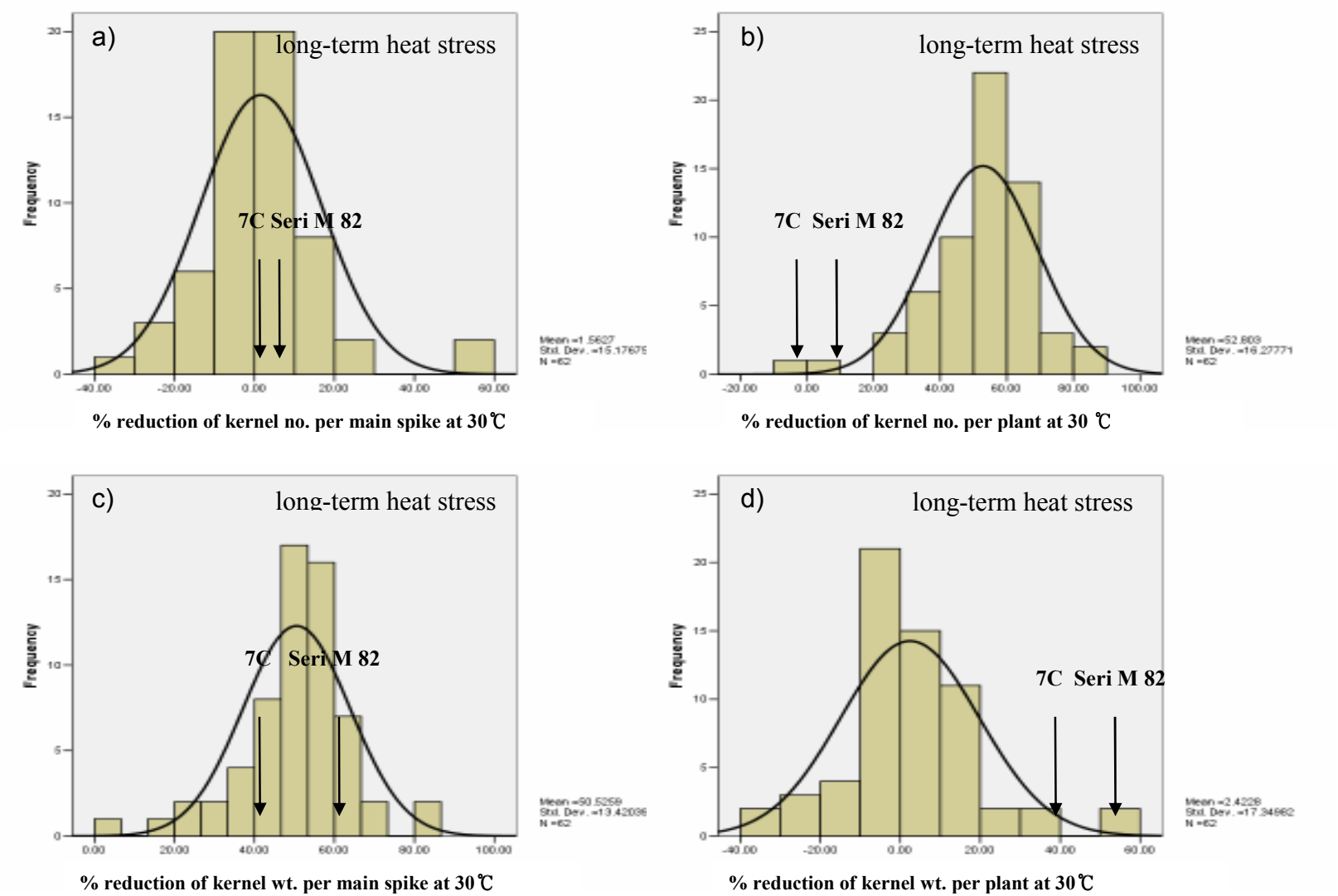


Fig.14. Histogram of the distribution of the % reduction of kernel number (no.) (a, b) and kernel weight (wt.) (c,d) per main spike and per plant for the 62 RILs after a long-term heat stress of 30℃ beginning 10 DAP until grain maturity in environmentally controlled growth rooms.

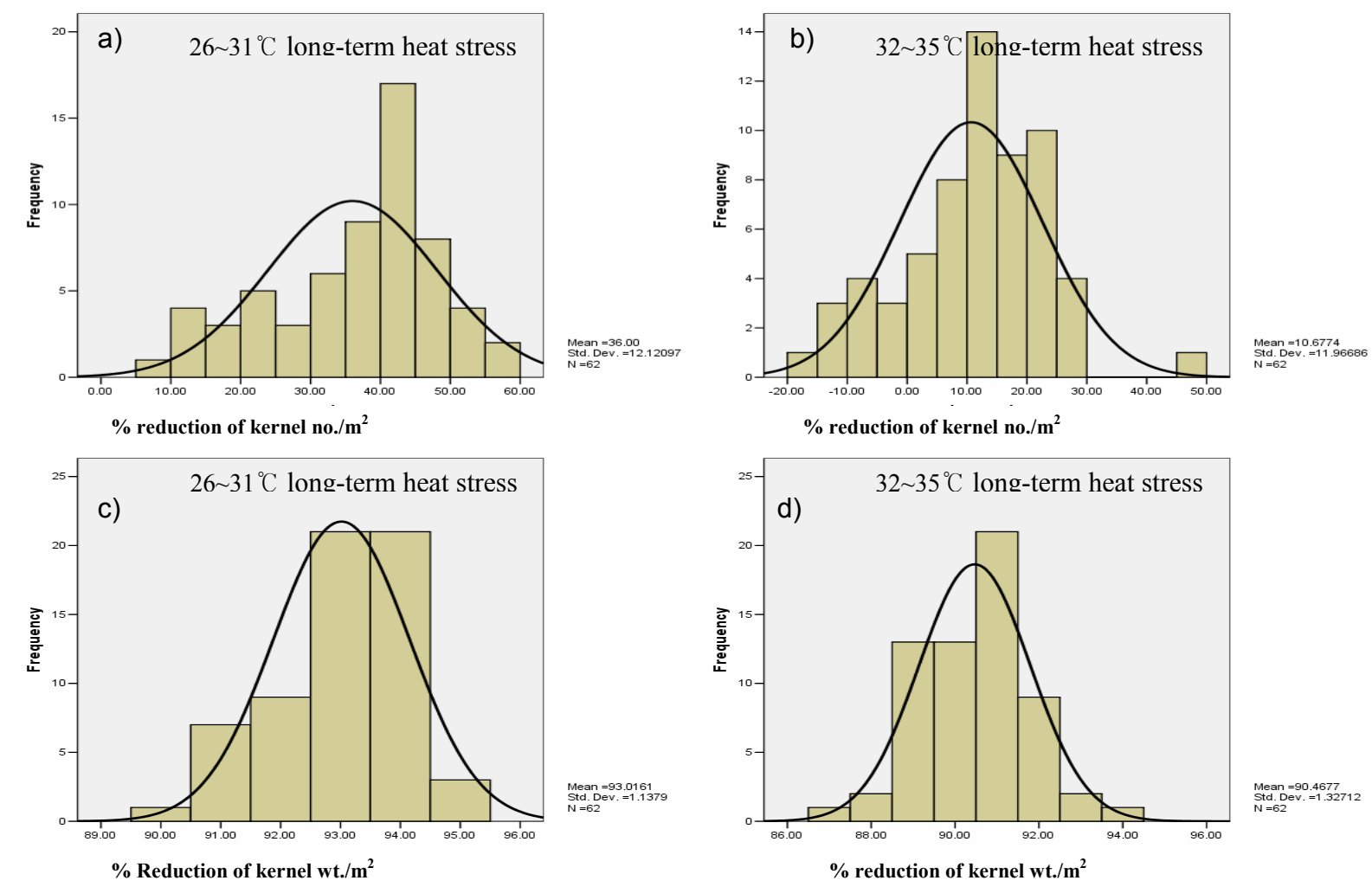


Fig. 15. Histogram of the distribution of the % reduction of kernel number (no)/m^2 (a, b) and kernel weight (wt)/m^2 (c, d) for the 62 RILs after long-term heat stress at 26~31℃ and 32~35℃ in the field. Data provided by CIMMYT (1996-1997 for control, 2002-2003 for heat-treated group).

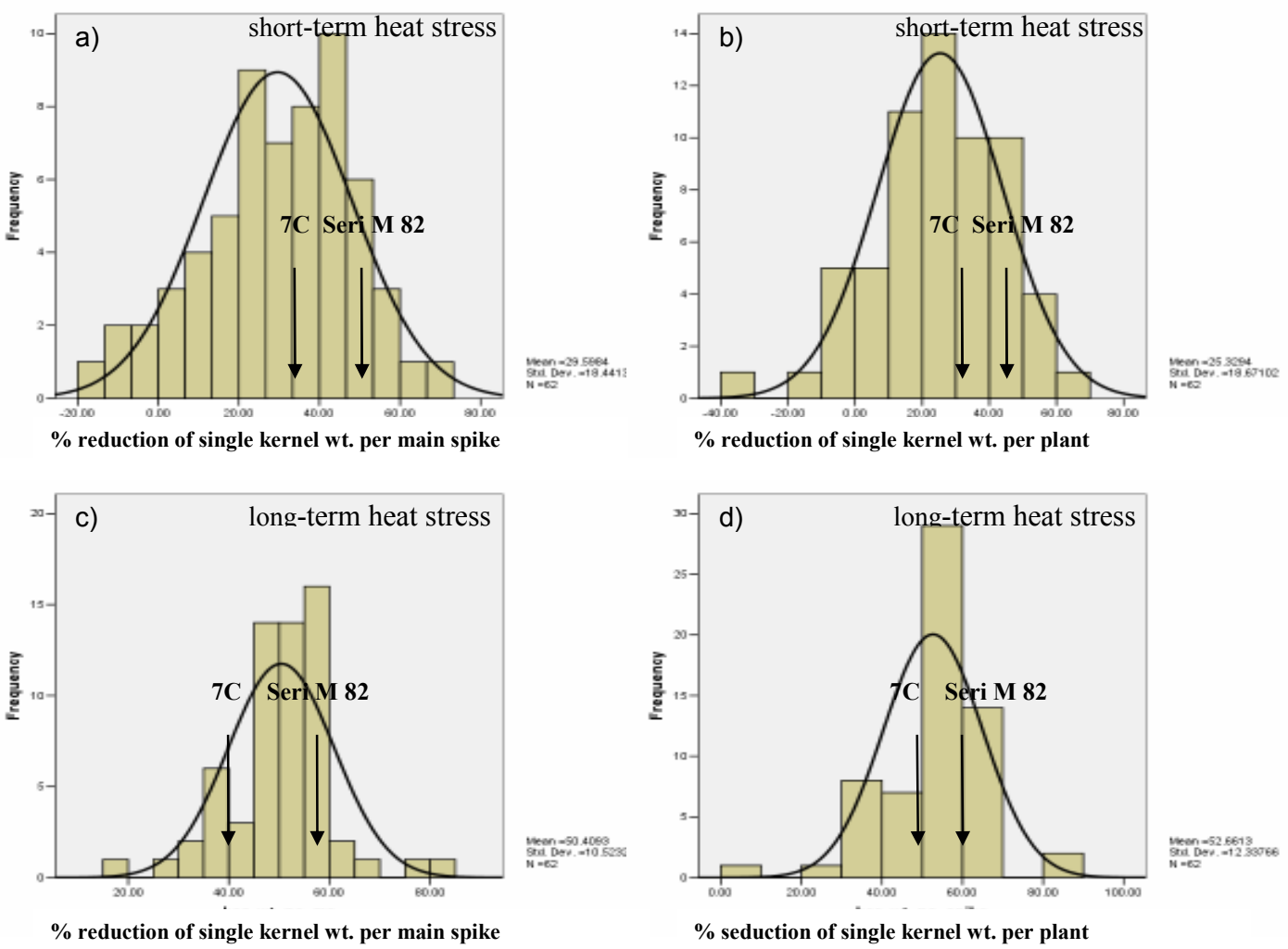


Fig. 16. Histogram of the distribution of the % reduction of the single kernel weight (kernel wt./ no.) per main spike and per plant for the 62 RILs after a short-term heat stress of 38℃ (a, b) for 2 days beginning at 10 DAP in growth chambers and long-term heat stress of 30℃ (c, d) beginning at 10 DAP until grain maturity in environmentally controlled growth rooms.

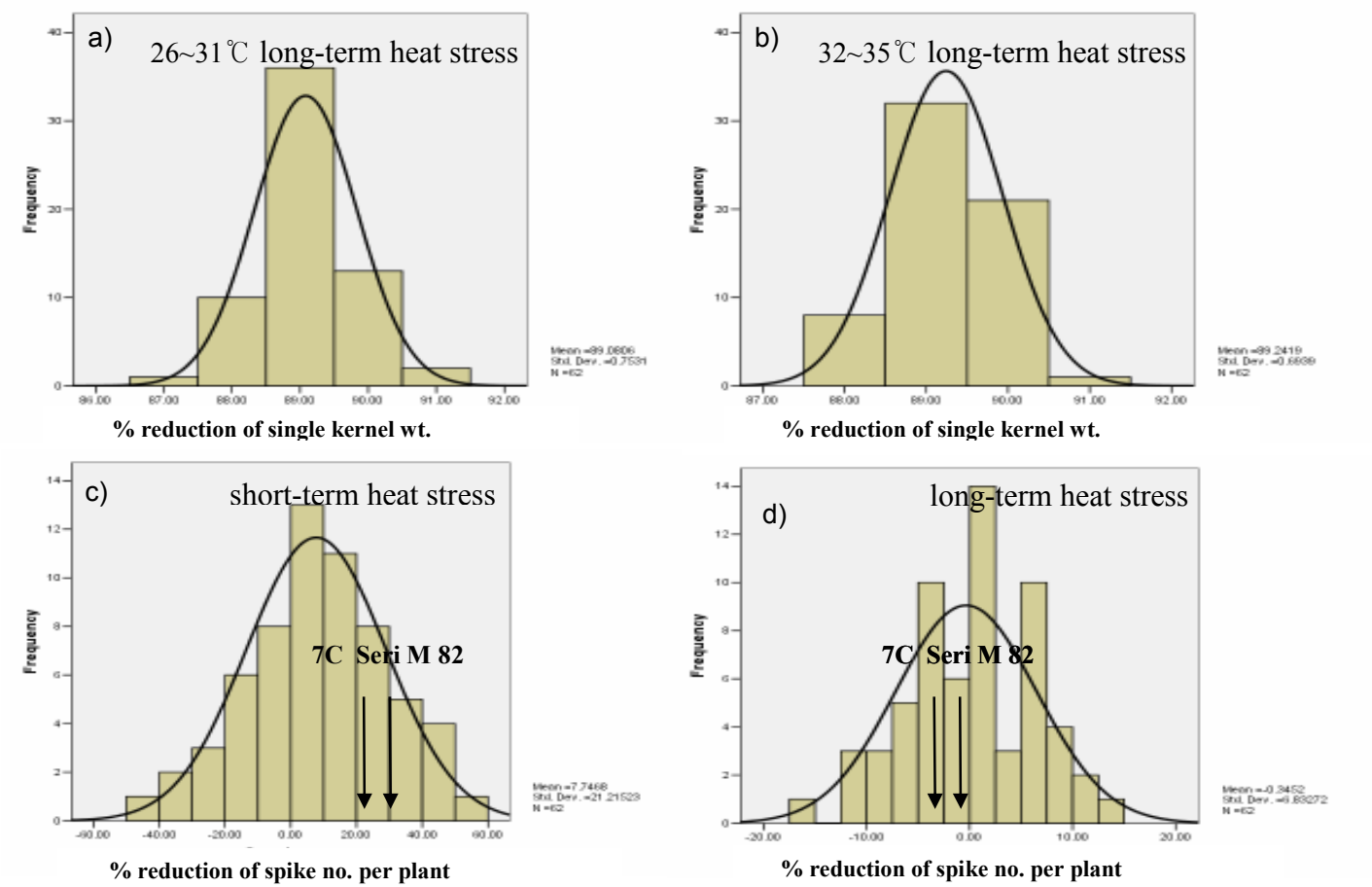


Fig. 17. Histogram of the distribution of the % reduction of the single kernel weight (kernel wt/ no.) per plant for the 62 RILs after long-term heat stress at 26~31℃ (a) and 32~35℃(b) in the field and spike no. per plant for the 62 RILs after short-term heat stress of 38℃ (a) for 2 days (c) beginning at 10 DAP in growth chambers and long-term heat stress of 30℃ from 10 DAP until grain maturity (d) in environmentally controlled growth rooms.

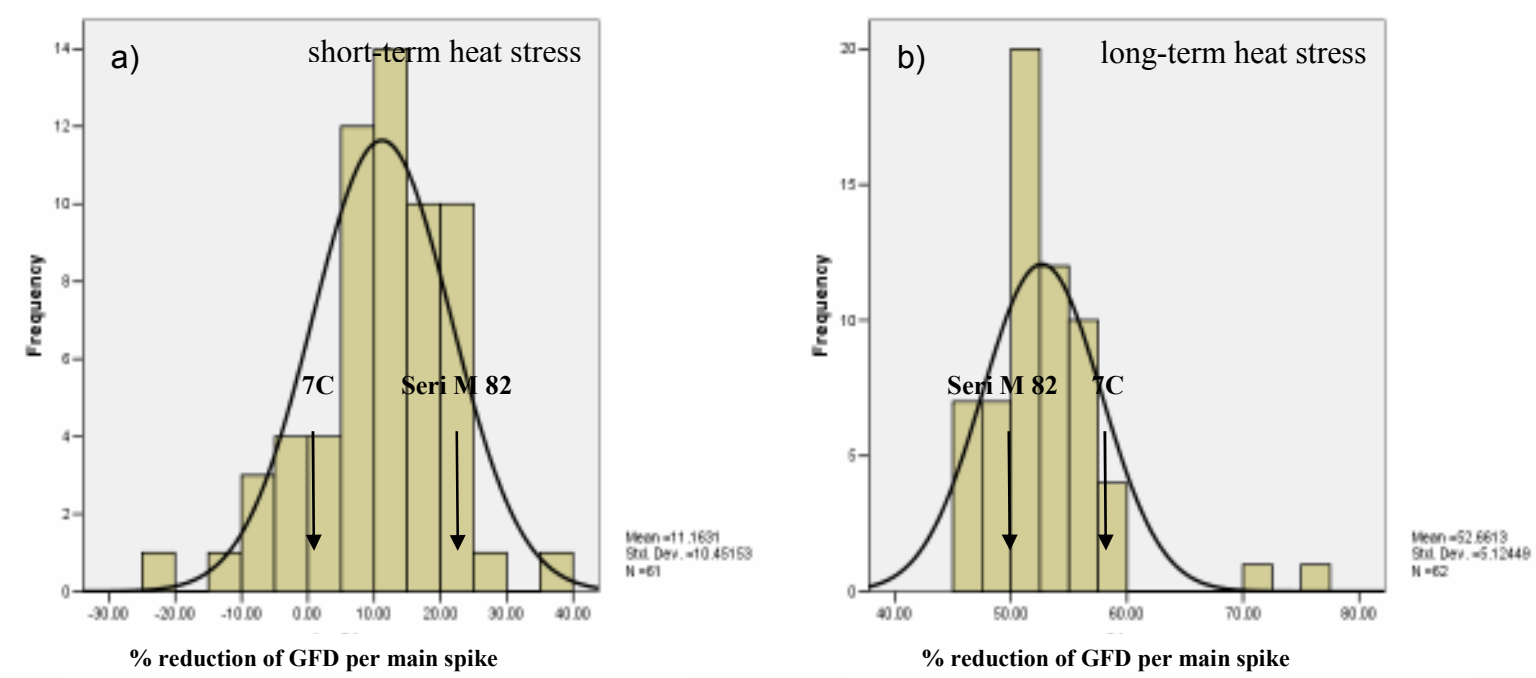


Fig. 18. Histogram of the distribution of the % reduction of grain filling duration (GFD) per main spike for the 62 RILs after short-term heat stress of 38℃(a) for 2 days beginning at 10 DAP in growth chambers and long-term heat stress of 30℃(b) from 10 DAP until grain maturity in an environmentally controlled growth room.

In this study, in addition to analysis of variation base on the distribution and standard deviation, the normality of the yield components was tested using Kolmogorov-Smirnov test. In the short-term heat stress experiment, the distribution of grain filling duration per main spike for control treat RILs and kernel number per main spike and spike number per plant for the heat treated RILs were normally distributed at α=0.01 and 0.05 (Table IV). Kernel number, single kernel weight and kernel weight per main spike and per plant for the control treated RILs and kernel weight and single kernel weight per main spike and per plant, and grain filling duration per main spike and kernel number per plant for the heat-treated population were also normally distributed for the short-term heat stress treated RILs at α=0.01 (Table IV). In case of the heat stressed RILs, only single kernel weight per main spike and kernel number per plant were not normally distributed at α=0.01 and 0.05.

In the long- term heat stress treatment, each yield component in the control treated RILs was not normally distributed (Table V). However, for the heat treated RILs, kernel weight per main spike, single kernel weight per main spike, kernel number and kernel weight per plant were normally distributed at both α=0.05 and α=0.01 and kernel number per main spike, single kernel weight per plant and grain filling duration per main spike were normally distributed at α=0.01(Table V). When the normality was tested for the same RILs grown in the field (Table VII) single kernel weight per plant and kernel number per plant were normally distributed at α=0.01 when grown in temperate temperatures. When the RILs were grown at moderate heat stress (26~ 31°C), only the kernel number per plant was normally distributed at both α=0.05 and α=0.01, and kernel

weight and single kernel weight per plant was normally distributed at α=0.01. However, when grown at 32 ~ 35°C, all yield components were not normally distributed (Table VII).

The normality test was also calculated on the basis of relative % reduction of yield components for the 62 RIL population. The relative % reduction of single kernel weight per main spike, single kernel weight per plant, spike number per plant and grain filling duration per main spike were normally distributed at both α=0.05 and α=0.01 in the response to short-term heat stress (Table VI). The relative % reduction of a kernel number per main spike, kernel weight per main spike were normally distributed at α=0.01 in response to short-term heat stress.

As for the long-term controlled heat stress, only the relative % reduction of kernel weight per plant and spike number per plant were distributed normally at both α=0.05 and α=0.01, which the relative % reduction of the kernel number per main spike, single kernel weight per main spike and kernel number per plant was also normally distributed at α= 0.01(Table VI).

In the field, only the relative % reduction of kernel number per plant in the long-term high temperature stress treatment of 32-35 ˚C exhibited a normal distribution at both $\alpha=0.05$ and $\alpha=0.01$(Table VII).

Traits with a normal distribution are often regulated by multiple QTLs with complex gene action as well as the degree of interaction between genes and environments resulting in a continuous variation (Kearsey and Pooni, 1996).

However it is unknown whether quantitative traits including yield components might be regulated by many genes in clustered or spread loci on the whole chromosomes with small genetic effects or specific major genes with large genetic effects among many genes.

Normal variation is required for identification of genes involved in quantitative traits. Thus in this study, all traits of yield components and their relative % reduction were analyzed to identify QTL for yield and yield components in response to heat stress.

Table. IV. Normality test for yield components from control-treated and heat-treated RILs after a short-term heat stress of 38˚C beginning at 10 DAP in growth chambers using by the Kolmogorov-Smirnov test.

	Short-Term Control			Short-Term Heat	
Standardized Residual for Taits	Kolmogorov-Smirnov (+)			Kolmogorov-Smirnov	
Traits	df	Statistic	Sig.	Statistic	Sig.
Kernel No. per Main Spike	62	.120	.026*	.067	.200
Kernel Wt. per Main Spike	62	.189	.000**	.121	.024*
Single Kernel Wt. per Main Spike	62	.121	.024*	.147	.010**
Kernel No. per Plant	62	.156	.000**	.137	.005**
Kernel Wt. per Plant	62	.211	.000**	.124	.020*
Single Kernel Wt. per Plant	62	.148	.002**	.116	.037*
Spike No. per Plant	62	.157	.001**	.107	.072
Grain Filling Duration of Main Spike	62	.089	.200	.113	.048*

(+) Lilliefors Significance Correction
*, ** indicate pair was statistically different from each other at the level of $p< 0.05$, $p< 0.01$

Table. V. Normality test for yield components from control-treated and heat-treated RILs after a long-term heat stress of 30˚C beginning at 10DAP until grain maturity in plant growth rooms using the Kolmogorov-Smirnov test.

	Long-Term Control			Long-Term Heat	
Standardized Residual for Taits	Kolmogorov-Smirnov (+)			Kolmogorov-Smirnov	
Traits	df	Statistic	Sig.	Statistic	Sig.
Kernel No. per Main Spike	62	.210	.000**	.123	.020*
Kernel Wt. per Main Spike	62	.160	.000**	.100	.222
Single Kernel Wt. per Main Spike	62	.219	.000**	.091	.200
Kernel No. per Plant	62	.146	.000**	.105	.200
Kernel Wt. per Plant	62	.168	.002**	.081	.118
Single Kernel Wt. per Plant	62	.192	.002**	.126	.016*
Spike No. per Plant	62	.157	.001**	.235	.000**
Grain Filling Duration per Main Spike	62	.156	.001**	.149	.016*

(+) Lilliefors Significance Correction
*, ** indicate pair was statistically different from each other at the level of $p < 0.05$, $p < 0.01$

Table. VI. Normality test for the % reduction of yield components after a short-term of 38˚C beginning at 10 DAP in growth chambers and long-term heat stress of 30˚C beginning at 10 DAP until grain maturity in plant growth rooms using Kolmogorov-Smirnov test.

	Short-Term			Long-Term	
Standardized Residual for Taits	Kolmogorov-Smirnov (+)			Kolmogorov-Smirnov	
% Reduction of Traits	df	Statistic	Sig.	Statistic	Sig.
Kernel No. per Main Spike	62	.210	.025*	.128	.014*
Kernel Wt. per Main Spike	62	.160	.028*	.137	.005**
Single Kernel Wt. per Main Spike	62	.219	.200	.123	.020*
Kernel No. per Plant	62	.146	.005**	.105	.033*
Kernel Wt. per Plant	62	.168	.007**	.117	.087
Single Kernel Wt. per Plant	62	.192	.200	.148	.002**
Spike No. per Plant	62	.044	.200	.109	.065
Grain Filling Duration per Main Spike	62	.092	.200	.139	.005**

(+) Lilliefors Significance Correction
*, ** indicate pair was statistically different form each other at the level of $p< 0.05$, $p<0.01$

Table VII. Normality test for the kernel no./m^2, kernel wt./m^2 and single kernel wt. of yield components and the % reduction of those yield components grown after a long-term heat stress of 26~31°C and 32~35°C in the field. Data provided by CIMMYT (1996-1997 for control, 2002-2003 for heat- treated group).

		Kolmogorov- Smirnov (+)					
		Control		26 ~31 ° C		32 ~ 35 ° C	
Standardized Residual for Taits	df	Statistic	Sig	Statistic	Sig	Statistic	Sig
Kernel No. per Plant	62	.126	.016*	.096	.200	.145	.002**
Kernel Wt. per Plant	62	.185	.000**	.123	.021*	.201	.000**
Single kernel Wt. per Plant	62	.085	.200	.123	.021*	.301	.000**
% Reduction of Kernel No. per Plant	62			.146	.002**	.108	.072
% Reduction of Kernel Wt. per Plant	62			.220	.000**	.188	.000**
% Reduction of Single Kernel Wt. per Plant	62			.301	.000**	.281	.000**

(+) Lilliefors Significance Correction
*, ** indicate pair was statistically different form each other at the level of $p< 0.05$, $p<0.01$

3-4. Correlation among traits of yield, its components and heat tolerance

The Pearson's correlation coefficients were analyzed between yield components to determine the co-segregation of yield traits in the RILs in response to both control unstressed and heat stressed treatments (Table VIII, Table IX). The highest correlation coefficients for both control and heat treated population were between kernel weight per main spike and kernel weight per plant (0.913 for control, 0.905 for heat treated population) in the short- term heat stress (Table VIII). Most of the other individual yield components including kernel number per main spike (0.632 and 0.548), single kernel weight per main spike (0.745 and 0.782), kernel number per spike (0.547 and 0.323), kernel weight per spike (0.845 and 0.786) and single kernel weight per plant (0.721 and 0.775) showed significant correlation with kernel weight per plant. However spike number per plant (0.100 and 0.121) and grain filling duration (0.133 and 0.311) per main spike were not significantly correlated with kernel weight per plant for either treatment (Table VIII). The kernel weight per plant and the kernel number per plant are components of yield. They showed significant correlation (0.853 and 0.804). However, the correlation between kernel numbers per plant with the individual yield components was not as significant as the correlation of kernel weight per plant. Grain filling duration correlated with yield in the heat treated RILs (0.311 for kernel weight and 0.227 for kernel number per plant) but not in the control treated population (0.133 for kernel weight and 0.069 per kernel number per plant).

In the long-term heat stress, the highest correlation for both control and heat treated population (0.964 for control and 0.956 for heat- treated population) was between

kernel weight per plant and kernel weight per spike (Table IX). Most of the yield components including kernel number per main spike (0.619 and 0.417), kernel weight per main spike (0.868 and 0.843) single kernel weight per main spike (0.651 and 0.743), kernel number per spike (0.748 and 0.573), single kernel weight per plant (0.759 and 0.845) exhibited a correlation with kernel weight per plant (Table IX). These results from long –term heat stress were similar to those for short-term heat stress. Grain filling duration also correlated more with yield in the heat treated RILs (0.317 for kernel weight and 0.018 for kernel number per plant) than in the control-treated RILs (-0.191 for kernel weight and -0.165 per kernel number per plant). This is similar to the pattern in the short-term heat stress, suggesting the important impact heat stress imposes on grain filling duration.

As in the short term heat stress, the correlation coefficients between yield components kernel number per plant and kernel weight per plant were significant (0.815 for control and 0.639 for heat treated RILs) (Table IX). However, the correlation between kernel numbers per plant with the rest of yield components was not as significant as the correlation of kernel weight per plant. A study of sorghum reported that productivity was more highly correlated with kernel number in the 249 RILs population and kernel weight in the other 379 RILs population (Rami et al., 1998). These results suggest that whether kernel number per plant or kernel weight per plant is the larger contributor to yield is genotypic specific. In this study, the yield components were more correlated with kernel weight than kernel number.

The Pearson correlation coefficients using the relative % reduction of the yield components were also analyzed to determine the co-segregation of the individual traits and the heritability of heat tolerance in the short-term and long- term heat stress (Table X). The relative % reduction of yield including kernel number per plant and kernel weight per plant had significant correlation with most of the other traits when RILs with a short-term or long-term heat stress. The relative % reduction of grain filling duration had a stronger correlation with the relative % reduction of kernel weight per plant (0.322 for short-term and 0.425 for long-term heat stress) than that of kernel number per plant (0.156 and 0.287) in both short-term and long-term heat stress treatments. In these results, the relative % reductions of all traits used in this study were correlated with heat tolerance leading to the conclusion that heat tolerance is quantitatively inherited for the individual components.

Table VIII. Pearson Correlation Coefficients between yield components after a short-term heat stress. The kernel number per main spike (Ker. no. / ms), kernel weight per main spike (Ker. wt. / ms), single kernel weight per main spike (Sin. ker. wt. / ms), kernel number per spike (Ker. no. / sp), kernel weight per spike (Ker. wt. / sp), single kernel weight per plant (Sin. Ker. wt. / pl), kernel number per plant (Ker. no. / pl), kernel weight plant (Ker. wt. / pl), spike number per plant (Sp. no. / pl), grain filling duration per plant (GFD / ms) of the 62 RILs from control (a) and heat-treated (b) RILs after a short-term heat stress of 38°C beginning at 10 DAP in growth chambers (N= 256 Prob > |r| under Ho: Rho=0).

Traits	Ker. no. / ms	Ker. wt / ms	Sin. ker. wt / ms	Ker. no. / sp	Ker. wt / sp	Sin. ker. wt. / pl	Ker. no. / pl	Ker. wt. / pl	Sp. no. / pl	GFD. / ms
Ker. no. / ms	1	.724**(a) .655**(b)	.233** .198**	.838** .805**	.704** .633**	.331** .226**	.716** .726**	.632** .548**	.177* .149*	.068 .058
Ker. wt. / ms	-	1	.814**(a) .833**(b)	.607** .429**	.925** .897**	.805** .824**	.751** .658**	.913** .905**	.132 .096	.197** .242**
Sin. Ker. wt / ms	-	-	1	.204**(a) .066(b)	.735** .750**	.893** .934**	.466** .375**	.745** .782**	.069 .040	.264** .328**
Ker. no. / sp	-	-	-	1	.741**(a) .624**(b)	.225** .063	.618** .537**	.547** .323**	.110 .148*	-.002 .018
Ker. wt. / sp	-	-	-	-	1	.793**(a) .787**(b)	.639** .555**	.845** .786**	.118 .124	.161** .248**
Sin. ker. wt. / pl	-	-	-	-	-	1	.375**(a) .321**(b)	.721** .775**	.070 .040	.258** .332**
Ker. no. / pl	-	-	-	-	-	-	1	.853**(a) .804**(b)	.077 .164	.069 .227**
Ker. wt. / pl	-	-	-	-	-	-	-	1	.100(a) .121(b)	.133** .311**
Sp. no. / pl	-	-	-	-	-	-	-	-	1	.012(a) .107(b)
GFD / ms	-	-	-	-	-	-	-	-	-	1

*, ** indicate pair was statistically different from each other at the level of $p< 0.05$, $p<0.01$

Table IX. Pearson Correlation Coefficients between yield components after a long-term heat stress. The kernel number per main spike (Ker. no. / ms), kernel weight per main spike (Ker. wt. / ms), single kernel weight per main spike (Sin.. Ker. wt. / ms), kernel number per spike (Ker. no. / sp), kernel weight per spike (Ker. wt. / sp), single kernel weight per plant (Sin. ker. wt. / pl), kernel number per plant (Ker. no. / pl), kernel weight plant (Ker. wt. / pl), spike number per plant (Sp. no. / pl), grain filling duration per plant (GFD / ms) of the 62 RILs from control (a) and heat-treated (b) RILs after a long-term heat stress of 30°C beginning at 10DAP until grain maturity in plant growth rooms (N= 256 Prob > |r| under Ho: Rho=0).

Traits	Ker. no. / ms	Ker. wt / ms	Sin. ker. wt / ms	Ker. no. / sp	Ker. wt / sp	Sin. ker. wt. / pl	Ker. no. / pl	Ker. wt. / pl	Sp. no. / pl	GFD. / ms
Ker. no. / ms	1	.740**(a) .532**(b)	.093 .038	.787** .638**	.637** .437**	.212** .156	.736** .593**	.619** .417**	.162* -.019	-.207** -.023
Ker. wt. / ms	-	1	.732**(a) .853**(b)	.667** .434**	.888** .845**	.735 ** .830**	.648** .410**	.868** .843**	.145* -.028	-.191** .316**
Sin. ker. wt. / ms	-	-	1	.189**(a) .138*(b)	.667** .734**	.887** .896**	.203** .135*	.651** .743**	.049 -.036	-.074 .362**
Ker. no. / sp	-	-	-	1	.788**(a) .652**(b)	.246** .173	.913** .854**	.748** .573**	.215** .069	-.202** -.045
Ker. wt. / sp	-	-	-	-	1	.781**(a) .842**(b)	.747** .563**	.964** .956**	.189** .042	-.215** .279**
Sin. ker. wt. / pl	-	-	-	-	-	1	.258**(a) .163*(b)	.759** .845**	.062 -.002	-.143* .400**
Ker. no. / pl	-	-	-	-	-	-	1	.815**(a) .639**(b)	.222** .025	-.165* .018
Ker. wt. / pl	-	-	-	-	-	-	-	1	.195(a) .017(b)	-.191** .317**
Sp. no. / pl	-	-	-	-	-	-	-	-	1	.100(a) -.076(b)
GFD / ms	-	-	-	-	-	-	-	-	-	1

*, ** indicate pair was statistically different from each other at the level of $p< 0.05$, $p<0.01$

Table X. Pearson Correlation Coefficients between the % reductions of yield components. The kernel number per main spike (Ker.no./ms), kernel weight per main spike (Ker.wt./ms), single kernel weight per main spike (Sin.ker.wt./ms), kernel number per spike (Ker.no./sp), kernel weight per spike (Ker.wt./sp), single kernel weight per plant (Sin.ker.wt./pl), kernel number per plant (Ker.no./pl), kernel weight plant (Ker.wt./pl), spike number per plant (Sp.no./pl), grain filling duration per plant (GFD./ms) of the 62 RILs after a short-term (a) heat stress of 38˚C beginning at 10 DAP in growth chambers and long-term (b) heat stress of 30˚C beginning at 10DAP until grain maturity in plant growth rooms (N= 256 Prob > |r| under Ho : Rho=0).

Traits	Ker. no. / ms	Ker. wt / ms	Sin. ker. wt / ms	Ker. no. / sp	Ker. wt / sp	Sin. ker. wt. / pl	Ker. no. / pl	Ker. wt. / pl	Sp. no. / pl	GFD. / ms
Ker. no. / ms	1	.706**(a) .723**(b)	.037 .537**	.826** .817**	.706** .630**	.150 .561**	.759** .630**	.626** .790**	.092 .064	.131 .503**
Ker. wt. / ms	-	1	.697**(a) .915**(b)	.542** .711**	.887** .931**	.722** .913**	.717** .920**	.904** .649**	.228 -.002	.313* .364**
Sin. ker. wt. / ms	-	-	1	-.001(a) .5 97** (b)	.539** .849**	.862** .931**	.321** .840**	.656** .538**	.272* -.039	.361** .465**
Ker. no. / sp	-	-	-	1	.730**(a) .760**(b)	.044 .588**	.602** .737**	.448** .918**	-.181 -.021	.021 .422**
Ker. wt. / sp	-	-	-	-	1	.674**(a) .929**(b)	.609** .977**	.804** .679**	-.003 -.029	.236 .270*
Sin. ker. wt. / pl	-	-	-	-	-	1	.317**(a) .920**(b)	.737** .533**	.250** -.021	.380* .382**
Ker. no. / pl	-	-	-	-	-	-	1	.799**(a) .739**(b)	.566** .310*	.156 .287*
Ker. wt. / pl	-	-	-	-	-	-	-	1	.442(a)** .140(b)	.322**(a) .425**(b)
Sp. no. / pl	-	-	-	-	-	-	-	-	1	.211(a) -.028(b)
GFD / ms	-	-	-	-	-	-	-	-	-	1

*, ** indicate pair was statistically different from each other at the level of $p< 0.05$, $p<0.01$

3-5. Analysis of variance (ANOVA) and heritability for yield and its components

An ANOVA was used to estimate effect of environments on yield components in the set of the 62 RILs, and the heritability of the yield components in the four heat stress environments (Table XI).

The ANOVA revealed significantly different genotype by environment effect for each yield component (α=0.01 and 0.05) (Table XI). The narrow sense heritability of the different traits ranged from 0.42 to 25% (Table XI). The heritability of kernel number per plant or yield was the highest at 25% and kernel number per main spike was second at 10%. The rest of traits had low heritability from 0.42~5% indicating the strong influence that environment exerts on these traits. In this study, the effect of genotype x environment was significant and heritability of all traits were very low. In comparison, the inheritance of similar traits such as kernel weight (55~74) and kernel number (45~75%) for sorghum and plant grain weight (11~13%), ear grain weight (31~70%) and ear grain number (21%) for spring barley were relatively high (Rami et al., 1998: Bezant et al., 1997). This might be due to significant genotype x environment effect derived from the marked differences between environments measured in this study. If their data will be used for the identification QTLs, the phenotypic variation for individual components should not be significant in their variation within replications of each individual RIL in the population. In this study, the variation of replications of all yields components was not significantly different at α=0.01 and 0.05 (Table XI).

As for the ANOVA used to estimate effect of various environments of high temperature on the heat tolerance of the different genotypes in the 62 RIL population

and the heritability of heat tolerance on the basis of the relative % reduction of the yield components, only the spike number per plant show no environmental variation (Table XII). This indicates that relative % reduction of spike number per plant in the 62 RILs was not affected by the heat treatment. The narrow sense heritability for heat tolerance was very low in the range of 0.1~2 % even though the correlation between the relative % reduction of each yield component with yield were very significant. This suggests that the environmental effect was higher than experimental error within replications. The heritability for heat tolerance in this study was very low compared to the results of 67 % and 89% by Blum et al. (2001) and Fokar et al.(1998), respectively. They calculated broad-sense heritability and heat tolerance based on cellular thermostability (CMS) and triphenyl tetrazolium chloride (TTC) assays at the seedling stage. Thus it appears that the higher narrow sense heritability of quantitative traits derives from using varied environments.

Table XI. Analysis of variance (ANOVA) and narrow sense heritability (h^2) for each yield component trait from parents and RILs between individual heat treatments (heat treatment during a short-term of 38°C for 2 days beginning at 10 DAP in growth chambers, long-term of 30°C beginning at 10 DAP until grain maturity in environmental controlled rooms, and long-term at 26~31°C and 32~35°C in the field).

Phenotype traits	Source (Mean Square)					
	Env.	Rep (env.)	Genotype	Genotype x Env.	Error	Heritability (h^2)
d.f.	3	8	61	183	488	
Ker.no. per ms	63442.7**	15.43	289.5**	118.4**	25.61	10 %
Ker.wt. per ms	242.6**	.2554**	.5296**	.3248**	.0662	5.0 %
Sin.ker.wt. per ms	.035233**	.000018	.000128**	.000059**	.000015	8.0 %
GFD per ms	47676.0**	17.3	81.46**	54.27**	7.67	4.4 %
Spike no. per pl.	69.103094**	2.115	4.1974**	2.3781**	1.2755	4.2 %
d.f.	6	14	61	366	854	
Sin.ker.wt. per pl.	2.5968**	.00166	.00244*	.002037*	.001714	0.4%
Ker.no. per pl.	8837773773**	273	101274419**	4418604**	216.55	25.5 %
Ker.wt. per pl.	659334792**	1	208688**	165258**	1	1.8 %

Table. XII. Analysis of variance (ANOVA) and narrow sense heritability (h^2) for heat tolerance using the % reduction between control group and heat-treated RILs of each yield component trait (from parents and progenies during a short-term of 38°C for 2 days beginning at 10 DAP in growth chambers, a long-term at 30°C from 10 DAP until grain maturity in environmental controlled growth rooms and long-term at 26~31°C and 32~35°C in the field).

% Reduction of phenotype traits	Source (Mean Square)					
	Env.	Rep (env.)	Genotype	Genotype x Env.	Error	Heritability (h^2)
d.f.	1	4	61	61	244	
Ker.no. per ms	1.01533**	.01190710	.117873**	.107138**	.032	1.3 %
Ker.wt. per ms	57960.3**	275.76	2262.6**	1976.59**	523.18	0.1 %
Sin.ker.wt. per ms	46504.0**	395.29	876.98**	737.48**	323.70	2.4 %
GFD per ms	145210.7**	43.07	278.319**	247.25**	59.92	2.0 %
Spike no. per pl.	21197.42**	977.29	981.834	934.446	906.13	0.4 %
d.f.	3	8	61	183	488	
Sin.ker.wt. per pl.	197393.6**	459.07	512.67**	473.47**	224.07	0.52 %
Ker.no. per pl.	37178.12**	274.4	1689.4**	1404.23**	226.55	1.9 %
Ker.wt. per pl.	169256.01**	32.55	1001.3**	914.22**	347.49	0.7 %

CHAPTER III

MAPPING QTL FOR YIELD AND ITS COMPONENTS

1. INTRODUCTION

Breeding efficiency and future development of new superior cultivars will depend on the use of efficient marker-assisted selection (MAS) strategies. MAS strategies are dependent on the identification of QTL controlling a significant amount of the phenotypic variation for a trait of interest, analysis of their genetic control, and the stability of these QTL across environments (Wan et al., 2005). The consistency of QTL across environments is very critical when determining the potential effectiveness of utilizing the QTL for MAS in a breeding program. Yields are significantly correlated with yield components such as kernel number, kernel size, kernel weight and 1000 kernel weight. Kernel weight is known to be an important contributor to yield. QTL mapping analysis for yield components and other agronomic traits has been reported in rice (Benmoussa 2005), sorghum (Rami et al., 1998) and barley (Romagosa et al., 1999; Thomas et al., 1995). In wheat, agronomically important traits including the dwarfing and vernalization response(Korzun et al., 1997), leaf rust resistance (Feuillet et al., 1995, 1997; Naik et al., 1998), kernel hardness(Sourdille et al., 1997), pre-harvest sprouting tolerance (Roy et al., 1998), protein content (Prasad et al., 1999; Mesfin et al., 1999), powdery mildew resistance (Qi et al., 1996), kernel traits(Campbell et al., 1999) and flour viscosity (Udall et al., 1999) have been studied intensely. Using grain filling duration as an in-direct measure of heat tolerance in wheat, a minimum of 1.4 genes were detected with both additive and dominant gene action associated with grain filling

duration. A high broad-sense heritability of grain filling duration of 80% in the F2 and F3 generation was calculated from a cross between 'Ventnor' (heat tolerant) and 'Karl 92' (heat susceptible) (Yang et al., 2002b). However, studies involving the identification and characterization of QTLs for yield components in wheat are still lacking from the current literature. This study aims to identify QTLs for yield components and the maintenance of these yield components under high temperature stress as a measure of heat tolerance.

The objective of this chapter is to identify and map QTLs for the kernel number per main spike and kernel weight per main spike and single kernel weight as yield components in addition to grain filling duration of the main spike as an indirect yield component. Yield components from seven environments are presented including three temperate conditions from two growth room and a field study with different light intensity, four high temperature conditions from growth rooms, with different heat periods, including short-term at 38°C and long-term at 30°C, and from field condition with long-term heat stress at 26~31°C and 32~ 35°C.

1-1. QTL analysis for yield and its components in wheat

Yield is known to be a complex trait which is quantitatively inherited and controlled by multiple genes with small individual effects (Benmoussa et al., 2005). This complex trait has a high correlation between the variation of the genetic background among the same varieties and phenotype difference (Cardon and Bell 2001). Therefore, if there are multiple genes segregating, but each gene exerts a small effect, the phenotypically different classes will be high with the difference between the classes

small. As such it is difficult to differentiate between genetic effects and experimental error (Falconer &Mackay 1996). An accurate observation or measurements with low experimental error are needed for an accurate characterization of QTLs for real genetic effects. Since QTL for grain yield are likely greatly affected by environmental factors, that explains low phenotypic stability, and heritability, the identification of high stability QTLs for marker assistant selection for crop breeding programs may be unlikely or require great care and attention to detail (Kato et al., 2000). In addition to grain yield, yield components such as kernel number, kernel weight, spike number, single kernel weight and 1000 grain weight also may be controlled by QTL with small genetic effects and hence small phenotypic variation (Yano & Sasaki 1997). However, some of the yield components, especially single kernel weight have been reported as having higher phenotypic stability and heritability than grain yield itself (Gandhi et al., 1963; Rasyad & Van Sanfold, 1992; Yano & Sasaki 1997). Giura and Saulescu (1996) insisted that kernel weight was highly heritable in most cultivars, suggesting greater stability. In a recent study using single chromosome recombinant substitution lines and restriction fragment length polymorphism(RFLP), QTLs for grain yield and other agronomic traits of interest were found to be distributed on various chromosomes, including 3A (Shah et al., 1999a; Campbell et al., 2003), 4A (Araki et al., 1999) and 5A (Kato et al., 2000). The QTLs on chromosome 5A have been shown to be highly correlated with adaptability and productivity (Kato et al., 2000). Kumar et al., (2006) reported that QTLs on chromosome 1A, 2B and 7A were important for high kernel weight. Microsatellite marker locus (Xwmc333) on chromosome 1A was verified as

having linkage with a QTL for kernel weight (Marza et al., 2006; Kumar et al., 2006). Kumar et al., (2006) reported 12 QTLs showing high association with grain weight, accounting for 6.57% to 10.76% of their individual phenotypic variation in individual environments. For yield and yield components, 10 QTL and 16 QTL, respectively, have been reported by Marza et al., (2006) in their study of a wheat population 'Ning 7840' x 'Clark'. In addition, it has been emphasized that kernel number per spike is one of the major contributors for wheat yield improvement (Slafer & Andrade 1989; Brancourt-Hulmet et al., 2003). Kirigwi et al.(2007) have reported in their study of QTL for grain yield of 127 RILs from a cross between common wheat cultivar 'Dharwar Dry' (drought tolerant and 'Sitta' under drought that Microsatellite locus Xwmc89 was associated with enhanced performance under drought stress, which this marker wmc89 may be major contributor for drought tolerance. Eight QTLs for yield traits including thousand kernel weight, spike number, sterile spikelet number, and fertile spikelet number per spike were detected in cluster on chromosome 7D near SSR markers Xwmc31, Xgdm67 and Xgwm428, controlling a high phenotypic variation of 27.53 to 67.63% (Li et al., 2007). Ma et al.,(2007) identified a major QTL tightly linked to two flanking markers Xcfd46 and Xwmd702 on the chromosome 7D for large spikes, which contribute toward increased kernel numbers and kernels weight and hence improvement of end-use yield production.

1-2. QTL and environmental interaction

Once a QTL is identified, the essential steps toward marker–assisted selection are to validate markers tightly linked to QTLs in an independent population of the same

parental lines and in different-genetic background, as well as to analyze the stability of QTL in various environments of different years and locations (Wan et al., 2005; Romagosa et al., 1999). However, complex traits such as grain yield, which are coordinated by the expression of multiple loci, present many challenges when trying to identify marker-QTL linkages stable in different genetic backgrounds and in different environments (Reyna & Sneller 2001). Often, detected QTL for grain yield in a single environment do not consistently appear in different environments (Shah et al., 1999b). This is often attributed to QTL x environmental interaction, which is the probability of different responses in different environments. This inconsistency of QTL detection has been reported in wheat (Araki et al., 1999; Kato et al., 2000) and other crops including rice (Zhuang et al., 1997) and barley (Hays et al., 1993). Campbell et al. (2003) reported a significant QTL by environmental interaction for grain yield and its components in their study of QTL for agronomic traits on chromosome 3A of wheat. Benmoussa et al. (2005) also reported that some QTLs were detected only in a single environment, while others were present in multiple environments in their study of QTL analysis for yield components in rice. Their study put an emphasis on the large effect QTLs, for traits of interest in one environment because the major QTL might have more probability to be detected in another environment.

2. MATERIALS AND METHODS

2-1. Phenotypic value

Sixty-two recombinant inbred lines derived from a cross between a parent exhibiting heat tolerance '7C' and another parent exhibiting heat susceptibility 'Seri M82' were provided by Mathew Reynolds (Cimmyt in Mexico). The following yield components were recorded; kernel number, kernel weight, and single kernel weight per main spike or primary tiller, per spike and per plant and grain filling duration per main spike after being expose to a short-term heat stress of 38°C for 2 days beginning at 10 DAP in growth chambers, a long-term heat stress of 30°C until grain maturity in the environmentally controlled rooms, and a long-term heat stress during reproductive development of 26~31°C and 32~35°C in two unique field studies. The detailed conditions for growth were previously described in the materials and methods of chapter II.

2-2. DNA isolation

Genomic DNA was isolated by harvesting fresh tissue (3g) of 10 seedlings from each of the 62 F_8 recombinant inbred lines (RILs) derived from the cross of 'Seri M82' and '7C' via single seed decent. The DNA extraction was performed using the DNeasy® Plant Mini Kit (Qiagen) with little modification. Extracted DNA was quantified at A260 and diluted to a standard concentration for screening polymorphism of SSRs markers in the two parental lines and their 62 RILs.

2-3. Genotypic value

Polymerase chain reactions (PCR) were performed in a 20 µl volume in MJ Tetrad Thermocycler. The PCR mixture was as follows: 24ng DNA, 1.5mM MgCl2, 50mM KCl, 0.8mMdNTPs, 5 pmol forward primer, 7 pmol reverse primer and 0.5 U Taq polymerase (Biolase). Thermal cycling included: 94 °C 2min; 35cycles of 95 °C, 1 min 61/51°C(different sets of annealing temperature), 73°C, 1min; 1cycle 73°C, 5min. The PCR products were separated by electrophoresis using 3% SFR agarose (AMRESCO), running 5X TBE buffer followed by the UV photography to visualize PCR products. The gels were reused four or five times each following re-melting and remolding. Two parental lines 'Seri M82' and '7C' were evaluated for polymorphism using 323 SSRs. 113 SSRs among the 323 SSRs screened showed polymorphism between the two parental lines. Genotypic data for each polymorphic SSR was obtained for every individual RIL in the same manner as that used for the parents.

2-4. Construction of linkage map of SSRs markers

A genetic linkage map was constructed using genotypic data obtained with the set of polymorphic SSRs, 113 among 323 evaluated, in each of individual lines of the 62 RILs population. Mapmaker/ Exp v.3.0 (Lander et al., Lincoln et al., 1999a) was used to construct the corresponding genetic maps.

2-5 Single marker analysis

This analysis is for fitting the data on the SSR markers as independent variable and the phenotypic data (all of each yield component of 62 RILs averaged over replication from seven individual environments) as dependant variable to the simple

linear regression model which is y= bo + b1x +e. The results from single marker analysis using QTL Cartographer v 2.0 give the estimates whether the marker is linked to a QTL through testing significance by determining if b1 is significantly different from zero. The F statistic compares the hypothesis H0: b1=zero to an alternative H1: b1 not zero. Significance of the associations between markers and phenotypic traits were detected at the 0.1% and 0.01% indicated by *** and ****, respectively.

2-6. Construction of QTL map

The estimated genetic map of SSRs markers was used as a framework for the positioning of QTL using composite interval mapping (Zeng, 1994; Basten et al., 2000) by associating the trait values from yield components. The percentage variation and additive effect of each of phenotypic traits caused by the presence of QTLs were also estimated using QTL Cartographer v 2.0. A LOD threshold of 2.5 was used to evaluate the presence of QTLs in each linkage group. Marker position with respect to chromosome was determined with the RIPPLE, and BUILD commands. Centimorgan units were calculated using Kosambi mapping function (Kosambi 1944)

3. RESULTS AND DISCUSSION

3-1. Genetic linkage map

The genetic map for the 62 RILs population was constructed using 102 SSR markers which represented a 32% polymorphism rate between the two parental lines for the SSR set used. The map covered 21 linkage groups of two or more markers. 11 markers were unlinked to linkage groups. Each individual of the 62 RILs population from a cross of two parental inbred lines ‘Seri M82’ and ‘7C’ was confirmed to be

highly inbred with 58% of '7C' homozygous alleles, 36% of 'Seri M82' homozygous alleles, 2% of heterozygous alleles from both of parents. Linkage groups were regarded as chromosome group number including 7 chromosomes with three genomes (AABBDD). The total linkage coverage and average interval distance were 1860.2 cM and 18.2 cM / marker, respectively. The A-, B- and D- genome chromosomes had 628.4, 617.5 and 614.3 cM with average interval distances of 18.5, 15.8 and 21.1cM /marker. Chromosome1A, 1B and 2B had few markers co-segregated (barc148 and gwm11 for chromosome 1A, barc8 and barc137 for chromosome 1B and barc18, gwm148, gwm429 and wmc154, barc159 for chromosome 2B). Most of linkage groups had large linkage distances, but chromosome 3D, 4A, 4B showed very close linkage distances with marker density of 9.2cM / marker. When the genetic linkage map was compared to existing high-density microsatellite consensus map and a microsatellite map for bread wheat, there was some disagreement in the order of closely linked markers and the position of markers on the chromosomes between maps (Somers et al 2004; Röder 1998). The markers localized on the present genetic linkage map were matched on average 62.8% with a high –density microsatellite consensus map for bread wheat (Somers et al 2004). Markers detected on chromosome 1B, 1D, 3B, 4A, 4B, 5D and 7D except for 1or 2 markers which were located on the other chromosome of the compared consensus map had perfect matches with a high–density microsatellite consensus map for bread wheat (Somers et al., 2004). Especially the position and interval distance of markers wmc48-wmc89 on the chromosome 4A was matched with those of the SSR/EST-STS marker map of 127 RILs from a cross between common wheat cultivars 'Dharwar Dry' (drought

tolerant) and ‘Sitta’(Kirigwi et al., 2007). Markers detected on chromosome 1A, 2A, 2B, 2D, 4D, 5A, and 5B matched an average of 87% with that high–density microsatellite consensus map even though their orders and interval distances on linkage groups had a few difference. These disagreements in exact positioning of markers and deviations of marker order have been found other in small populations, different populations used, and lower saturated maps due to the use of fewer markers (Somers et al., 2004; Röder 1998).

3-2. Single marker analysis and differences of means

The single marker analysis allows estimating the existence of potential QTL by identifying markers segregating with phenotypic traits. This simple analysis of each of yield components with a total of 102 SSR markers was conducted to assess the association of a phenotypic trait with a marker genotype using data individually pooled from seven individual environments. The results of regression analysis of each of yield components on individual markers showed significance from 5% to 0.01% levels. There were so many markers showing association with phenotypic traits in the above range of significance levels. This study narrowed the significance levels into 0.1% and 0.01% which were indicated by ***, **** (Table XVI in Appendix). One hundred sixty-two markers showed significant associations with yield and its components. If it supposes the markers as potential QTLs, it is much greater number of markers compared to 81 QTLs for yield and its components from composite interval mapping analysis (Table XIII). In addition, it showed partially common markers between two analyses. This indicates possibility of false-positive or false-negative QTL in single marker analysis which also can be adjusted by multiple statistical tests (Rebecca, 2001).

Among 162 markers, 19 markers showing 0.01% significance level from two or more phenotypic traits and heat stress environments were selected to contrast the mean of yield and its components (Table XVIII in Appendix). This result allowed confirming positive marker alleles by significant difference between means of separately grouped alleles from two parental lines. Most of positive marker alleles contributing to the more yield and its components were derived from alleles of 'Seri M82' in the short-term heat stress environment except for two markers (gwm604 and wmc667 on the chromosome 5B, 6A). Specifically, these markers showed significant positive effect in the kernel weight per plant. In contrast to short-term, in the long-term heat stress environment, more marker alleles derived from '7C' showed positive effect but not significant. This may indicate different genotypic response according to different environments.

Table XIII. Summary of QTLs identified by composite interval mapping for yield and its components in the 62 RILs in seven individual environments. They includ a short-term heat stress (env2) with control (env1) at 38°C for 2 days beginning at 10 days after pollination (DAP), long-term heat stress (env4) with control (env3) at 30°C from 10DAP until grain maturity in environmentally controlled growth rooms and long- term heat tress in the field at both of 26 ~31°C (env6) and 32 ~35°C(env7) with control (env5) at temperate temperatures.

Marker Interval	Traits	Locus	Chr.	Int. Dis. (cM)	LOD	R2 (%)	Additive	Env.
wmc336 - cfd48	Ker_no_ms	*QS_*kno_ms_con*	c1d	29.3	4.93	26	5.59	env1
wmc283 - gwm389	Ker_no_ms	**Q*S_kno_ms_heat*	c3b	32.6	3.06	16	2.83	env2
barc17 - wmc232	Ker_no_ms	*Q*S_kno_ms_con*	c6b	20.2	5.52	19	4.03	env1
wmc336 - cfd48	Ker_no_pl	Q*S_kno_pl_con*	c1d	29.3	5.65	36	15.21	env1
barc124 - gwm312	Ker_no_pl	Q*F_kno_pl_con*	c2a	41.8	2.81	10	-829.75	env5
gwm372 – wmc154 barc159	Ker_no_pl	**Q*F26_kno_pl*	c2b	20.0	3.44	19	818.03	env6
gwm192 - wmc285	Ker_no_pl	Q*S_kno_pl_heat*	c4d	45.9	3.06	17	7.52	env2
	Ker_no_pl	Q*L_kno_pl_con*	c4d	45.9	2.66	12	24.32	env3
wmc632 – barc 54	Ker_no _pl	Q*F_Kno_pl_con*	c6d	48.5	3.54	17	690.03	env5
barc111 – gwm469	Ker_no_pl	Q*S_kno_pl_con*	c7b	32.3	2.57	15	8.87	env1
	Ker_no_pl	Q*S_kno_pl_heat*	c7b	32.3	2.66	12	4.88	env2
gwm642 - wmc626	Ker_wt_ms	**Q*L_kwt_ms_heat*	c1d	35.8	3.75	24	0.13	env4
gwm493 – cfd223	Ker_wt_ms	*Q*S_kwt_ms_heat*	c3b	24.2	3.11	21	0.1	env2
gwm192 - wmc285	Ker_wt_ms	*Q*S_kwt_ms_heat*	c4d	45.9	3.89	20	0.09	env2
	Ker_wt_m	Q*L_kwt_ms_con*	c4d	45.9	4.58	24	0.54	env3
gwm443 – barc232	Ker_wt_ms	Q*S_kwt_ms_con*1	c5a	22.6	2.83	8	0.24	env1
		QL_kwt_ms_con	c5a	22.6	3.31	16	0.5	env3
wmc667 – wmc658	Ker_wt_ms	Q*S_kwt_ms_con2*	c6a	43.0	8.03	38	0.28	env1
barc77 – wmc632	Ker_wt_ms	Q*S_kwt_ms_con3*	c6d	56.4	2.55	7	0.22	env1
wmc634 – wmc606	Ker_wt_ms	Q*S_kwt_ms_heat*	c7d	31.0	4.37	22	0.09	env2
cfa2129 – bard148 gwm11	Ker-wt_pl	Q*S_kwt_pl_con*	c1a	17.3	2.55	7	0.22	env1
wmc336 – cfd48	Ker_wt_pl	Q*S_ker_wt_pl_con*	c1d	29.3	3.66	12	0.3	env1
	Ker_wt_pl	*Q*F26_kwt_pl*	c1d	29.3	8.35	43	49.21	env6
barc124 – gwm312	Ker_wt-pl	Q*F_kwt_pl_con*	c2a	41.8	3.32	12	319.01	env5
wmc154 – barc159	Ker-wt_pl	Q*S_kwt_pl_con*	c2b	20.8	2.5	13	0.23	env1
gwm372 – wmc154 barc159	Ker_wt_pl	*Q*F26_kwt_pl*	c2b	20.0	3.32	18	33.87	env6
barc62 – wmc418	Ker_wt_pl	Q*F_kwt_pl_con*	c3a	36.9	3.93	14	347.8	env5
gwm493 – cfd223	Ker-wt_pl	Q*S_kwt_pl_con*	c3b	24.2	5.92	20	0.33	env1
	Ker-wt_pl	*Q*S_kwt_pl_heat*	c3b	24.2	4.32	17	0.18	env2
wmc283 –gwm389	Ker_wt_pl	Q*L_kwt_pl_con*	c3b	32.6	2.66	12	1.17	env3
wmc48 wmc89	Ker_wt_pl	Q*F_kwt_pl_con*	c4a	1.5	4.18	11	311.5	env5
gwm192 – wmc285	Ker_wt_pl	*Q*S_kwt_pl_heat*	c4d	45.9	4.24	20	0.16	env2
	Ker_wt_pl	Q*L_kwt_pl_con*	c4d	45.9	3.59	10	1.78	env3
	Ker_wt_pl	Q*F_kwt_pl_con*	c4d	45.9	4.11	15	341.1	env5
barc4 – gwm371	Ker-wt_pl	Q*F_kwt_pl_con*	c5b	42.0	2.58	20	479	env5
wmc231 – wmc765	Ker_wt_pl	*Q*S_kwt_pl_heat*	c5d	18.5	2.58	11	0.14	env2
	Ker_wt_pl	Q*L_kwt_pl_con*	c5d	18.5	2.98	15	1.71	env3
wmc658 – wmc622	Ker_wt_pl	Q*F32_kwt_pl*	c6a	16.2	2.7	40	116.9	env7

Table XIII (continued)

Marker Interval	Ttraits	Locus	Chr	Int. Dis. (cM)	LOD	R2 (%)	Additive	Env.
cfd75 – barc17	Ker_wt_pl	*Q*S_kwt_pl_heat*	c6b	29.5	3.4	11	0.1	env2
cfd102 – wmc532	Ker_wt_pl	*Q*S_kwt_pl_heat*	c6b	17.9	4.19	18	0.15	env2
barc77 – wmc632	Ker-wt_pl	Q*S_kwt_pl_con*	c6d	56.4	5.17	15	0.29	env1
	Ker-wt_pl	Q*F_kwt_pl_con*	c6d	56.4	2.58	20	356.9	env5
	Ker_wt_pl	Q*F32_kwt_pl*	c6d	56.4	2.51	23	50.67	env7
wmc264 – wmc764 wmc382	Ker_wt_pl	Q*F_26_kwt_pl*	c7a	24.9	2.58	13	21.2	env6
barc111 – gwm469	Ker_wt_pl	Q*F_kwt_pl_con*	c7b	32.4	3.49	10	302.5	env5
wmc634 – wmc606	Ker_wt_pl	Q*F26_kwt_pl*	c7d	31.0	2.52	10	20.87	env6
gwm111 – cfd31	Ker_wt_pl	Q*F26_kwt_pl*	c7d	21.8	2.64	13	26.33	env6
wmc418 – gwm369	Sker_wt_ms	Q*L_skwt_ms_con*	c3a	37.3	3.19	19	0.001	env3
gwm192 – wmc285	Sker_wt_ms	Q*L_skwt_ms_con*	c4d	45.9	2.98	13	0.001	env3
cfd18 – gwm190	Sker_wt_ms	*Q*S_skwt_ms_con*	c5d	9.2	4.18	20	0.001	env1
wmc765 – gwm595	Sker_wt_ms	**Q*L_skwt_ms_heat*	c5d	9.2	3.03	14	0.001	env4
wmc667 – wmc658	Sker_wt_ms	*Q*S_skwt_ms_con*	c6a	43.0	4.71	26	0.001	env1
barc77 – wmc632	Sker_wt_ms	Q*S_skwt_ms_heat*	c6d	56.4	3.09	19	0.001	env2
wmc632 – barc54	Sker_wt_ms	**Q*L_skwt_ms_heat*	c6d	48.5	2.77	14	0.001	env4
wmc475 – gwm273	Sker_wt_ms	**Q*L_skwt_ms_heat*	c7b	32.8	2.57	10	0.001	env4
wmc634 – wmc606	Sker_wt_ms	Q*S_skwt_ms_heat*	c7d	31.0	4.33	26	0.001	env2
barc8 – barc184 barc137	Sker_wt_pl	Q*L_skwt_pl_con*	c1b	28.7	6.37	49	0.001	env3
wmc336 – cfd48	Sker_wt_pl	Q*S_skwt_pl_con*	c1d	29.3	4.79	30	0.001	env1
barc62 – wmc418	Sker_wt_pl	Q*F_skwt_pl_con*	c3a	36.9	5.1	19	0.001	env5
gwm493 – cfd223	Sker_wt_pl	*Q*S_skwt_pl_heat*	c3b	24.2	2.55	14	0.001	env2
gwm389 – barc147	Sker_wt_pl	*Q*L_skwt_pl_heat*	c3b	9.2	2.57	10	0.001	env4
gwm604 – barc4	Sker_wt_pl	Q*F_skwt_con*	c5b	0.8	4.33	15	0.001	env5
wmc231 – wmc765	Sker_wt_pl	Q*L_skwt_pl_con*	c5d	18.5	2.77	17	0.001	env3
gwm292 – cfd18	Sker_wt_pl	*Q*L_skwt_pl_heat*	c5d	19.7	2.72	10	0.001	env4
wmc765 - gwm595	Sker_wt_pl	*Q*L_skwt_pl_heat*	c5d	31.6	2.62	12	0.001	env4
wmc622 – wmc332	Sker_wt_pl	*Q*L_skwt_pl_heat*	c6a	10.1	2.68	10	0.001	env4
wmc632 – barc54	Sker_wt_pl	Q*F_skwt_pl_con*	c6d	48.5	5.25	25	0.001	env5
	Sker_wt_pl	Q*F32_skwt_pl*	c6d	48.5	5.11	39	0.001	env7
wmc517 – barc182	Sker_wt_pl	*Q*F26_skwt_pl*	c7a	8.6	2.67	16	0.001	env6
gwm273 – barc111	Sker_wt_pl	Q*S_skwt_pl_con*	c7b	31.9	3.22	20	0.001	env1
wmc634 – wmc606	Sker_wt_pl	Q*F_skwt_pl_con*	c7d	31.0	4.64	13	0.001	env5
barc148 – wmc128 gwm11	Sp_no_pl	Q*L_spno_pl_heat*	c1a	22.2	2.58	12	0.12	env4
wmc154 – barc159	Sp_no_pl	Q*L_spno_pl_heat*	c2b	20.8	3.72	20	0.71	env4
gwm493 – cfd223	Sp_no_pl	Q*S_spno_pl_con*	c3b	24.2	3.43	18	0.65	env1
gwm190 – cfd57	Sp_no_pl	Q*S_spno_pl_con*	c5d	32.9	2.51	12	0.62	env1
	Sp_no_pl	**Q*S_spno_pl_heat*	c5d	24.2	4.19	16	0.53	env2
wmc418 – gwm369	GFD_ms	*Q*L_gfd_ms_heat*	c3a	37.3	3.28	21	1.45	env4
wmc89 – wmc491	GFD_ms	Q*L_gfd_ms_con*	c4a	7.2	2.62	14	4.99	env3
gwm192 – wmc285	GFD_ms	*Q*L_gfd_ms_heat*	c4d	45.9	3.16	20	1.08	env4
wmc667 – wmc658	GFD_ms	*Q*L_gfd_ms_heat*	c6a	43.0	3.13	16	0.85	env4
barc77 – wmc632	GFD_ms	Q*S_gfd_ms_heat*	c6d	56.4	2.68	15	2.05	env2

*, ** indicate traits showing normal distribution at the level s of α=0.05 and 0.01

3-3. Detection and localization of QTLs for yield components

The QTLs for yield and its components in addition to grain filling duration per main spike as an indirect yield component were detected and localized based on linear ordering of SSRs markers in the linkage groups and associating the phenotypic yield components to the map. The yield components included kernel number, kernel weight and single kernel weight per main spike and per plant. Phenotype data from seven environments were used. These include 3 temperate or control (2 growth rooms and 1 field) and 4 high temperature treatments (1 short-term growth room heat stress treatment of 38 ˚C beginning at 10 DAP for 2days, 1 long-term growth room heat stress treatment of 30 ˚C beginning at 10 DAP maintained through to maturity, and 2 field long-term heat stress treatments during reproductive development of 26~31°C and 32~35°C. For QTL detection and localization on the chromosomes, composite interval mapping (CIM) was constructed with the 62 RILs population (Fig. 19 and Table XIII). Seventy one QTLs for yield and its components were detected on most chromosomes with one or more QTLs per chromosome, except for chromosome 2D, 3D and 4B. QTLs with high LOD values were found evenly for traits with both normal distribution and non-normal distribution. This suggests a good utility of using discontinuous quantitative traits for identification of QTLs of interest (Table XIII, *mark for normal distribution).

Among 81QTLs, 3QTLs were detected for kernel number per main spike with a high percentage of the phenotypic variation of 16~26%, with a LOD value of 3.06~5.52 and an additive effect of 2.83~5.59, 8 QTLs for kernel number per plant with 10~36%, 2.57~5.65 and -829~818.03, 9 QTLs from kernel weight per main spike with 7~38%,

2.55~8.03 and 0.09~0.54, 27 QTLs for kernel weight per plant with 7~43%, 2.55~8.35 and 0.1~356.9, 9 QTLs and 15 QTLs from single kernel weight per main spike with 10~26%, 2.57~4.33 and 0.001, and per plant with 10~49%, 2.62~6.37, respectively, 5 QTLs for spike number per plant with 12~20%, 2.51~4.19 and 0.12~0.71, and 5 QTLs for grain filling duration which were detected with 14~20%, 2.61~3.28 and 0.85~4.99 phenotypic variation, LOD value and additive effect, respectively (Table XIII).

Two QTLs among 9 QTLs for single kernel weight per main spike including marker regions between wmc632-barc54 and between wmc634-wmc606 on chromosome 6D and 7D matched with 2 QTLs of 15 QTLs for single kernel weight per plant. Two QTLs among 4 QTLs for spike number per plant were in the same regions for 2 QTLs for kernel weight per plant. These included markers between wmc154 and barc159 and between gwm493and cfd223 on chromosomes 2B and 3B, respectively.

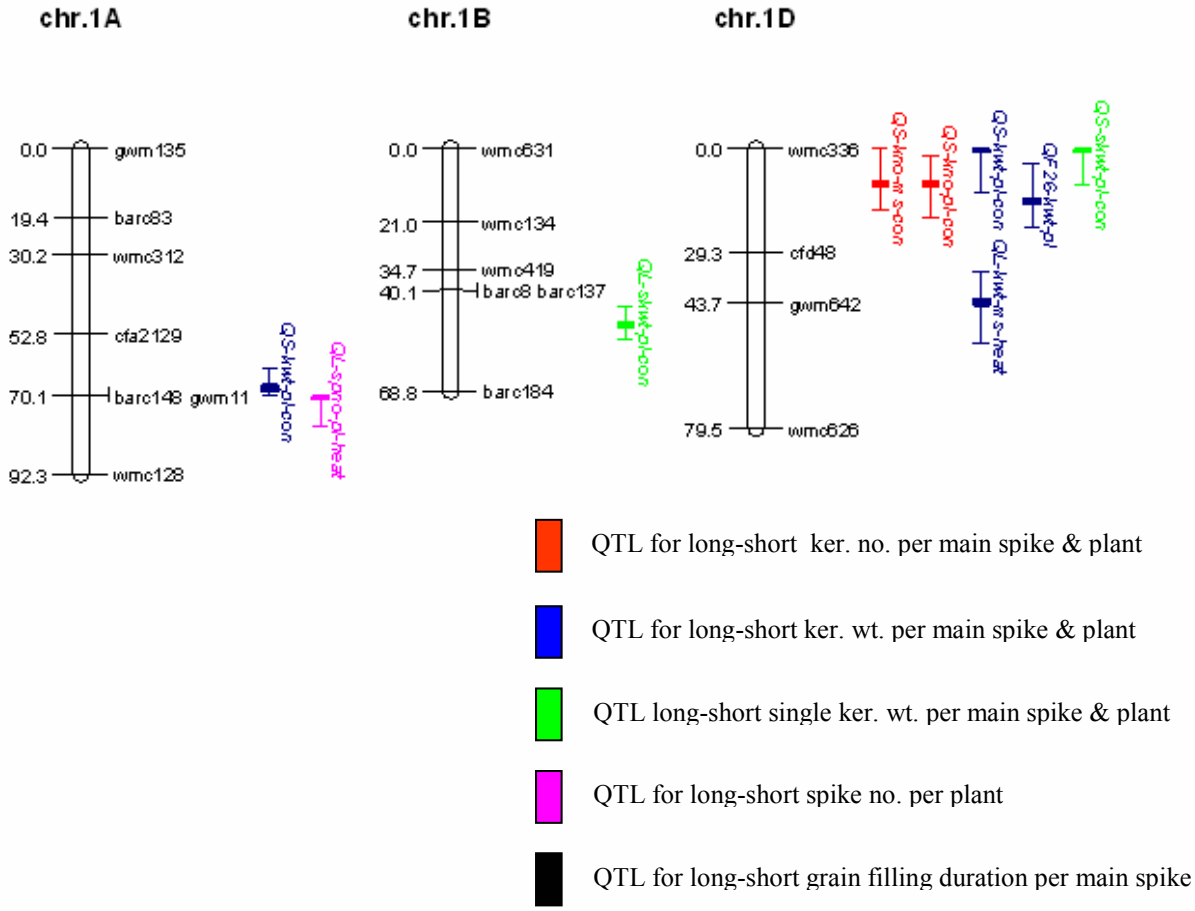


Fig. 19. Composite interval mapping of QTLs for yield and its components for the 62 RILs population.

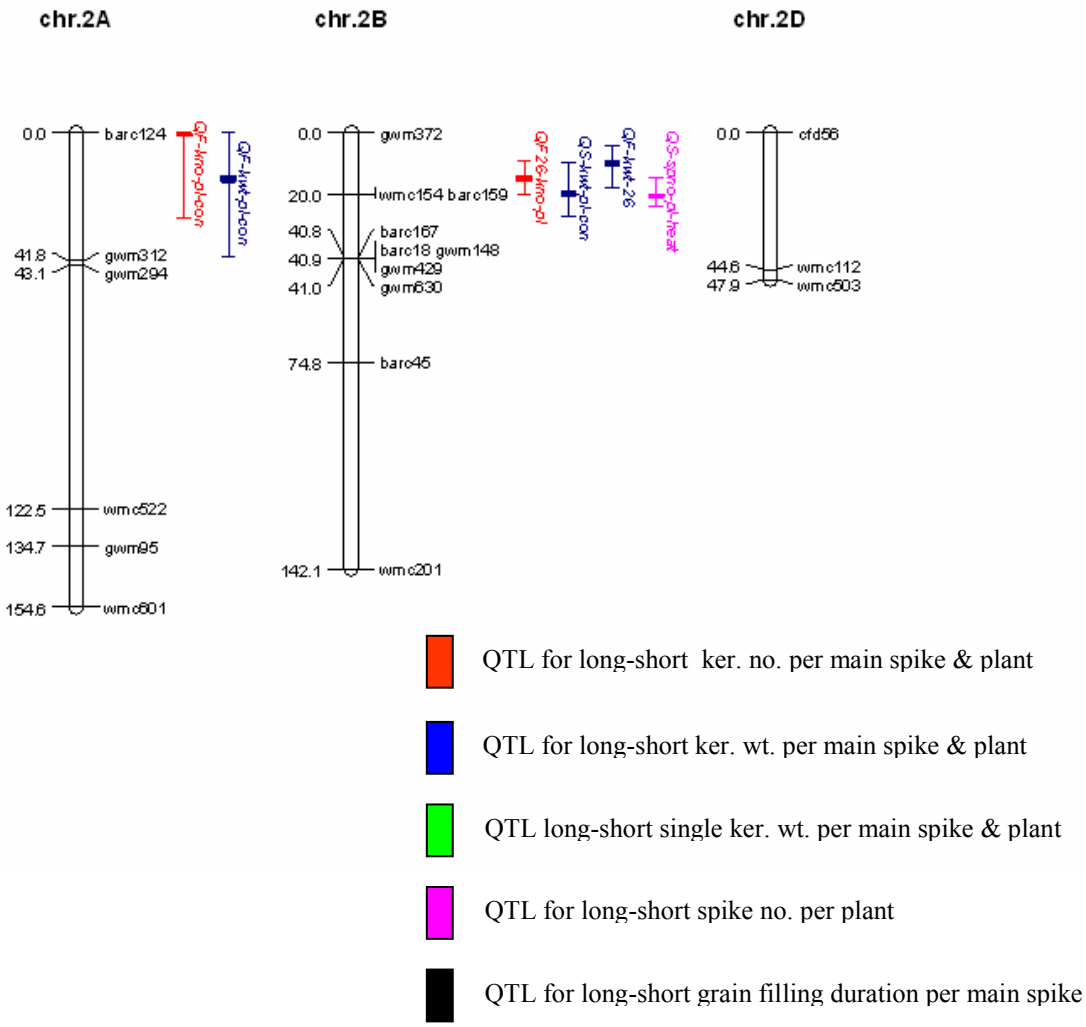


Fig. 19 (continued)

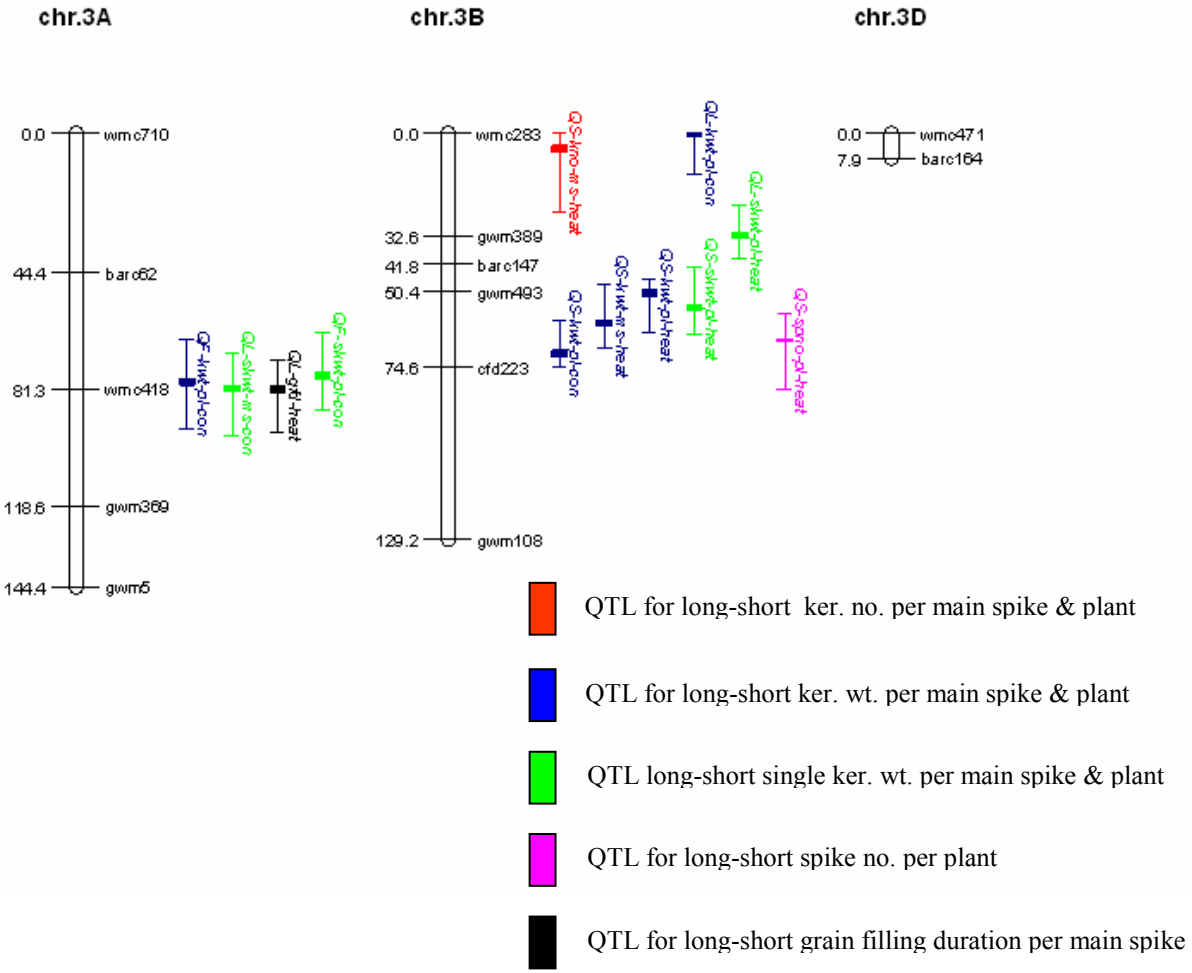


Fig. 19 (continued)

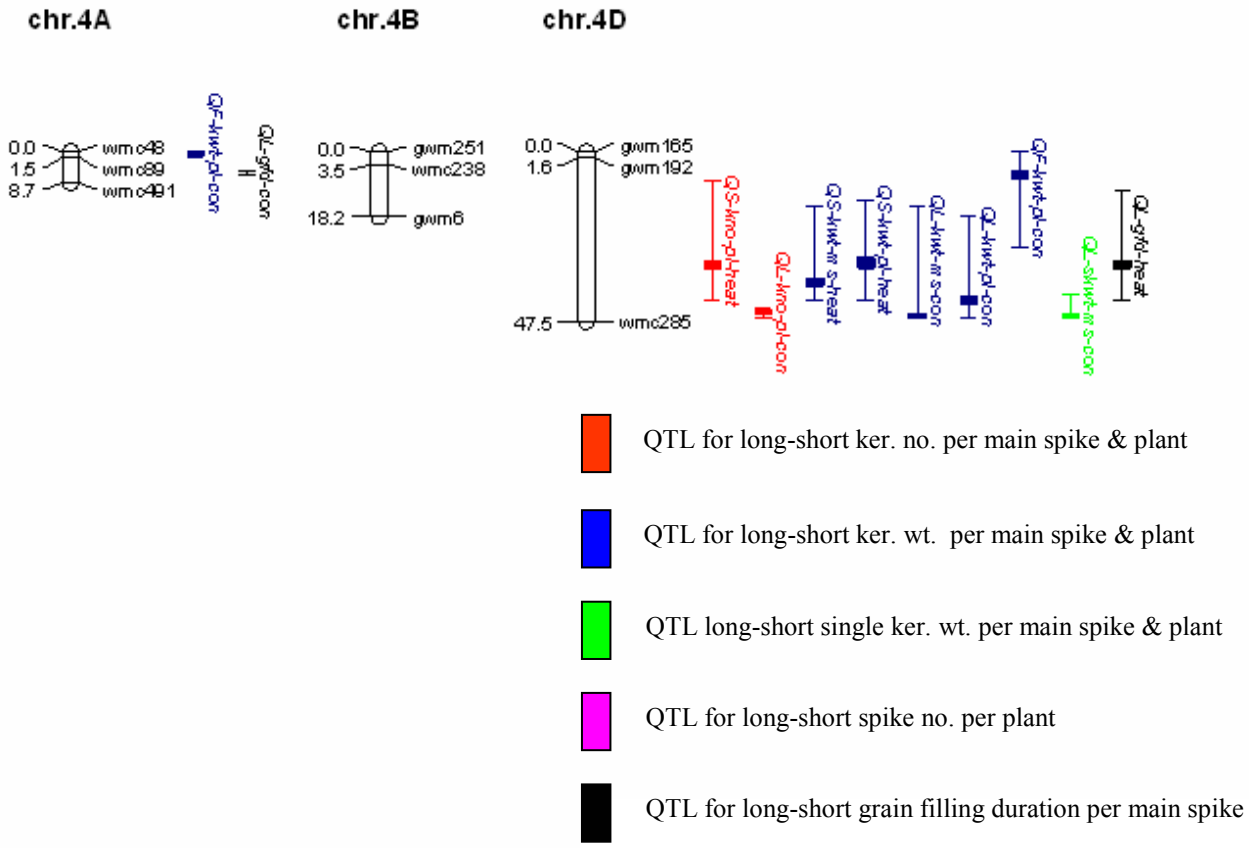


Fig. 19 (continued)

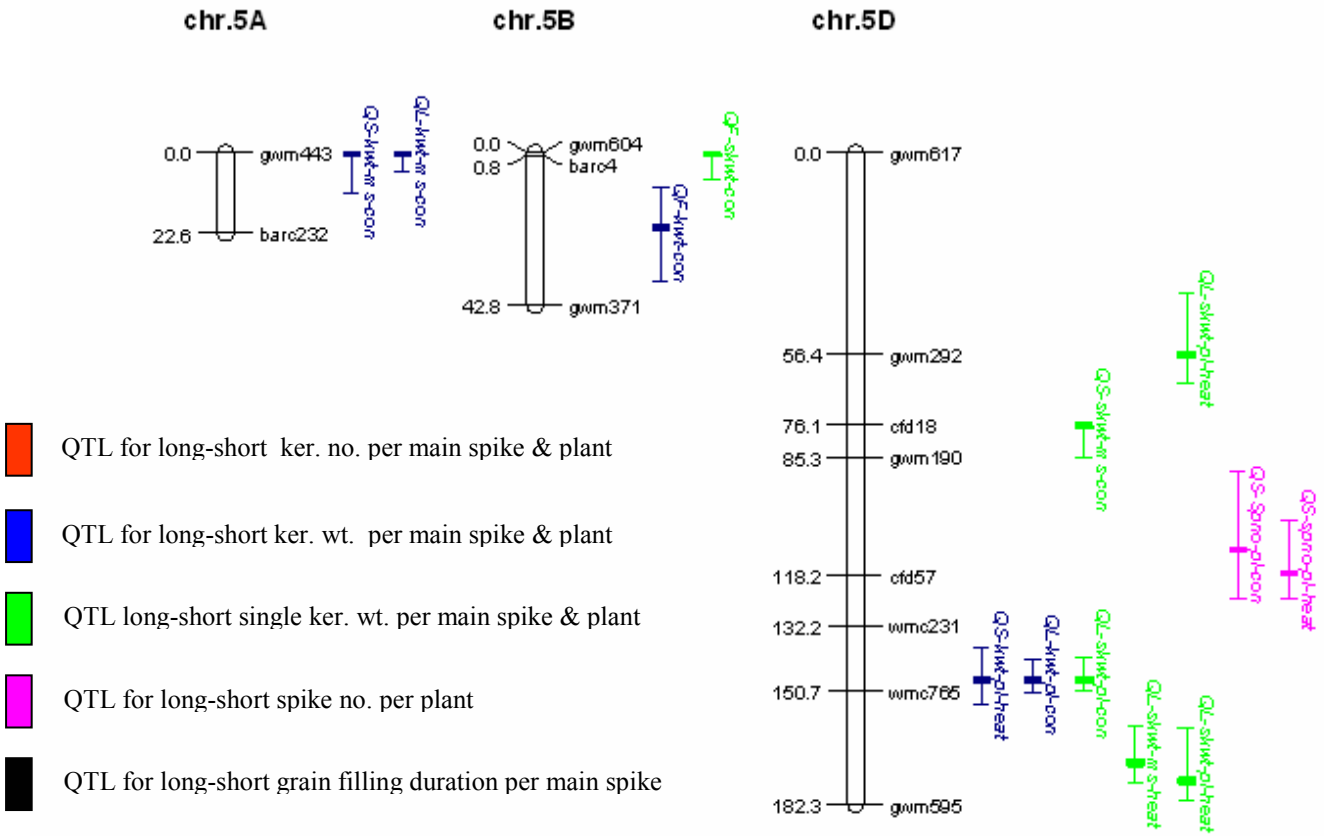


Fig. 19 (continued)

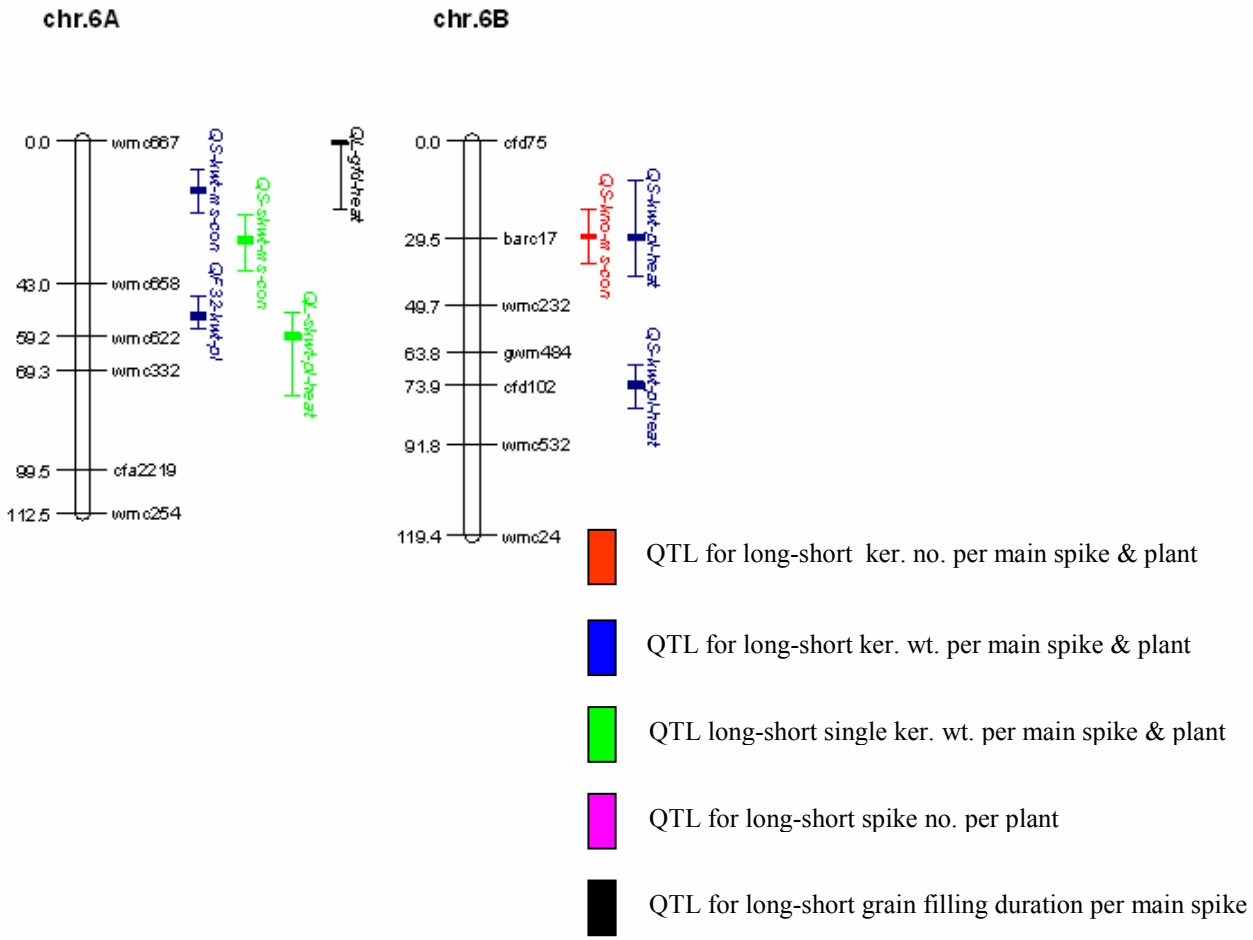


Fig. 19 (continued)

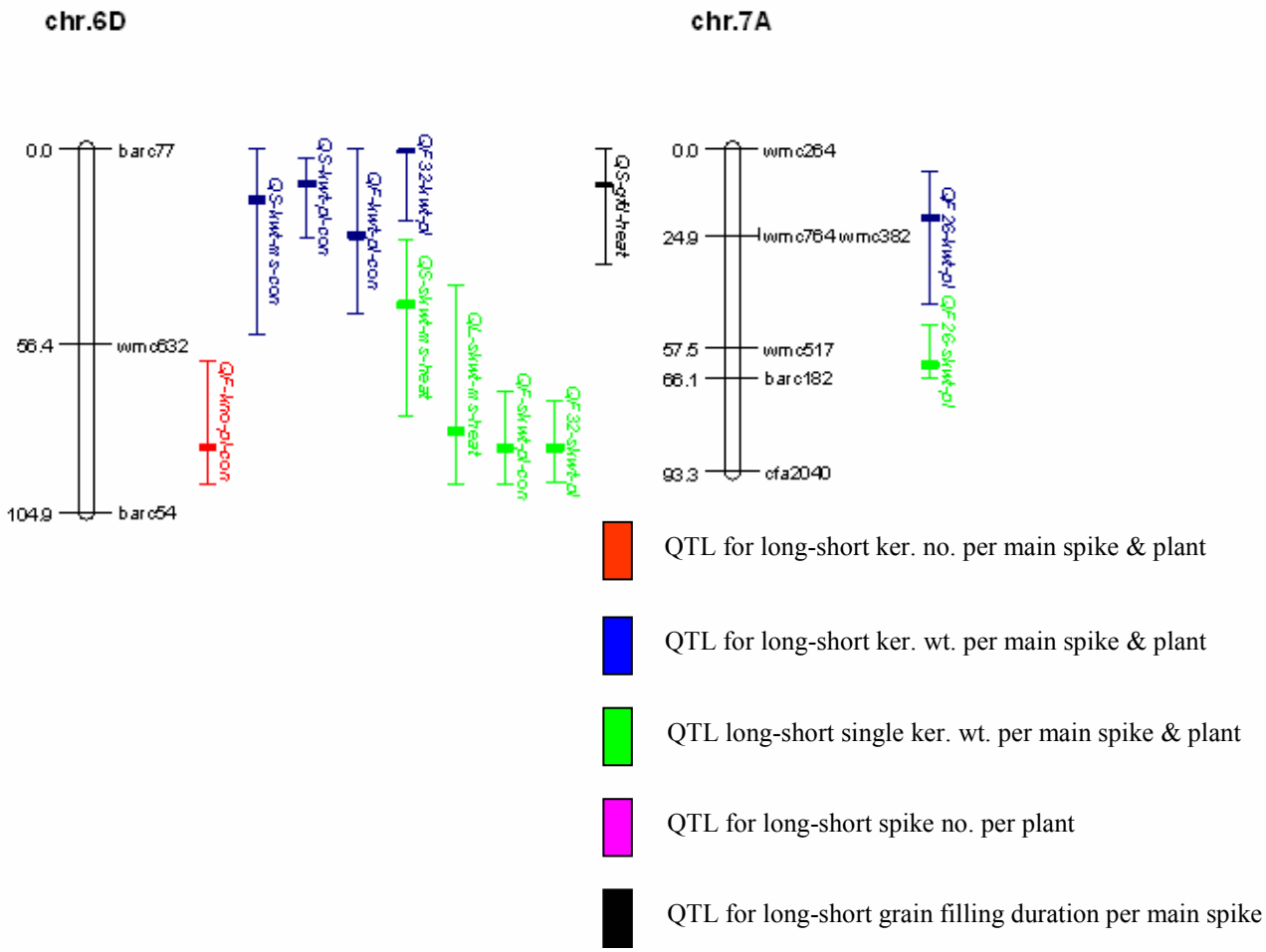


Fig. 19 (continued)

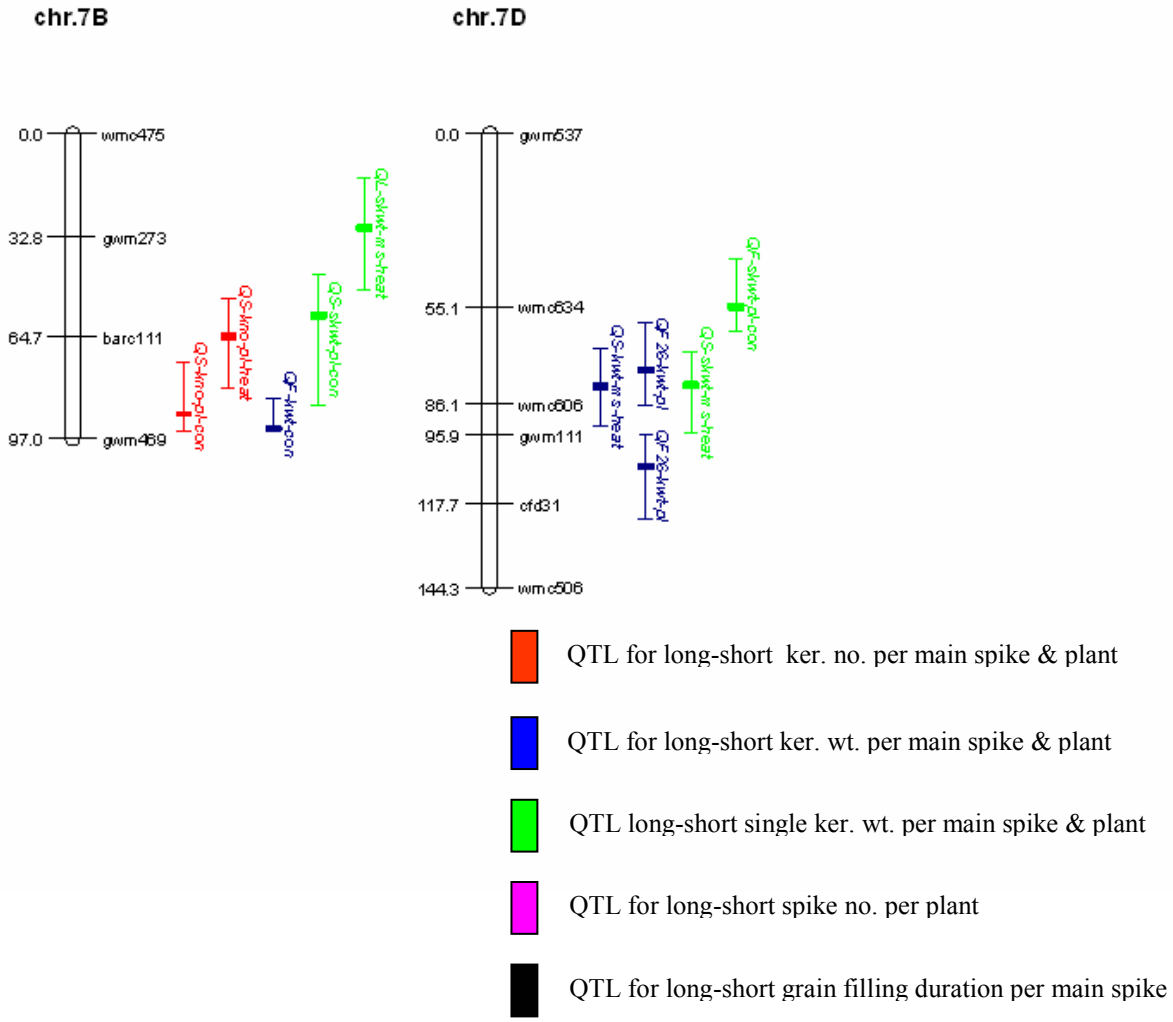


Fig. 19 (continued)

One QTL including markers interval between gwm192 and wmc285 on the chromosomes 4D and 2 QTLs including markers interval between gwm192 and wmc285 and between barc77 and wmc632 on the chromosomes 4D and 6D, respectively among 5 QTLs for grain filling duration overlapped with QTLs for kernel number per plant and kernel weight per plant, respectively. These results indicate that quantitative traits for main spike, spike number and grain filling duration yield components do not represent all yield QTLs including kernel number and kernel weight per plant. Four QTLs among 6QTLs for kernel number per plant (Yield) including between wmc336-cfd48, between baec124-gwm312, between gwm372-wmc154(barc159) and between gwm192-wmc285 on chromosomes 1D, 2A, 2B and 4D, respectively matched with one of 27 QTLs for kernel weight per plant(another yield). These locations might be very important positions for total yield production. In addition, it suggested that most of genes or loci controlling kernel number are overlap with genes or loci controlling kernel weight. The regions between markers of gwm192-wmc285 and barc77-wmc632 on the chromosome 4D and 6D respectively were very important for kernel weight on the basis of several QTLs detected for kernel weight from both environmentally controlled growth room and field studies. Four QTLs of 9 QTLs for single kernel weight per main spike had matched with 4 QTLs of 9 QTLs for kernel weight per main spike. Four QTLs of 15 QTLs of single kernel weight per plant had matched with 4 QTLs of 27 QTLs for kernel weight per plant. This genetic correlation between single kernel weight per main spike or per plant and kernel weight per main spike or per plant supports the high correlation between them based on the Pearson's correlation coefficients (Table VIII, Table IX).

The heat resistant QTLs for yield components were estimated based on the high phenotypic variation and additive effect from only heat stress conditions (4 environments) among the 7 environments. The heat tolerance QTLs for kernel number per plant were within markers interval between gwm192 and wmc285 and between gwm372 and wmc154 (barc159) on the chromosome 4D and 2B, respectively. They explained high phenotypic variation of 17% and 19 %, and had large additive effect with 7.5 and 818 for the kernel number per plant in both short-term heat condition in environmental controlled growth chambers and the long-term heat stress condition in the field, respectively. Heat resistant QTLs for kernel weight per plant revealed markers intervals between gwm493 and cfd223, and wmc658 and wmc622 on the chromosome 6D and 6A, respectively. They also showed high phenotypic variation of 17 % and 40%, and 2.05g and 116.9 g increase in weight by additive effects in the short-term heat stress treatment and field treatment, respectively. Another heat resistant QTL for grain filling duration per main spike was markers interval between barc77 and wmc632 on the chromosome 6D with 15% variation and 2.05 increase in days by an additive effect from the short-term heat stress treatments. These QTLs exhibited both the higher phenotypic variation and additive effect compared to the rest of QTLs for yield components. This indicates that these heat resistant QTLs exhibit better environmental stability for high kernel number and kernel weight even under unique heat stress treatments. On the other hand, the QTL on the markers interval between barc124 and gwm312 on the chromosome 2A showed 829.75 decreases in kernel number per plant by an additive effect in the field control condition. This may result from significant effect of QTL x

environment from complicated environmental factors in the field. Alternatively, this QTL may confer heat susceptibility.

Multiple QTL clusters also were identified throughout the 21genomes. Chromosomes 1D, 3B, 4B, 6D and 7D were identified with clusters of four and more QTLs (Fig. 19). Specifically, chromosomes 1D, 3B, 4D, 5D, 6A, 6D, 7B and 7D had several distributed QTLs for yield components and yield. However, the interval distances between markers of the chromosomes were very high. Wan et al. (2005) reported in their study of stability of QTLs for rice grain dimension and endosperm chalkiness characteristics that high stability of QTLs across multiple environment is attributed to major loci effects associated with high LOD scores (average 6.8), high heritability of phenotypic traits in the range of 85-95%, and minimum levels of genetic interaction between newly recombinated alleles as well as slight interactions between QTLs and the environment. In this study, most of the QTLs for yield and its components were detected from one or 2 environments among 7 environments suggesting significant instability of those QTLs to environments. The exception to this was QTLs between markers interval between gwm192and wmc285, and between Barc77 and wmc632 on the chromosomes 4D and 6D, respectively which were detected from 4 environments (Fig. 19). It has been reported that QTLs for grain yield and agronomic trait of interest were distributed on chromosome 3A (Shah et al., 1999; Campbell et al., 2003), 4A (Araki et al., 1999) and 5A (Kato et al., 2000). Especially chromosome 5A has been suggested to be correlated with adaptability and productivity (Kato et al., 2000). Kumar et al., (2006) have reported that chromosome 1A, 2B and 7A are important for high

kernel weight. Microsatellite marker locus (Xwmc333) on chromosome 1A was verified having linkage with QTLs for kernel weight (Marza et al., 2006; Kumar et al., 2006). But in this study, chromosome 1D, 3B, 4D, 5D, 6A, 6D, 7B and 7D were more associated with QTLs for yield and its components. In addition, regions between gwm192 and wmc285 and between barc77 and wmc632 on the chromosomes 4D and 6D respectively were more important for high kernel weight, although they showed great interval distances between them. These results did not agree with reported results mentioned above. These disagreements might be due to many reasons including different genetic backgrounds, population size, different environments and an unsaturated linkage map. Therefore, the success of QTL analysis will depend on further investigation of tight linkage between markers and QTLs, significant QTLs effects in larger populations with more environments (Rebecca, 2001, Wan et al., 2005).

3-4. Composite interval mapping and differences and means

Among 81 QTLs, 17 QTLs involving flanking markers, which were commonly detected in two and more phenotypic traits and different environments within small interval distance (<10cM) between flanking markers, were selected to contrast the mean of yield and its components per main spike and per plant (Table XIX and Table XX in Appendix). These results allowed confirming positive QTLs for yield and its components by significant difference between means of separately grouped alleles of two flanking markers from two parental lines. Most of positive QTLs between flanking marker alleles contributing to each of yield and its components were derived from QTLs between flanking marker alleles of ‘Seri M82’ in the short-term heat stress environment

except for a few QTLs. Specifically, QTLs showed significant positive effect in the kernel weight per plant. In addition, in the long-term heat stress environment, more QTLs derived from ‘Seri M82’ showed positive effect than those derived from ‘7C’, although their positive effect was not significant. The QTLs between gwm192 and wmc285 and between wmc231and wmc765 on the chromosome 4D and 5D, respectively showed greater differences of means between two different alleles than those of other QTLs. This result supports the previous result in this study that QTLs on the chromosome 4D and 5D were more associated with QTL for yield components (Fig. 19).

CHAPTER IV

MAPPING QTL FOR HEAT TOLERANCE

1. INTRODUCTION

In wheat, many studies have been carried out evaluating the physiological and quantitative results of plant responses to high temperature stress. Examples include high temperature effects on dry matter accumulation, carbohydrate metabolism (Jenner 1991b), yield and grain development (Johnson and Kanemasu, 1983; Tashiro and Wardlaw 1989), grain growth (Nicolas et al, 1984), pollen tube growth during floral development (Saini and Aspinall 1983), grain filling duration (Sofield et al., 1977; Stone and Nicolas 1995), and flour quality (Stone and Nicolas 1995). Using QTL analysis to dissect heat tolerance in maize, 6 QTL accounting for 53% of the genetic variability for cellular membrane thermo stability (CMS) were detected in recombinant inbred lines (RILs). Using the same population, 5 QTL were detected controlling pollen germination and thermo-tolerance (Frova and Sari-Gola, 1993; Frova, 1996). In wheat, Yang et al., (2002) reported in their study of F1, F2, and F3 population derived from two cultivars, 'Ventnor' (heat-tolerant) and 'Karl 92' (heat-susceptible) that two markers, Xgwm11 and Xgwm293 were linked to GFD in the F2 population. The GFD was used as a measure of heat tolerance due to a negative correlation of GFD with heat stress treatment (Reynolds et al., 1994: Stone & Nicolar, 1995).

Heat during reproductive development is now becoming recognized as a primary constraint to wheat production in the U.S. Southern Great plains. Wheat is a cool season species with an optimum temperature for maximum dry weight per grain at maturity of

15/10 °C day/ night (Chowdhury and Wardlaw, 1978). While wheat exhibits great adaptive plasticity in its ability to thrive in marginal environments, seed development and maturation of many elite genotypes are compromised in economic levels in response to adverse environmental stresses. For example, the Southern Great Plains hard red winter wheat growing region (as in many wheat growing regions of Australia, the Middle East, Asia, Europe, Africa, and South America) experiences extreme heat stress on a periodic basis and low level heat stress on a yearly basis during pollination and grain filling. A reduction in the yield per spike of 3 to 4 % for every 1°C increase in temperature over 15°C is typical for most wheat varieties (Wardlaw et al., 1989).

Several physiological traits such as stomatal conductance (Jones 1977), epicuticular waxes (Johnson et al 1983), photosynthetic rate (Kaul & Crowle, 1974), chlorophyll fluorescence (Moffatt et al., 1990), and canopy temperature depression (Blum et al., 1982) have been studied in conjunction with heat tolerance. Recently, heat hardening or acquired thermotolerance, which explains the ability of plants to have heat tolerance in the extreme temperature after being acclimated in non-lethal heat stress, has become a topic of interest (Blum et al., 2001). The quantification for acquired cellular thermotolerance in plants was estimated by cell membrane thermostability (CMS) and triphenyl tetrazolium chloride (TTC) cell variability (Blum, 1988).Several CMS-related heat tolerance traits have been reported showing significant correlation between high cell membrane thermostability (CMS) and grain yield or kernel weight in wheat (Shanahan et al., 1990; Fokar et al., 1998; Reynolds et al., 1994). Blum et al. (2001) also found that biomass (r=0.60**) and yield (r=0.53**) were significantly ($p < 0.01$) correlated with

CMS and a high broad-sense heritability for CMS (h^2=.67) under heat stress. Fokar et al. (1998) evaluated the inheritance of thermotolerance by measuring broad-sense heritability of triphenyl tetrazolium chloride (TTC) cell viability which has been used to quantify thermotolerance in plants (Blum, 1988) This enables selection for improvement of heat tolerance. Ibrahim and Quick (2001) also have confirmed the selection for heat tolerance based on the result of high heritability of triphenyl tetrazolium chloride (TTC) in early generation. Heat tolerance is also inherited quantitatively based on phenotypic traits like higher seed set, grain weight and extended the grain filling duration at elevated temperatures (Yang et al., 2002).

Other than the before mentioned studies, very little work has been carried out involving the identification and characterization of QTL for heat tolerance in wheat. The objective of this chapter is to construct a map for heat tolerance QTLs in wheat based on the relative % reduction of each trait of direct and indirect yield components in different temperature conditions. The mapped traits include kernel number, kernel weight, and the single kernel weight of the main spike and the grain filling duration of the main spike when exposed to short-term and long-term heat stress in environmentally controlled growth rooms as well as long term heat stress in the field. These QTL for heat tolerance were analyzed in terms of logarithmic odds (LOD) score, the location on the chromosome, and average interval distance among markers, genetic action associated with additive effect, and the phenotypic variation explained by the QTL.

2. MATERIALS AND METHODS

2-1. Phenotypic value

A set of 62 recombinant inbred lines derived from a cross between a parent exhibiting heat tolerance '7C' and another parent exhibiting heat susceptibility 'Seri M82' were provided by Cimmyt in Mexico. The relative % reduction was calculated from each yield component which was counted and measured for the kernel number, kernel weight, and single kernel weight per main spike (or primary tiller), per spike and per plant and the grain filling duration per main spike after exposure to a short-term heat stress of 38°C for 2 days beginning at 10DAP in growth chambers, a long-term heat stress at 30°C beginning at10 DAP until grain maturity in environmentally controlled growth rooms and two long-term reproductive stage heat stress field trials of 26~31°C in 2002 and 32~35°C in 2003. A simple score of heat tolerance was on a scale of 0 or 1 for yield components (where 0 for an increased or unchanged yield component after heat treatment compared to control, 1 for a yield component that decreased) was also used. The detailed conditions for growth were previously described in the materials and methods of chapter II.

2-2. DNA isolation

Genomic DNA was isolated by harvesting fresh tissue (3 g) of 10 seedlings from each of the 62 F_8 recombinant inbred lines (RILs) derived from the cross of 'Seri M82' and '7C' via single seed decent. The DNA extraction was performed using the DNeasy® Plant Mini Kit (Qiagen) with a little modification. Extracted DNA was quantified at 260

nm absorbance in UV spectro-photometer and diluted to a standard concentration for screening polymorphism of SSRs markers in the two parental lines and their 62 RILs.

2-3. Genotypic value

Polymerase chain reactions (PCR) were performed in a 20 µl volume in Perkin-Elmer (Norwalk, CT) thermocycler. The PCR mixture contained as follows: 24 ng DNA, 1.5 mM $MgCl_2$, 50 mM KCl, 0.8 mM dNTPs, 5 pmol forward primer, 7 pmol reverse primer and 0.5 U Taq polymerase (Biolase). Thermal cycling included: 94°C 2 min; 35 cycles of 95 °C, 1 min 61/51°C (different sets of annealing temperature), 73°C, 1min; 1cycle 73°C, 5 min. The PCR products were separated by electrophoresis using 3% SFR agarose, running 5X TBE buffer followed by UV photography to visualize the PCR products. The gels were reused four or five times each following re-melting and remolding. In each lane, a standard ladder with known size was included. Two parental lines 'Seri M82' and '7C' were evaluated for polymorphism using 323 SSRs. 113 among the 323 SSRs screened showed polymorphism between the two parental lines. Genotypic data for each polymorphic SSR was obtained for every individual RIL in the same manner as that used for the parents.

2-4. Linkage map construction of SSRs markers

A linkage genetic map was constructed using genotypic data obtained with the 113 SSRs polymorphic evaluated in each RIL using Mapmaker/ Exp v.3.0 (Lander et al., Lincoln et al., 1999a). A list of all primer sequences and mapped microsatellites are presented in the appendix.

2-5. Single marker analysis

This analysis is for fitting the data on the SSR markers as independent variable and the phenotypic data (all % reduction of each yield component of 62 RILs averaged over replication from four individual environment) as dependant variable to the simple linear regression model which is y= bo + b1x +e. The results from single marker analysis using QTL Cartographer v 2.0 give the estimates whether the marker is linked to a QTL through testing significance by determining if b1 is significantly different from zero. The F statistic compares the hypothesis H0: b1=zero to an alternative H1: b1 not zero. Significance of the associations between markers and phenotypic traits were detected at the 0.1% and 0.01% indicated by *** and ****, respectively.

2-6. Construction of QTL map

The estimated genetic map of SSR markers was used as a framework for the positioning of QTL using composite interval mapping (Zeng, 1994; Basten et al., 2000) by associating the trait values with yield components. The percentage variation and additive effect of each of phenotypic traits caused by the presence of QTL were also estimated using QTL Cartographer v 2.0. A LOD threshold of 2.5 was used to evaluate the presence of QTLs in each linkage group. Marker positions with respect to chromosome were determined with the RIPPLE and BUILD commands. Centimorgan units were calculated using the Kosambi mapping function (Kosambi 1944)

3. RESULTS AND DISCUSSION

3-1. Single marker analysis and differences of means

Single marker analysis of each of the relative % reduction of yield and its components with a total of 102 SSR markers was conducted to assess the association of a phenotypic trait with a marker genotype for heat tolerance using data individually pooled from four individual environments. The results of regression analysis of each of the relative % reduction of yield and its components on individual markers showed significance from 5% to 0.01% levels. There were so many markers showing association with phenotypic traits in the above range of significance levels. This study narrowed the significance levels into 0.1% and 0.01% which were indicated by ***, **** (Table XVI in Appendix). Forty-two markers showed significant associations with the relative % reduction of yield and its components from different individual environments. This is much lesser number of markers compared to 68 QTLs for the relative % reduction of yield and its components which is measure of heat tolerance from composite interval mapping analysis (Table XIII). It also showed partially common markers between two analyses. This indicates possibility of false-negative and false-negative QTL in single marker analysis which also can be adjusted by multiple statistical tests (Rebecca, 2001).

Among 42 markers, 19 markers showing 0.01% significance level from two or more phenotypic traits and different heat stress environments were selected to contrast the mean of the relative % reduction of yield and its components (Table XVIII in Appendix). This result allowed confirming positive marker alleles by significant difference between means of separately grouped alleles from two parental lines. Positive

marker alleles contributing to the lesser % reduction of yield and its components were derived from alleles of '7C' and 'Seri M82' equally in the short-term heat stress environment.

In contrast to short-term, in the long-term heat stress environment, more marker alleles derived from '7C' showed positive effect in the % reduction of yield and its components for the estimation of heat tolerance although not significant positive effect for short-term heat stress environments. This result indicate that it is very complicated to estimate exact positive markers associated with heat tolerance due to different genotypic response according to different environments.

3-2. Detection and localization of QTLs for heat tolerance

The map was constructed to identify QTLs for heat tolerance based on the relative % reduction and a simple score of each direct and indirect yield components. The yield components mapped were kernel number per main spike, kernel weight per main spike, single kernel weight per main spike and the grain filling duration in response to short term- and long term- heat stress in environmentally controlled growths room and long term heat stress in the field. The QTLs for heat tolerance identified using different temperature conditions were compared in terms of the number of QTLs, the location on the chromosome, average interval distance among markers, and the genetic action and phenotypic variation (Table XIV). Using QTL analysis to dissect heat tolerance in maize, 6 QTLs accounting for 53% of the genetic variability for cellular membrane thermo stability (CMS) were detected in recombinant inbred lines (RILs). Using the same population, 5 QTLs were detected controlling pollen germination and thermo-tolerance

(Frova and Sari-Gola, 1993; Frova, 1996). In this study, 68 QTLs and 33QTLs for heat tolerance on the basis of the relative % reduction and simple score were detected, respectively. The relative percent reduction was based on the reduction in each component in response to temperature stress versus the same components when grown in ideal conditions for each RIL. The simple score was based on a scale of 0 or 1 for yield components (where 0 for an increased or unchanged yield component after heat treatment compared to control and 1 for yield component that decreased). QTLs were detected on all chromosomes except for chromosome 1B and 3A. QTLs were evenly distributed for normally and non-normally distributed traits. This suggests that QTLs for discontinuous quantitative traits can also be identified (Table XIV, *mark for normal distribution). Among 68 QTLs for heat tolerance based on the relative % reduction of traits, 3 QTLs were detected for kernel number per main spike that explained 2-22% of phenotypic variation. Fourteen QTLs were detected for kernel number per plant explaining a variation of 2-25%. Six QTLs were detected for kernel weight per main spike with 11-20% variation. Fifteen QTLs were detected for kernel weight per plant with 6-33% variation. Five QTLs were detected for single kernel weight per main spike with 3-12% variation. Eleven QTLs were detected for single kernel weight per plant with 11-40% variation. Five QTLs were detected for spike number per plant with 20-25% variation and 9 QTLs were detected for grain filling duration per main spike with 5-14% variation (Table XIV).

Table XIV. Summary of QTLs identified by composite interval mapping for heat tolerance based on relative % reduction of yield and its component traits in the 62 RILs in four individual environments. They include a short-term heat stress (env1) of 38°C for 2days beginning at 10 days after pollination (DAP) in growth chambers, a long-term heat stress (env2) of 30°C from 10 DAP until grain maturity in the environmentally controlled growth rooms and two long-term heat stress trials in the field of 26 ~31°C (env3) and 32 ~35°C (env4). The relative percent reduction was based on the reduction in each component in response to temperature stress versus the same components when grown in ideal conditions for each RIL.

Marker Interval	Traits	Locus	Chr	Int. Dis. (cM)	LOD	R2 (%)	Additive	Env.
gwm192 - wmc285	Ker_no_ms	*Q*L_red_kno_ms*	c4d	45.9	2.76	14	7.19	env2
barc4 - gwm371	Ker_no_ms	*Q*L_red_kno_ms*	c5b	42	3.04	22	11.05	env2
wmc475 - gwm273	Ker_no_ms	*Q*S_red_kno_ms*	c7b	32.8	2.51	2	3.81	env1
wmc312 - cfa2129	Ker_no_pl	Q*F26_red_kno_pl*	c1a	22.6	5.08	22	10.74	env3
cfa2129 - barc148 gwm11	Ker_no_pl	Q*F26_red_kno_pl*	c1a	17.3	3.71	15	7.62	env3
barc124 - gwm312	Ker_no_pl	Q*F26_red_kno_pl*	c2a	41.8	2.83	12	5.01	env3
cfd56 – wmc112	Ker_no_pl	**Q*F32-red_Kno_pl*	c2d	44.6	2.70	15	5.5	env4
wmc47 - barc164	Ker_no_pl	Q*F26_red_kno_pl*	c3d	7.9	5.25	21	7.13	env3
wmc48 – wmc89	Ker_no_pl	**Q*F32_red_kno_pl*	c4a	1.5	5.39	25	6.91	env4
gwm251 - wmc238	Ker_no_pl	*Q*L_red_kno_pl*	c4b	3.5	2.81	14	10.43	env2
gwm443 - barc232	Ker_no_pl	QS*_red_kno_pl*	c5a	22.6	4.04	10	12.29	env1
	Ker_no_pl	Q*F26_red_kno_pl*	c5a	22.6	3.19	17	7.48	env3
wmc231 - wmc765	Ker_no_pl	*Q*L_red_kno_pl*	c5d	18.5	2.52	17	10.10	env2
wmc667 - wmc658	Ker_no_pl	*Q*L_red_kno_pl*	c6a	43	2.58	12	8.07	env2
wmc332 – cfa2219	Ker_no_pl	**Q*F32_red_kno_pl*	c6a	30.2	2.59	2	17.03	env4
barc77 - wmc632	Ker_no_pl	Q*F26_red_kno_pl*	c6d	56.4	3.71	11	5.13	env3
barc111 - gwm469	Ker_no_pl	*Q*L_red_kno_pl*	c7b	32.3	3.92	10	7.22	env2
wmc336 – cfd48	Ker_wt_ms	Q*L_red_kwt_ms*	c1d	29.3	5.42	21	7.92	env2
gwm372 – wmc154 barc159	Ker_wt_ms	*Q*S_red_kwt_ms*	c2b	20	2.94	15	12.48	env1
wmc48 – wmc89	Ker_wt_ms	*Q*S_red_kwt_ms*	c4a	1.5	4.01	20	13.71	env1
gwm251 – wmc238	Ker_wt_ms	*Q*S_red_kwt_ms*	c4b	3.5	3.15	17	12.14	env1
wmc238 – gwm6	Ker_wt_ms	Q*L_red_kwt_ms*	c4b	14.7	2.64	11	6.25	env2
wmc634 – wmc606	Ker_wt_ms	*Q *S_red_kwt_ms*	c7d	31	2.63	13	12.70	env1
barc45 – wmc201	Ker_wt_pl	Q*F26_red_kwt_pl*	c2b	67.3	6.35	33	1.0	env3
gwm493 – cfd223	Ker_wt_pl	Q*F26_red_kwt_pl*	c3b	24.2	5.02	21	0.87	env3
wmc48 – wmc89	Ker_wt_pl	Q*S_red_kwt_pl*	c4a	1.5	3.07	13	11.28	env1
	Ker_wt _pl	Q*F26_red_kwt_pl*	c4a	1.5	3.44	14	0.54	env3
	Ker_wt_pl	Q*F32_red_kwt_pl*	c4a	1.5	3.38	14	0.57	env4
wmc238 – gwm6	Ker_wt_pl	Q*F32_red_kwt_pl*	c4b	14.7	3.11	10	0.55	env4
gwm604 – barc4	Ker_wt_pl	*Q*L_red_kwt_pl*	c5b	0.8	5.12	26	15.08	env2
wmc765 – gwm595	Ker_wt_pl	*Q*L_red_kwt_pl*	c5d	31.6	2.66	14	8.43	env2
gwm190 – cfd57	Ker_wt_pl	Q*F26_red_kwt_pl*	c5d	32.9	3.47	7	0.41	env3
	Ker_wt_pl	Q*F32_red_kwt_pl*	c5d	32.9	2.55	10	0.51	env4
wmc658 – wmc622	Ker_wt_pl	Q*F32_red_kwt_pl*	c6a	16.2	2.92	19	1.05	env4
gwm484 – cfd102	Ker_wt_pl	Q*F26_red_kwt_pl*	c6b	10.1	5.80	13	0.69	env3
barc77 – wmc632	Ker_wt_pl	Q*F26_red_kwt_pl*	c6d	56.4	3.0	8	0.41	env3
wmc475 – gwm273	Ker_wt_pl	Q*S_red_kwt_pl*	c7b	32.8	2.81	6	10.29	env1
barc111 – gwm469	Ker_wt_pl	Q*F32_red_kwt_pl*	c7b	32.3	3.73	19	0.72	env4

Table XIV (continued)

Marker Interval	Ttraits	Locus	Chr	Int. Dis. (cM)	LOD	R2 (%)	Additive	Env.
wmc336 - cfd48	Sker_wt_ms	*Q*L_red_skwt_ms*	c1d	29.3	3.59	12	4.57	env2
gwm192 – wmc285	Sker_wt_ms	*Q*L_red_skwt_ms*	c4d	45.9	3.55	11	4.69	env2
gwm604 – barc4	Sker_wt_ms	*Q*L_red_skwt_ms*	c5b	0.8	2.52	10	6.71	env2
barc111 – gwm469	Sker_wt_ms	**Q*S_red_skwt_ms*	c7b	32.3	2.55	3	4.79	env1
	Sker_wt_ms	*Q*L_red_skwt_ms*	c7b	32.3	2.86	11	4.58	env2
cfd223 – gwm108	Sker_wt_pl	Q*F32_red_skwt_pl*	c3b	54.6	3.73	19	0.35	env4
wmc48 – wmc89	Sker_wt_pl	Q*F26_red_skwt_pl*	c4a	1.5	4.24	25	0.36	env3
gwm251 – wmc238	Sker_wt_pl	**Q*S_red_skwt_pl*	c4b	3.5	4.44	30	14.31	env1
wmc238 – gwm6	Sker_wt_pl	**Q*S_red_skwt_pl*	c4b	14.7	3.09	25	13.06	env1
gwm192 – wmc285	Sker_wt_pl	**Q*S_red_skwt_pl*	c4d	45.9	2.68	12	7.49	env1
gwm604 – barc4	Sker_wt_pl	Q*L_red_skwt_pl*	c5b	0.8	5.17	22	10.53	env2
gwm617 – gwm292	Sker_wt_pl	Q*F32_red_skwt_pl*	c5d	56.4	2.74	12	0.26	env4
wmc658 – wmc622	Sker_wt_pl	Q*F26_red_skwt_pl*	c6a	16.2	3.65	40	1.05	env3
wmc622 – wmc332	Sker_wt_pl	Q*F26_red_skwt_pl*	c6a	10.1	3.39	31	0.81	env3
cfd75 – barc17	Sker_wt_pl	**Q*S_red_skwt_pl*	c6b	29.5	2.85	15	9.11	env1
barc17 – wmc232	Sker_wt_pl	Q*F32_red_skwt_pl*	c6b	20.2	2.85	11	0.29	env4
gwm604 – barc4	Sp_no_pl	**Q*S_red_spno_pl*	c5b	0.8	3.54	20	15.03	env1
gwm617 – gwm292	Sp_no_pl	**Q*L_red_spno_pl*	c5d	56.4	3.7	25	4.62	env2
wmc622 – wmc332	Sp_no_pl	**Q*L_red_spno_pl*	c6a	10.1	3.23	21	6.79	env2
	Sp_no_pl	**Q*L_red_spno_pl*	c6a	10.1	3.85	25	8.34	env2
barc77 – wmc632	Sp_no_pl	**Q*L_red-spno_pl*	c6d	56.4	2.94	24	4.73	env2
wmc336 – cfd48	GFD_ms	Q*L_red_gfd_ms*	c1d	29.3	5.71	12	2.43	env2
gwm372 – wmc154	GFD_ms	Q*L_red_gfd_ms*	c2b	20	5.12	11	3.51	env2
barc147 – gwm493	GFD_ms	Q*L_red_gfd_ms*	c3b	8.6	6.27	13	2.52	env2
wmc238 – gwm6	GFD_ms	Q*L_red_gfd_ms*	c4b	14.7	9.37	14	2.7	env2
barc4 – gwm371	GFD_ms	Q*L_red_gfd_ms*	c5b	42.0	6.92	9	2.5	env2
wmc231 – wmc765	GFD_ms	**Q*S_red_gfd_ms*	c5d	18.5	3.18	14	5.08	env1
	GFD_ms	Q*L_red_gfd_ms*	c5d	18.5	6.04	5	1.64	env2
wmc765 – gwm595	GFD_ms	Q*L_red_gfd_ms*	c5d	31.6	7.58	9	2.27	env2
wmc634 – wmc606	GFD_ms	Q*L_red_gfd_ms*	c7d	31	7.32	7	1.91	env2

*, ** indicate traits showing normal distribution at the level s of α=0.05 and 0.01

As for heat tolerance using simple score, 33QTLs were detected including 1 QTL for kernel number per main spike which explained 22% of the variations, 7 QTLs for kernel number per plant explained 3-100% of the variation, 2 QTLs for kernel weight per main spike explained 6 and 100% of the variation, 5 QTLs for kernel weight per plant explained 1-100% of the variation, 3 QTLs for single kernel weight per main spike explained 26-100% of the variation, 5QTLs for single kernel weight per plant with 20-100% variation, 3QTLs for spike number per plant with 18-22% variation and 7QTLs for grain filling duration per main spike with 10-25% variation were found (Table XV). The number of QTLs for heat tolerance was more when based on the relative % reduction than based on simple score of traits. In addition, additive effects of QTLs base on the relative % reduction were mostly high and in the positive direction, but when based on simple score very low close to 0 or 1. This is expected due to the lack of variation.

No QTLs for heat tolerance based on the relative % reduction of kernel number per main spike matched QTLs identified for the relative % reduction of kernel number per plant. Two QTLs among 6 QTLs for heat tolerance for kernel weight per main spike matched two of the 15 QTLs for kernel weight per plant. These included QTLs between wmc48 and wmc89 and between wmc238 and gwm6 on the chromosome 4A and 4B, respectively. In addition, 2 QTLs among 5 QTLs for maintenance of single kernel weight of the main spike overlapped two QTLs of the 11QTLs for single kernel weight per plant. These were located on chromosome 4D and 5B, respectively. One QTL among 5 QTLs for maintenance of spike number per plant during heat stress overlapped with 1

QTL of 14 QTLs for maintenance of kernel number per plant on chromosome 5B. Two QTLs for maintenance of spike number per plant also overlapped 2 QTLs of 15 QTLs for maintenance of kernel weight per plant on chromosome 6D. Two QTLs among 9 QTLs for maintenance of grain filling duration overlapped with 2 QTLs for kernel weight per plant on the chromosomes 4B and 5D. Three QTLs among 14 QTLs for heat tolerance based on the relative % reduction of kernel number per plant (yield) had matches with three QTLs of the 15 QTLs for % reduction of kernel weight per plant (another yield). These are located between wmc48 and wmc89 and between barc77 and wmc632 and barc111and gwm469 on the chromosome 4A, 6D and 7B, respectively. These may be important loci for heat tolerance based on various measurements for heat tolerance. There were 3QTLs or more for heat tolerance clustered on some chromosomes based on the relative % reduction of traits of yield and its components including chromosome 1D (3traits), 4A (4traits), 4B (5traits), 5B (5traits), 6A (3traits), 6D (3traits), and 7B (3traits) which included marker regions between wmc336 and cfd48, between wmc48 and wmc89, between gwm251and wmc238, and between gwm604and barc4, and between wmc658 and wmc622, and between barc77and wmc632, and between barc111and gwm469, respectively (Fig. 20). These QTLs might be very important regions for heat tolerance because those QTLs for heat tolerance were commonly detected from three or more traits with more than one environment of different high temperatures and their interval distances between QTLs were within 10 cM which could been regarded as the same QTLs (Collard et al., 2003). In addition, these QTLs had high correlation coefficients for the relative % reduction of the traits (Table X, Fig. 20). Three

QTLs for relative % reduction of kernel number per plant and kernel weight per plant on 4A, 5A and 5D were stable across 2 of 4 heat stress environments (Table XIV). The rest of the QTLs for heat tolerance might be greatly affected by the effect of QTL x environment based on detection of QTL in only one individual environment. QTLs for maintenance of kernel weight, single kernel weight and grain filling duration of the main spike during heat stress were closely clustered on the chromosome 1D, 2B, 4A, 4B, 5B, 6A, 7B and 7D. This clustered localization of QTLs for the relative % reduction of traits corresponds to the high correlation coefficients between the relative % reductions of these traits. This indicates a high probability of the co-segregation between these traits (Fig. 20, Table X). QTLs for the maintenance of grain filling duration per main spike during heat stress clustered with the QTLs for kernel weight per main spike and per plant. This is in agreement with the high correlation or variation between these two traits (Fig. 20, Table X).

Table. XV. Summary of QTLs identified by composite interval mapping for heat tolerance based on simple score of yield and its component traits in the 62 RILs in four individual environments. They include short-term heat stress (env1) at 38°C for 2days beginning at 10 days after pollination (DAP) in growth chambers, long-term heat stress (env2) at 30°C from 10DAP until grain maturity in the environmentally controlled growth rooms and long- term heat tress in the field at both of 26~31°C (env3) and 32~35°C(env4). The simple score was based on the stability of the individual yield components in response to heat stress versus control-treated conditions.

Marker Interval	Traits	Locus	Chr	Int. Dis. (cM)	LOD	R2 (%)	Additive	Env.
wmc154 – barc167 gwm11	Ker_no_ms	O*L_ss_kno_ms*	c2b	20.8	3.91	22	0.24	env2
cfa2129 – barc148 gwm11	Ker_ no_pl	Q*S_ss_kno_pl*	c1a	17.3	10.76	3	1	env1
gwm642 – wmc626	Ker_no_pl	Q*S_ss_kno_pl*	c1d	35.8	17.14	77	1	env1
gwm630 – barc45	Ker_no_pl	Q*F32_ss_kno_pl*	c2b	67.3	8.6	12	0.22	env4
wmc48 – wmc89	Ker_no_pl	Q*F32_ss_kno_pl*	c4a	1.5	5.0	26	0.23	env4
cfd18 – gwm626	Ker_no_pl	Q*L_ss_kno_pl*	c5d	9.2	2.65	14	0.23	env2
wmc622 – wmc332	Ker_no_pl	Q*F26_ss_kno_pl*	c6a	10.1	323.0	100	0.01	env3
wmc332 –cfa2219	Ker_no_pl	Q*F32_ss_kno_pl*	c6a	30.2	2.53	10	0.20	env4
wmc622 – wmc332	Ker_wt_ms	Q*L_ss_kwt_ms*	c6a	10.1	323.0	100	0.01	env2
wmc475 – gwm273	Ker_wt_ms	Q*S_ss_kwt_ms*	c7b	32.8	3.13	6	0.09	env1
cfa2129 – barc148 gwm11	Ker_wt_pl	Q*S_ss_kwt_pl*	c1a	17.3	15.27	72	0.52	env1
gwm642 – wmc626	Ker_wt_ pl	Q *S_ss_kwt_pl*	c1d	35.8	6.83	28	0.44	env1
wmc622 – wmc332	Ker_wt _pl	Q*F26_ss_kwt_pl*	c6a	10.1	321.0	100	0.01	env3
	Ker_wt_pl	Q*F32_ss_kwt_pl*	c6a	10.1	5.32	100	0.01	env4
barc111 – gwm469	Ker_wt_pl	Q*L_ss_kwt_pl*	c7b	32.3	323.2	1.0	-0.02	env2
Wmc658 – wmc622	SKer_wt_ms	Q*S_ss_skwt_ms*	c6a	16.2	4.51	50	0.47	env1
Wmc622 – wmc332	SKer_wt_ms	Q*L_ss_skwt_ms*	c6a	10.1	322.0	100	0.01	env2
Wmc517 – barc182	SKer_wt_ms	Q*S_ss_skwt_ms*	c7a	8.6	2.79	26	0.26	env1
Wmc658 – wmc622	Sker_wt_pl	Q*S_ss_skwt_pl*	c6a	16.2	9.34	67	0.50	env1
Wmc622 – wmc332	Sker_wt_pl	Q*L_ss_skwt_pl*	c6a	10.1	322.6	100	0.01	env2
	Sker_wt_pl	Q*F26_ss_skwt_pl*	c6a	10.1	321.7	100	0.01	
env3								
	Sker_wt_pl	Q*F32_ss_skwt_pl*	c6a	10.1	321.0	100	0.01	env4
Wmc634 – wmc606	Sker_wt_pl	Q*S-ss_skwt_pl*	c7d	31.0	2.70	20	0.21	env1
gwm443 – barc232	Sp_no_pl	Q*L_ss_spno_pl*	c5a	22.6	3.45	18	0.27	env2
wmc622 – wmc332	Sp_no_pl	Q*S_ss_spno_pl*	c6a	10.1	3.54	21	0.33	env1
	Sp_no_pl	Q*L_ss_spno_pl*	c6a	10.1	3.38	22	0.39	env2
cfd48 – gwm642	GFD_ms	Q*S_ss_gfd_ms*	c1d	14.4	2.50	10	0.48	env1
	GFD_ms	Q*L_ss_gfd_ms*	c1d	14.4	6.82	20	0.01	env2
wmc48 –wmc89	GFD_ms	Q*S_ss_gfd_ms*	c4a	1.5	2.69	14	0.16	env1
gwm604 – barc4	GFD_ms	Q*L_ss_gfd_ms*	c5b	0.8	23.64	25	0.01	env2
wmc231 – wmc765	GFD_ms	Q*S_ss_gfd_ms*	c5d	18.5	3.02	19	0.2	env1
cfd57 – wmc231	GFD_ms	Q*L_ss_gfd__ms*	c5d	14	7.36	25	0.01	env2
wmc622 – wmc332	GFD_ms	Q*L_ss_gfd_ms*	c6a	10.1	321.67	25	0.01	env2

3-3. Composite interval mapping and differences and means

Among 68 QTLs, 17 QTLs involving flanking markers, which were commonly detected in two and more phenotypic traits and different environments within small interval distance (<10cM) between flanking markers, were selected to contrast the mean of the relative % reduction of yield and its components per main spike and per plant (Table XIX and Table XX in Appendix). These results allowed confirming positive QTLs for heat tolerance by significant difference between means of separately grouped alleles of two flanking markers from two parental lines. The more positive QTLs between flanking marker alleles contributing to the lesser % reduction of yield and its components for estimation of heat tolerance were derived from QTLs between flanking marker alleles of 'Seri M82' in the short-term heat stress environment. In contrast, in the long-term heat stress environment, the more QTLs derived from '7C' showed positive effect than those derived from 'Seri M82'. QTLs between wmc336 and cfd48, between wmc48and wmc89, between gwm251 and wmc238, and between wmc622 and wmc332 on the chromosome 1D, 4A, 4B and 6A, respectively, showed greater differences of means of kernel weight between two different alleles than those of other QTLs. These QTLs involving flanking marker alleles contributing to the lesser % reduction of yield and its components were derived from QTLs between flanking marker alleles derived from '7C' in both short-term and long term heat stress environments. This result supports the previous result in this study that there were 3QTLs or more for heat tolerance clustered on some chromosomes based on the relative % reduction of traits of

yield and its components including chromosome 1D (3traits), 4A (4traits), 4B (5traits), 6A (3traits), 7D (Fig. 20).

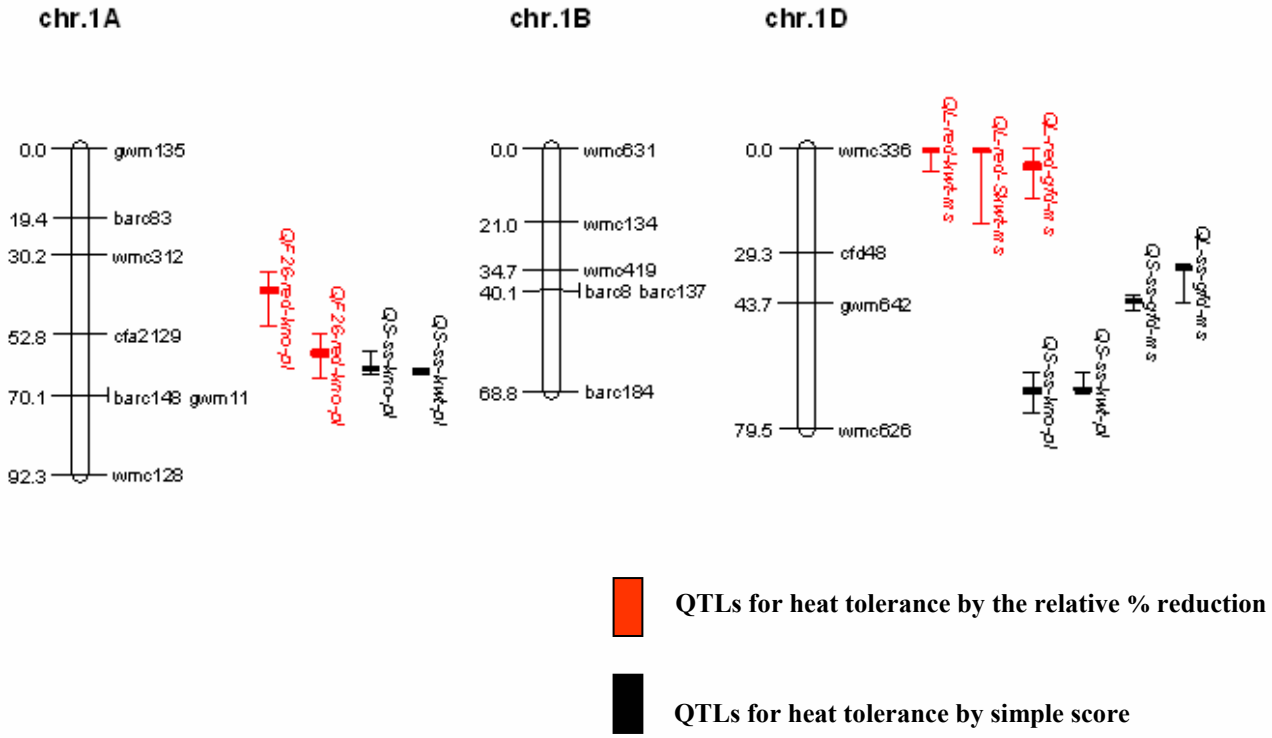
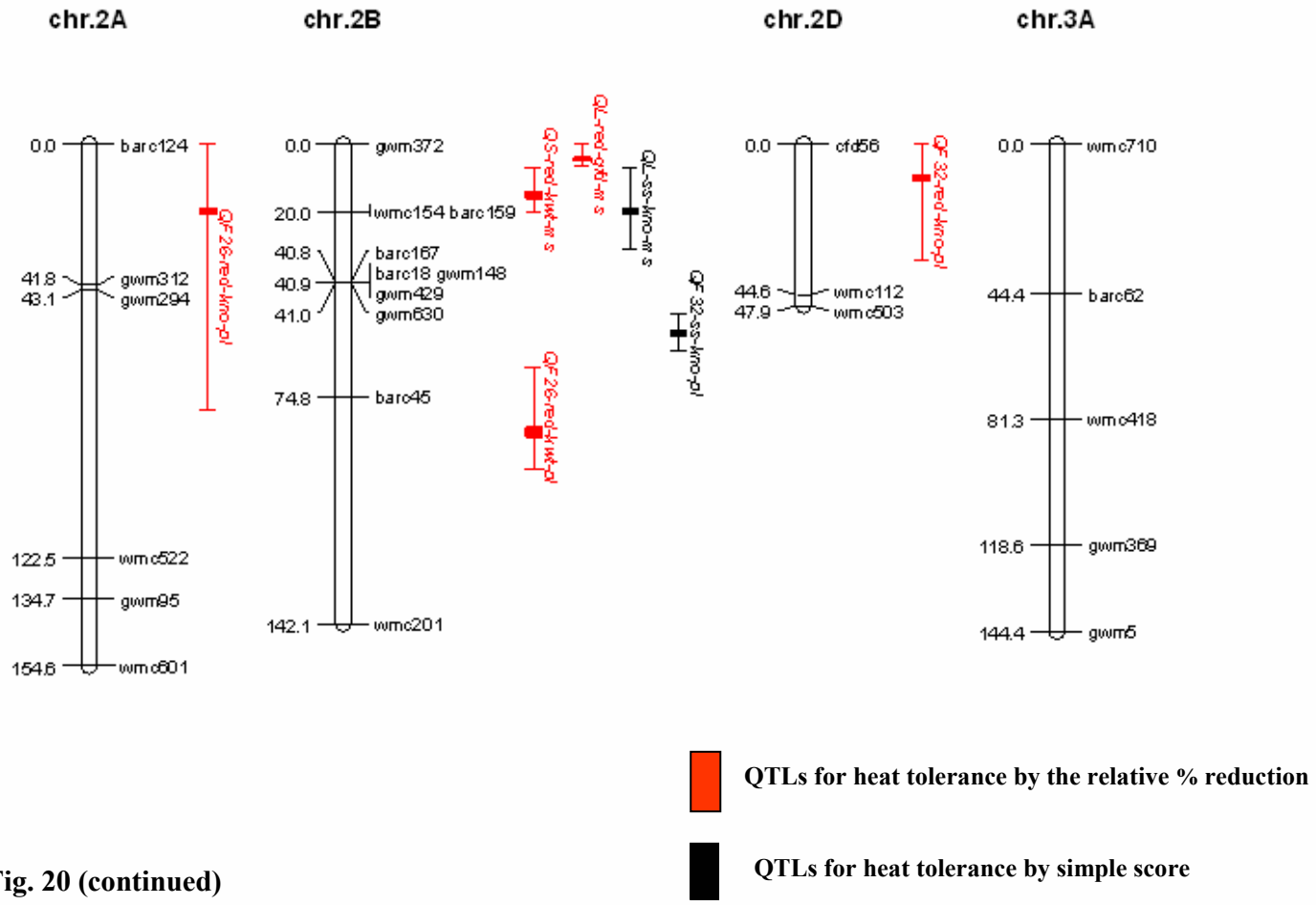


Fig. 20. Composite interval mapping of QTLs for heat tolerance on the basis of the relative % reduction and simple score of yield and its components in the 62 RIL population.



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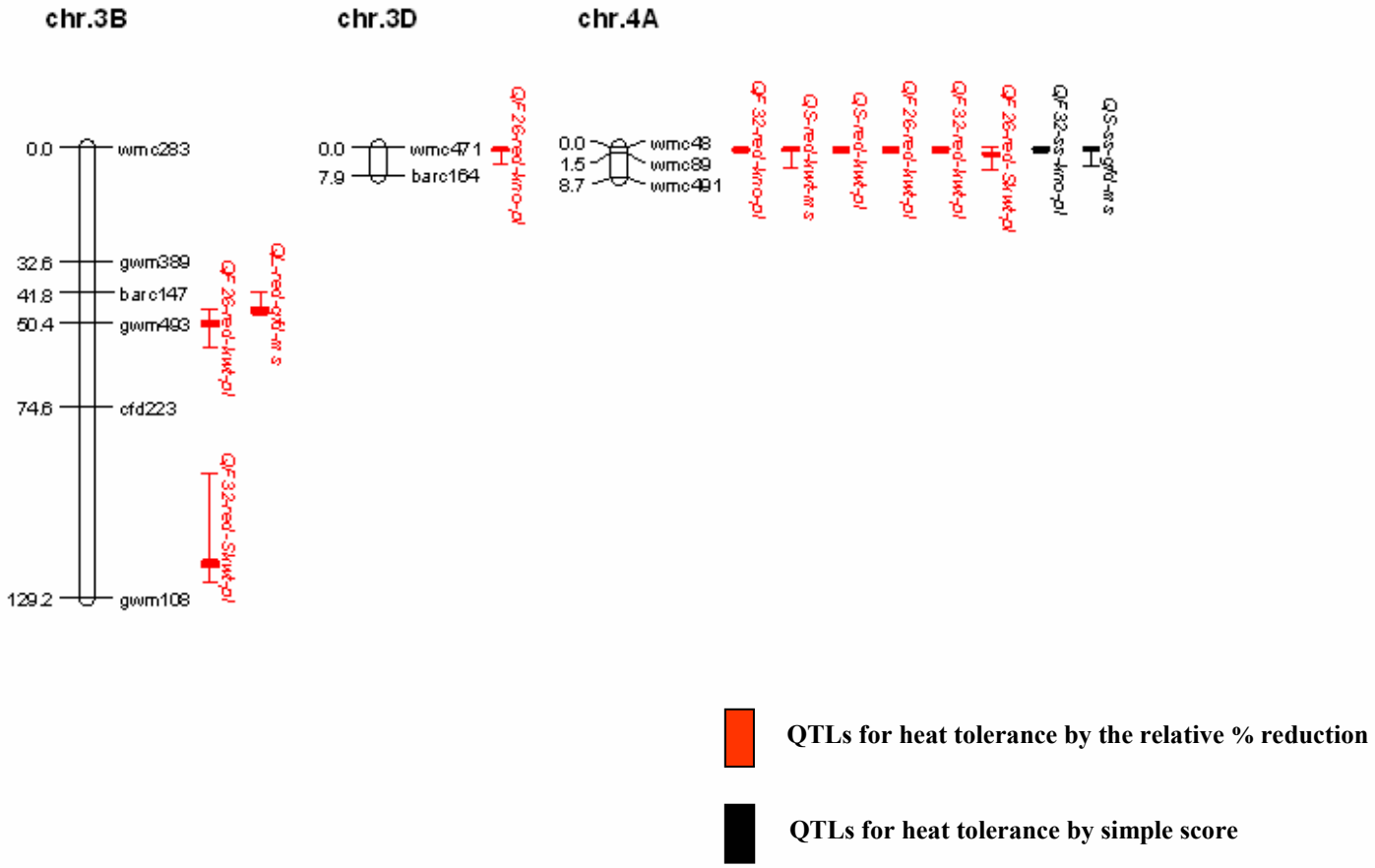

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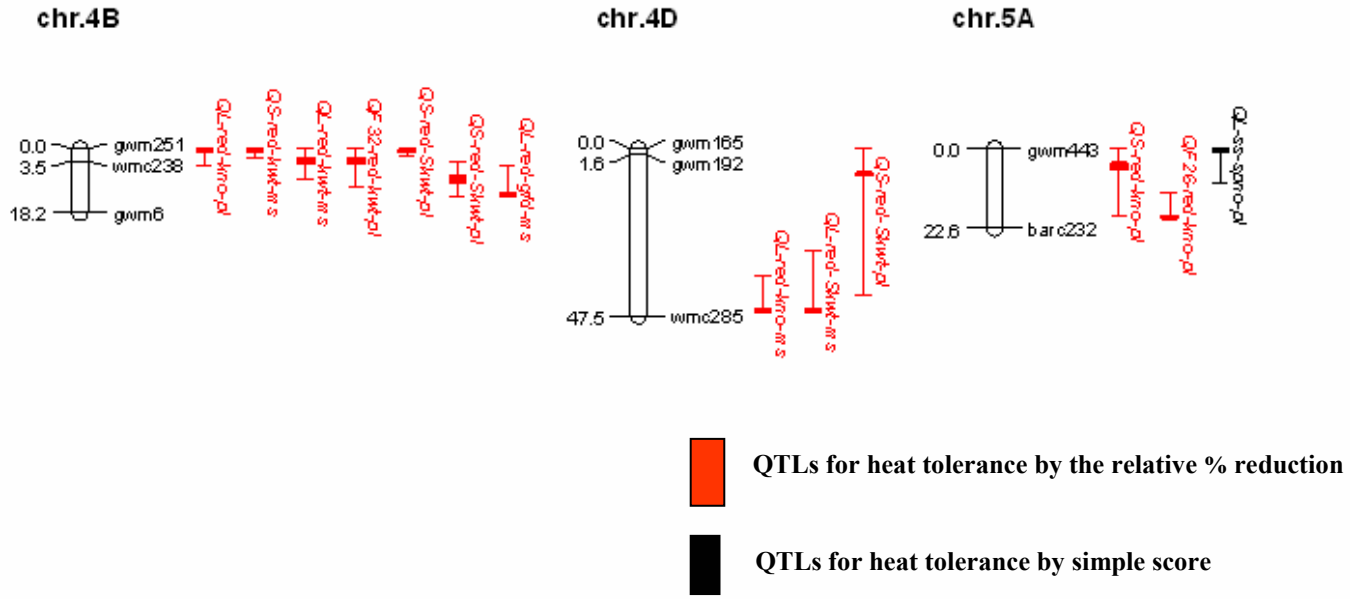

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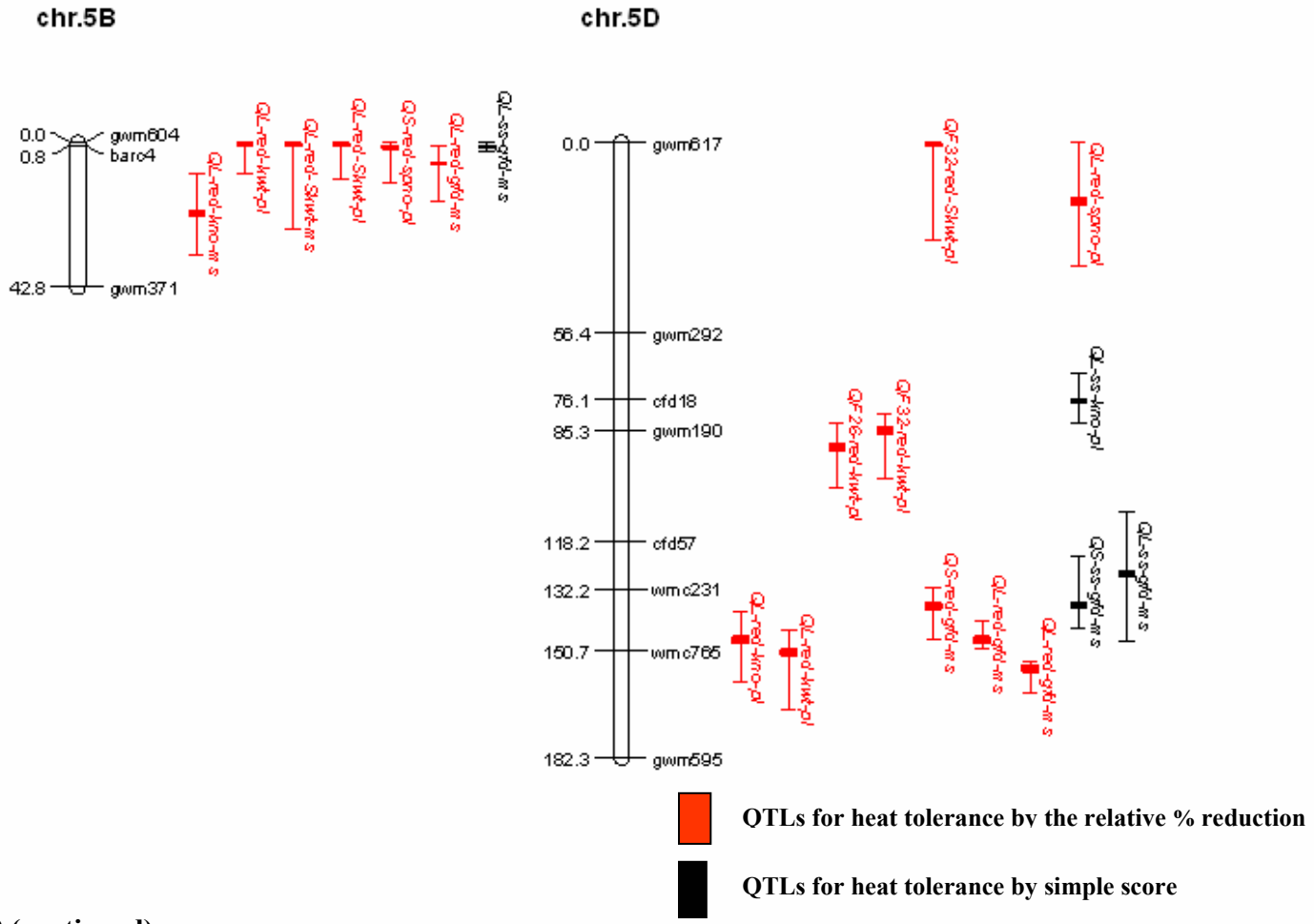

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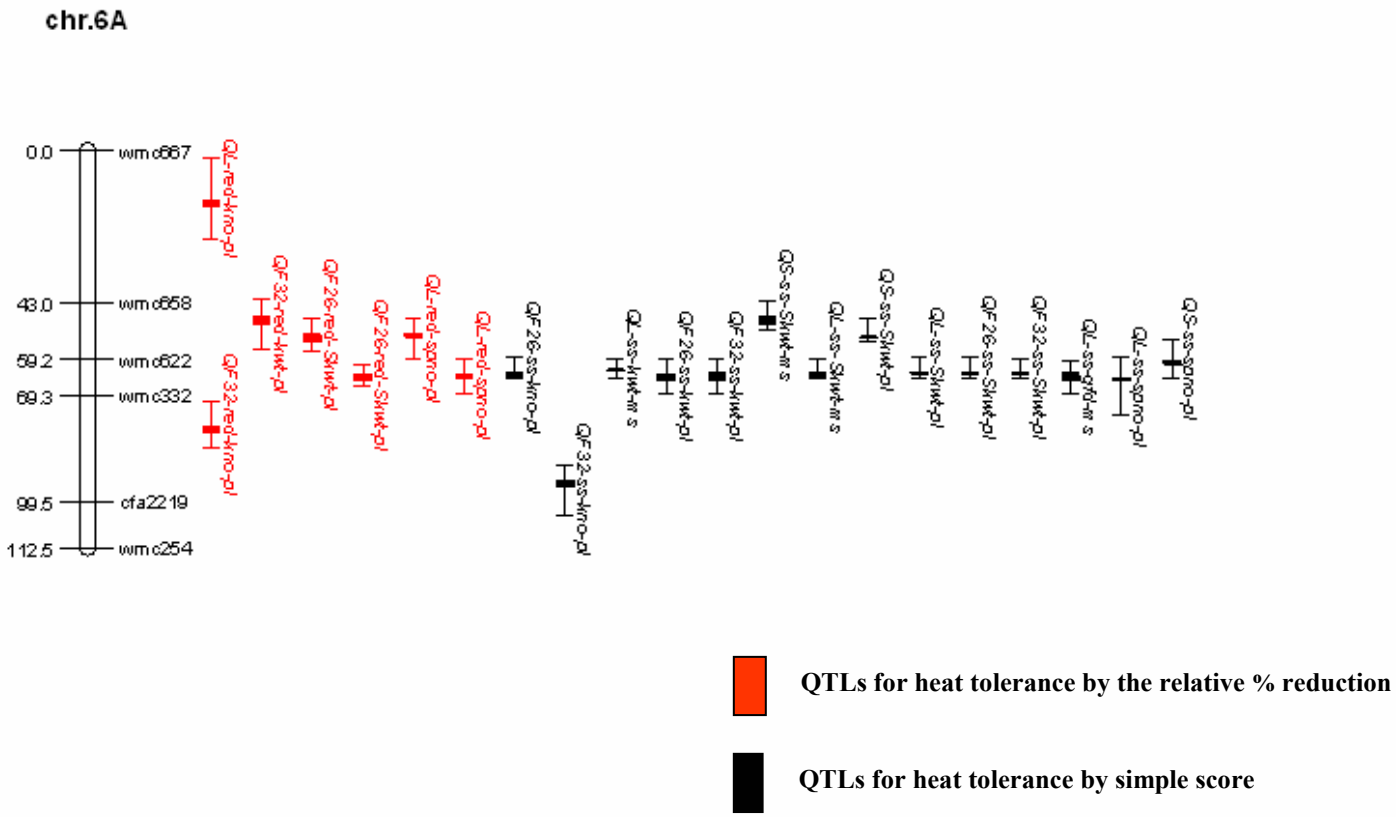

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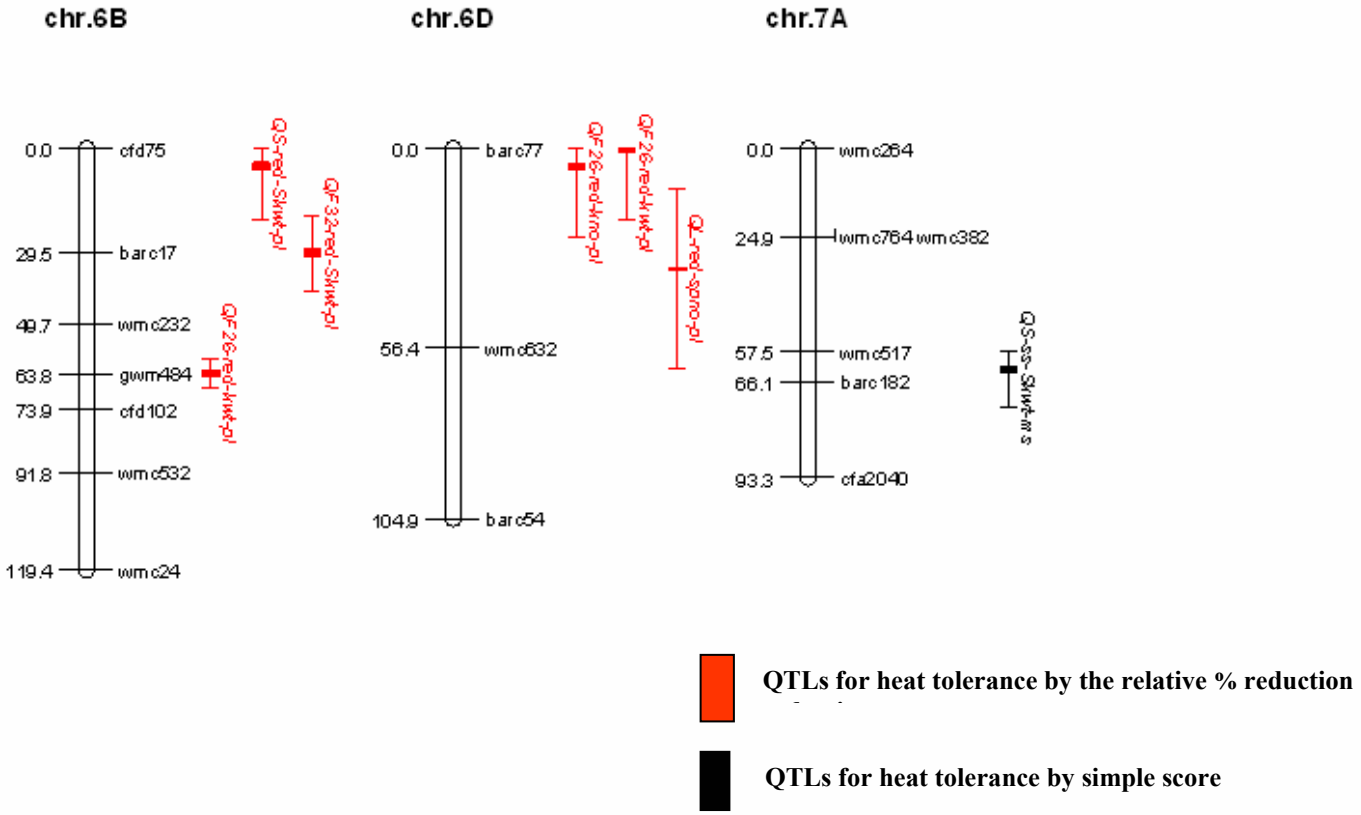

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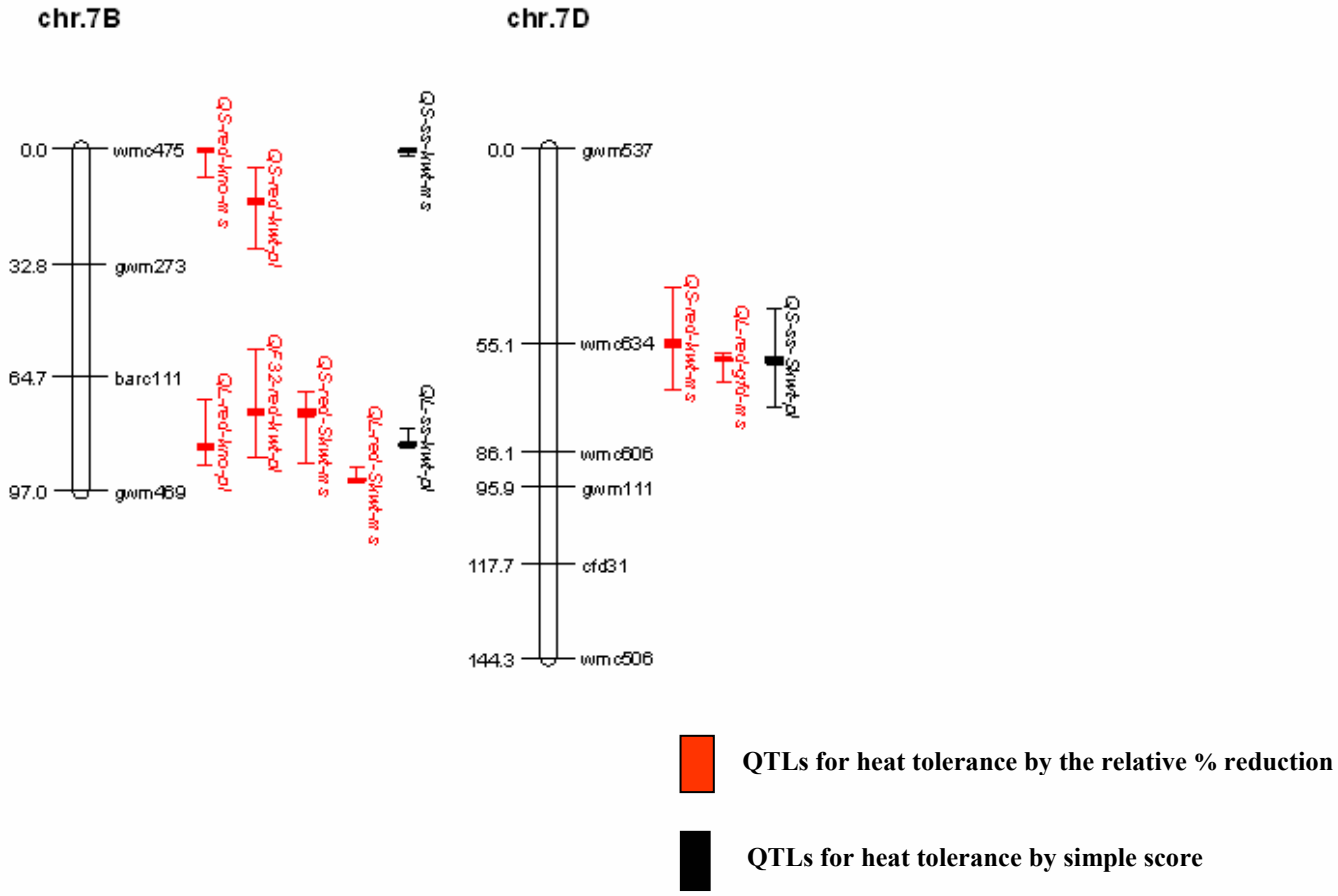

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CHAPTER V

CONCLUSION

Two suitable parents were selected for the study. 'Seri M82' (moderate heat susceptible variety) and '7C' (moderate heat tolerant variety) exhibited contrasting yield response to long and short-term reproductive stage heat stress based on the analysis of individual yield components (Table I). The difference in the relative % reduction of yield and its components between the two parental lines was more significant after short-term than long-term heat stress. Kernel weight was also more sensitive to heat stress than kernel number. '7C' increased in kernel number per main spike and per plant even after two days of heat stress, but not kernel number per main spike after long- term heat stress (Table I). However kernel weight reduced in both of parental lines after both heat stresses.

Significant differences for mean yield and mean yield components were observed between the control population and the heat treated population for the 62 RILs of which most of individual lines were greatly reduced after those heat stresses (Table II, Table III).

The degree of decrease of yield including kernel number per plant and kernel weight per plant was much higher in the field than in the environmentally controlled growth rooms (Table II and Table III). These results can be explained by the likelihood that multiple environmental factors occurred in conjunction with high temperature in the field than environmentally controlled growth room.

The population of 62 RILs from a cross of heat tolerant variety '7C' and heat susceptible variety 'Seri M 82' exhibited a broad segregation and significant trangressive segregation compared to the two parental lines for most yield components.

This resulted from an optimal recombination of desirable alleles and their different additive effects on the yield components (Fig. 2~Fig. 18).

The normality analysis of yield components revealed that the RIL distributed more normally when heat treated compared to control conditions (Tables IV~VII).This suggests adverse environment exerted a greater influence on inducing gene regulation.

The Pearson correlation coefficients identified significant correlation between yield components as well as yield components using the relative % reduction (Table VIII, Table IX and Table X). Most of the yield components significantly correlated with both kernel number per plant and kernel weight per plant in response to long and short-term reproductive stage heat stress. This indicates that each component for yield plays a role as an individual contributor to yield productivity through their coordinated operation in response to heat stress treatment. In addition, this significant correlation between each yield component and yield also suggests a quantitative inheritance of yield components and heat tolerance.

The ANOVA analysis revealed significant differences between the 62 RIL for each yield component and heat tolerance based on the relative % reduction of yield components (Table XI and Table XII). The heritability for yield components and heat tolerance was very low. This may have resulted from the significant genotype x environment effects derived from marked differences between environments measured in this study from environmentally controlled growth room with extremely different temperature to field condition with various environmental factors. Thus it appears that the higher heritability depend on using more various environments, replications and less experimental error.

On the foundation of various quantitative analyses for yield components in response to heat stress treatments, yield components and the relative % reduction of the yield components may have important meaning as quantitative traits for heat tolerance.

The genetic map for the 62 RILs population was composed of 102 SSR markers (Fig. 19). Eleven SSR markers were not linked to linkage groups. Most of the linkage groups had large linkage distances. These results demand more SSR markers localized on the chromosomes for fine mapping. The present genetic map did not completely match with existing high –density microsatellite consensus map for a bread wheat (Somers et al., 2004). This may derive from the small population, the different genetic backgrounds used, and not enough polymorphic markers.

Eighty-one QTLs for yields components were detected on most chromosomes with one or more QTLs except for chromosome 2D, 3D and 4B. The QTLs for yield components and heat tolerance based on the relative % reduction were found evenly from both normal distribution and non-normal distribution (Table XIII and Table XIV, '*' mark indicates normal distribution). These QTLs had high LOD values and phenotypic variations. This suggests good utility of discontinuous quantitative traits for identification of QTLs of interest.

Sixty-eight QTLs for yield components using the relative % reduction of traits were detected with high LOD value, phenotypic variation, and additive effect (Table XIV). As for yield components based on simple score, 33QTLs were detected with high phenotypic variation, LOD value and additive effects (Table XV). These results indicate that the multiple QTLs for yield components and heat tolerance have great meanings as

major alleles affecting phenotype variation by regulations of the major genes in response to heat stress.

Most of loci controlling kernel number were overlapped or closely clustered with loci controlling kernel weight (Fig. 21). In addition, the loci controlling the other yield components also were overlapped or closely clustered one another on the same linkage group. These correspond to high correlation coefficients between them in response to heat stress treatments (Table VIII, Table IX and Table X). These results indicate that the loci associated with yield components were co-segregated or each gene was involved in pleiotropic effect. A few QTLs for each yield component overlapped with QTLs for total yield including kernel number per plant and kernel weight per plant. This indicates that total yield is associated with incorporation of QTLs for each yield component.

Twelve QTLs among 15 QTLs for heat tolerance base on the % reduction of the kernel weight per plant showed less % reduction than 1% or so in kernel weight per plant by additive effect and high phenotypic variation (above 10%) under heat stress environments (Table XIV). These QTLs could be more heat resistant QTLs than the rest of QTLs for heat tolerance based on the less % reduction in yield components by additive effects.

In many interval regions of markers on the chromosome 1D, 2A, 2B, 3B, 4A, 4D, 5A, 5B, 6A, 6B, 6d, 7B, 7D exhibited QTLs for both yield components and heat tolerance (Fig 21). This indicates that these QTLs involve common yield and heat tolerance responses. Specifically the interval regions of markers between wmc48 and wmc89 and between wmc622 and wmc332 on the chromosome 4A and 6A, respectively possessed QTLs for yield components and heat tolerance based on both the relative %

reduction and simple score of yield components from various environments (Fig. 21). This indicates the possible importance of these loci for stable yield even under extreme heat stress. In addition, the marker region between wmc48 and wmc89 on the chromosome 4A has been reported to be significant QTL for drought tolerance of common wheat (Kirigwi et al., 2007). This suggests that the marker region between wmc48 and wmc89 may be useful for MAS to enhance drought and heat tolerance, simultaneously.

On the contrary, on chromosome, 3A, and 4B, only QTLs for yield components and heat tolerance were detected, respectively. This suggests the presence of distinct genes controlling heat tolerance separately from genes controlling yield components.

The QTLs for heat tolerance on the basis of the simple score of traits on chromosome 1D were localized separately with those based on the relative % reduction. This may indicate that there is a poor relationship between two methods for measurements of heat tolerance. The reason is unknown. However, based on the QTLs on chromosome, 4A and 6A where 2 or more QTLs co-localized for heat tolerance based on both the relative % reduction and the simple score of traits with high LOD values and high phenotypic variations (Fig. 21). And QTLs using the simple score were clustered on the chromosome 1A, 1D and 6A (Fig. 21) which corresponded with the results from the Pearson's correlation (Table X). Thus, use of the two methods for measurements of heat tolerance may be useful in the identification of major QTLs for heat tolerance.

In comparison of both single marker analysis and composite interval mapping analysis, the partially common markers between two analyses suggest that there may be possibility of false-positive and false-negative markers requiring multiple adjustment

tests. In the contrast of mean of yield components and their % reduction, positive marker alleles contributing to the greater yield components and the lesser % reduction of yield components from were differently derived from two parental lines according to short-term and long-term heat stress environments. This suggest that it is very complicated to estimate exact positive markers or QTLs associated with yield components and heat tolerance due to different genotypic response to various environments. But in this study, it has been found that the positive alleles for high yield components were mostly derived from 'Seri M82' and the positive alleles for great heat tolerance were nearly derived from '7C'. This indicates that the wheat variety having high yield performance does not always show the great heat tolerance.

Further confirmatory and complementary studies to identify QTLs for yield components and heat tolerance will be required prior to the application of molecular markers to wheat breeding program. These include the construction of 'high resolution mapps' using a larger population size (> 1000 individuals), greater numbers of markers (at least < 5cM but ideally < 1cM interval between genes) (Blair et al., 2003; Chunwongse et al., 1997; Li et al., 2003), validation of markers in independent populations and different genetic backgrounds (Cakir et al., 2003; Collins et al., 2003; Jung et al., 1999; Langridge et al., 2001; Li et al., 2001; Sharp et al., 2001) and finally, the verification of the putative QTLs by evaluating the difference in yields between individuals with and without alleles of the putative QTLs in more than 3 environments (Romagosa et al., 1999). These processes will enable the utilization those putative QTLs in marker assisted selection (MAS) (Sharp et al., 2001). In addition, the stability of QTLs across unique heat stress environments with optimal numbers of locations and years is

crucial in enhancing the efficiency of selection for heat tolerant genotypes in early generation (Collard et al., 2003).

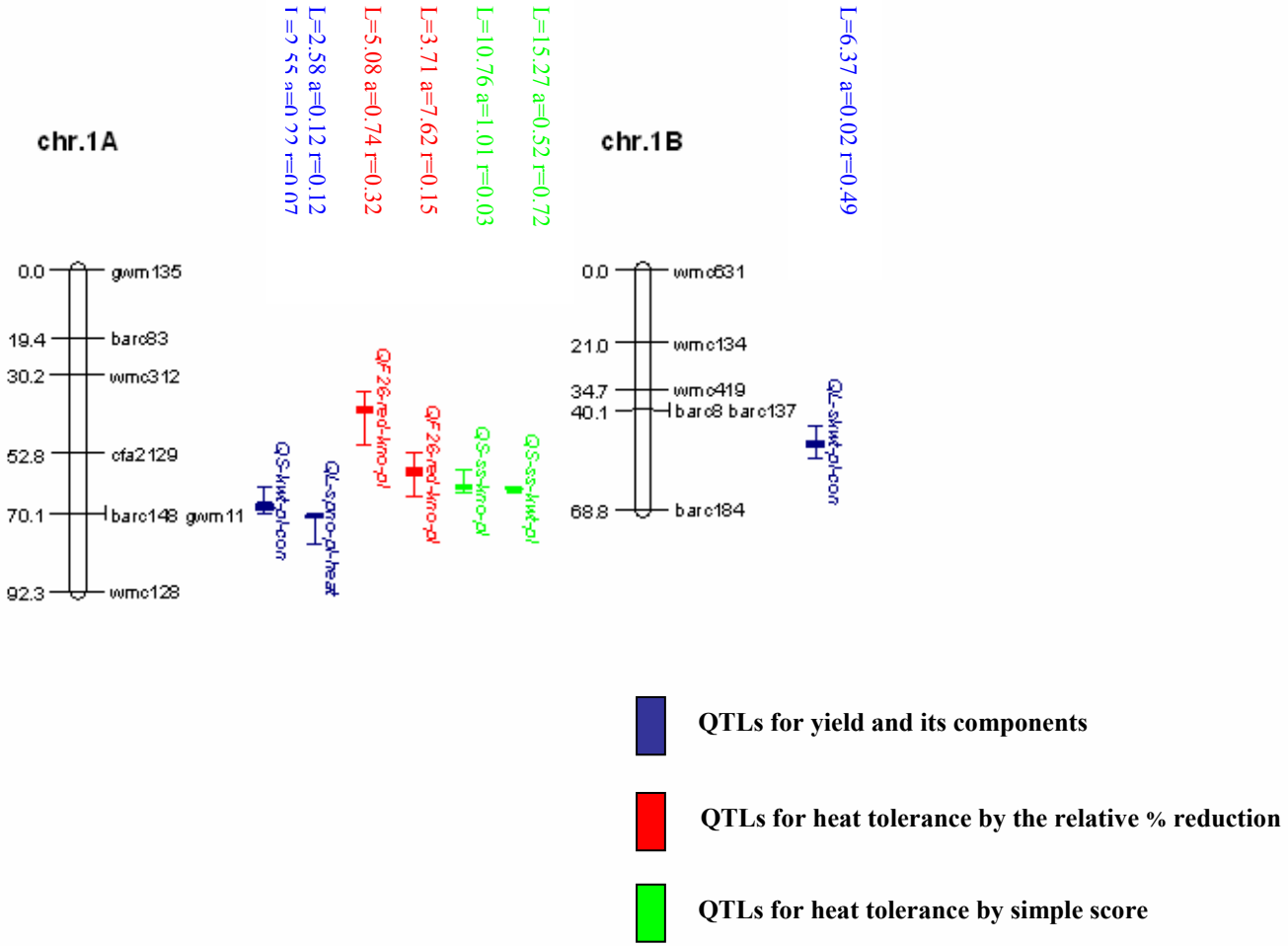
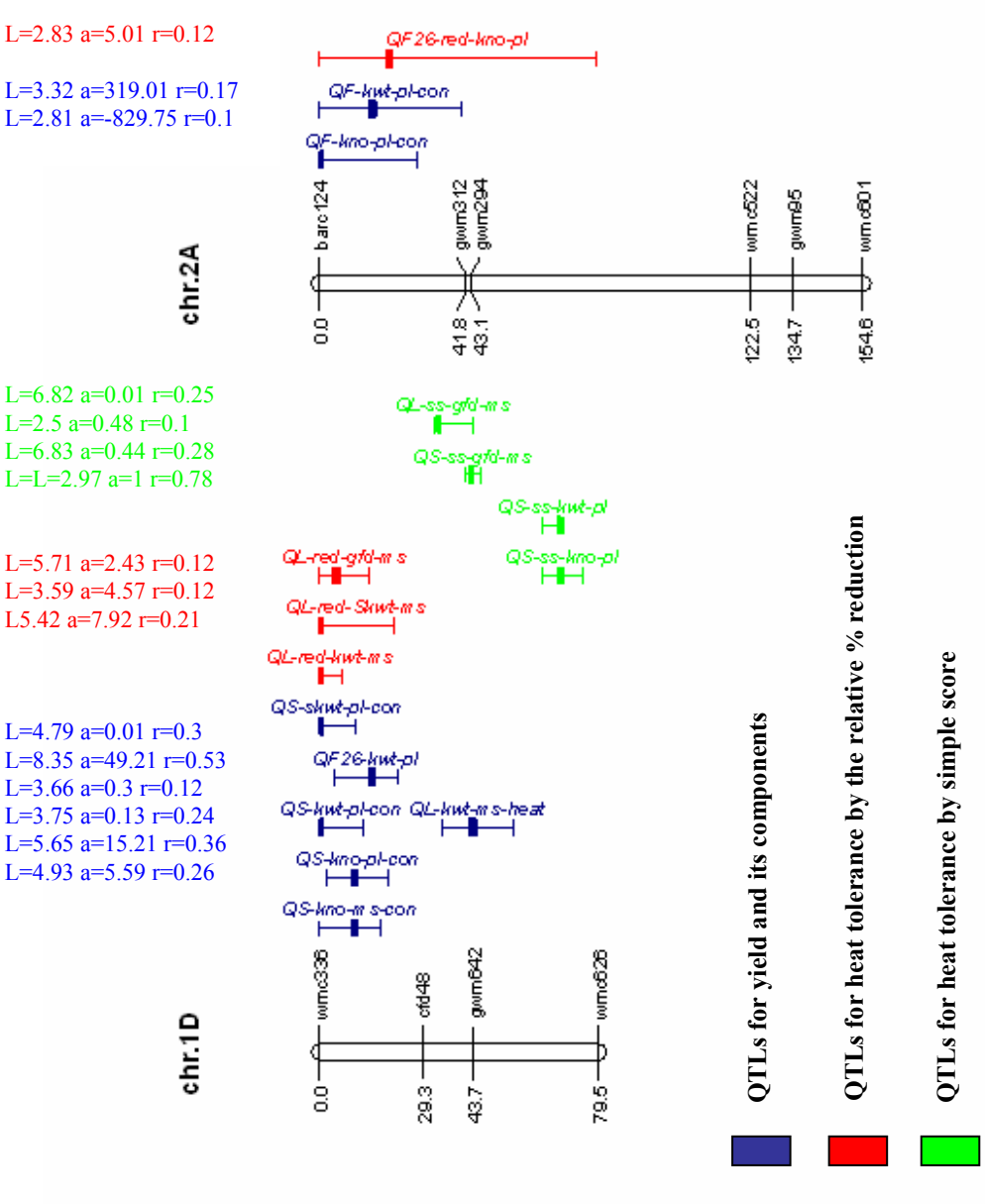


Fig. 21. Composite interval mapping of QTL for yield, its component, and heat tolerance on the basis of the relative % reduction and simple score of yield and its components in the 62 RIL population. Designations to the top of the chromosome represent l=lod, a=additive effect and r= phenotypic variation.



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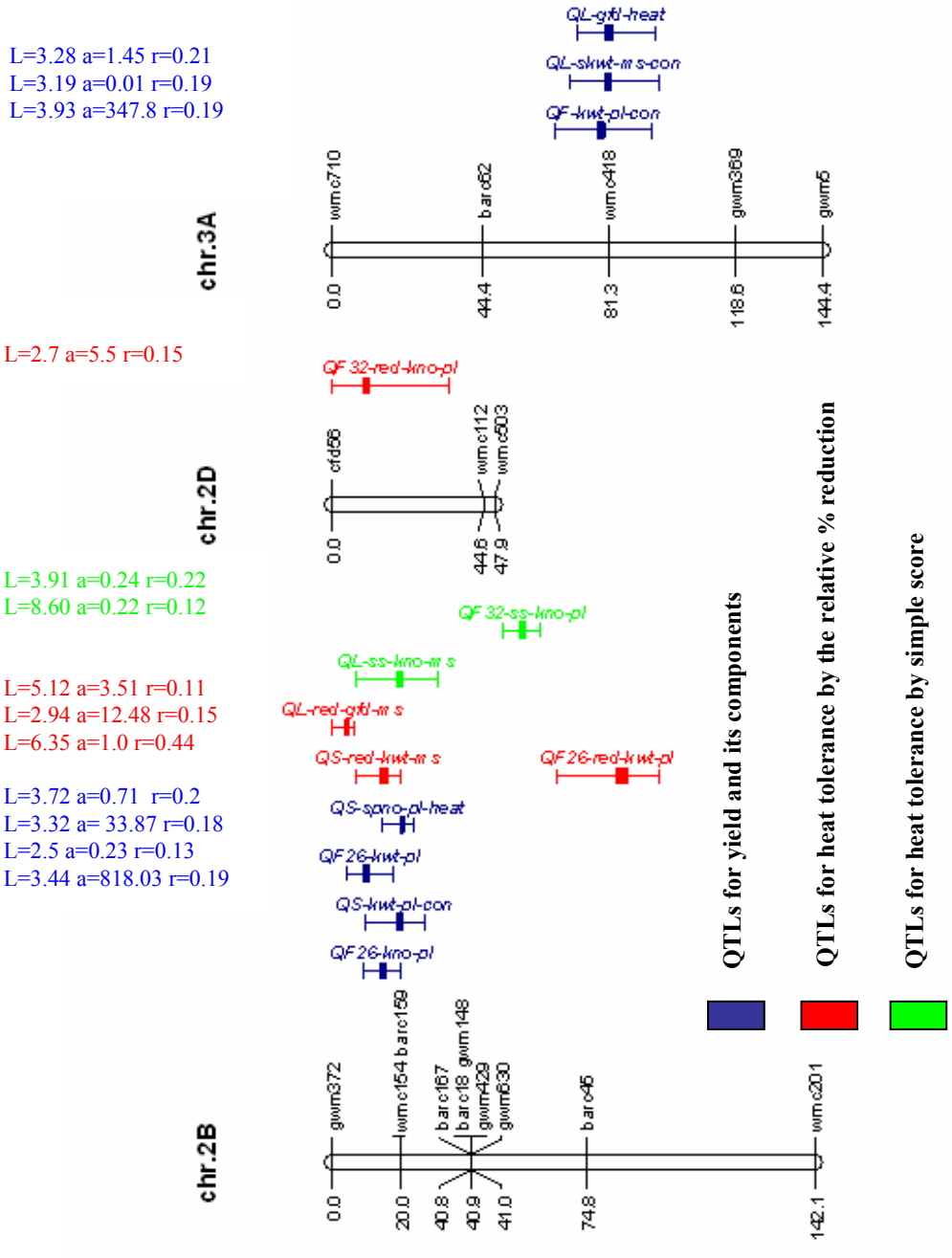

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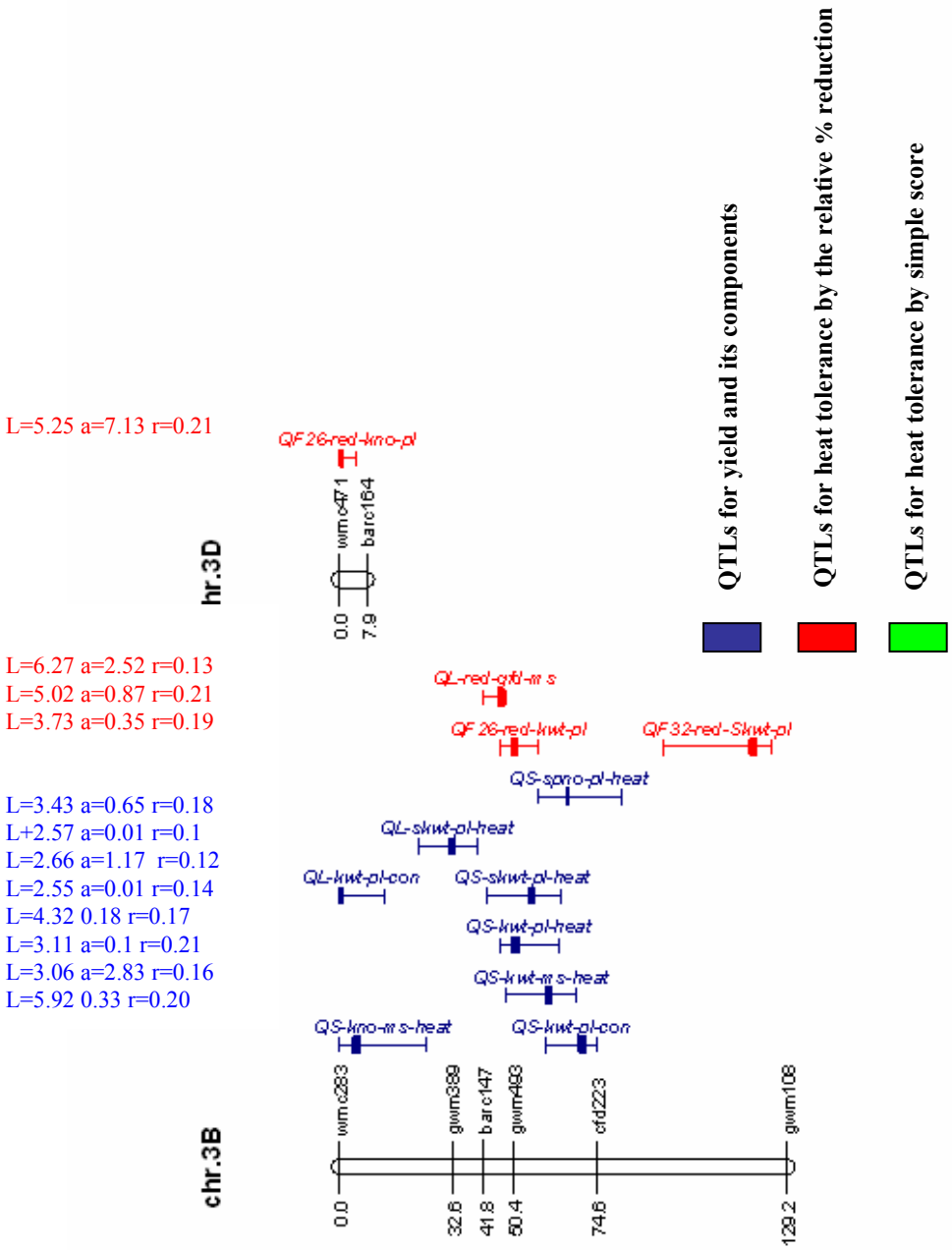

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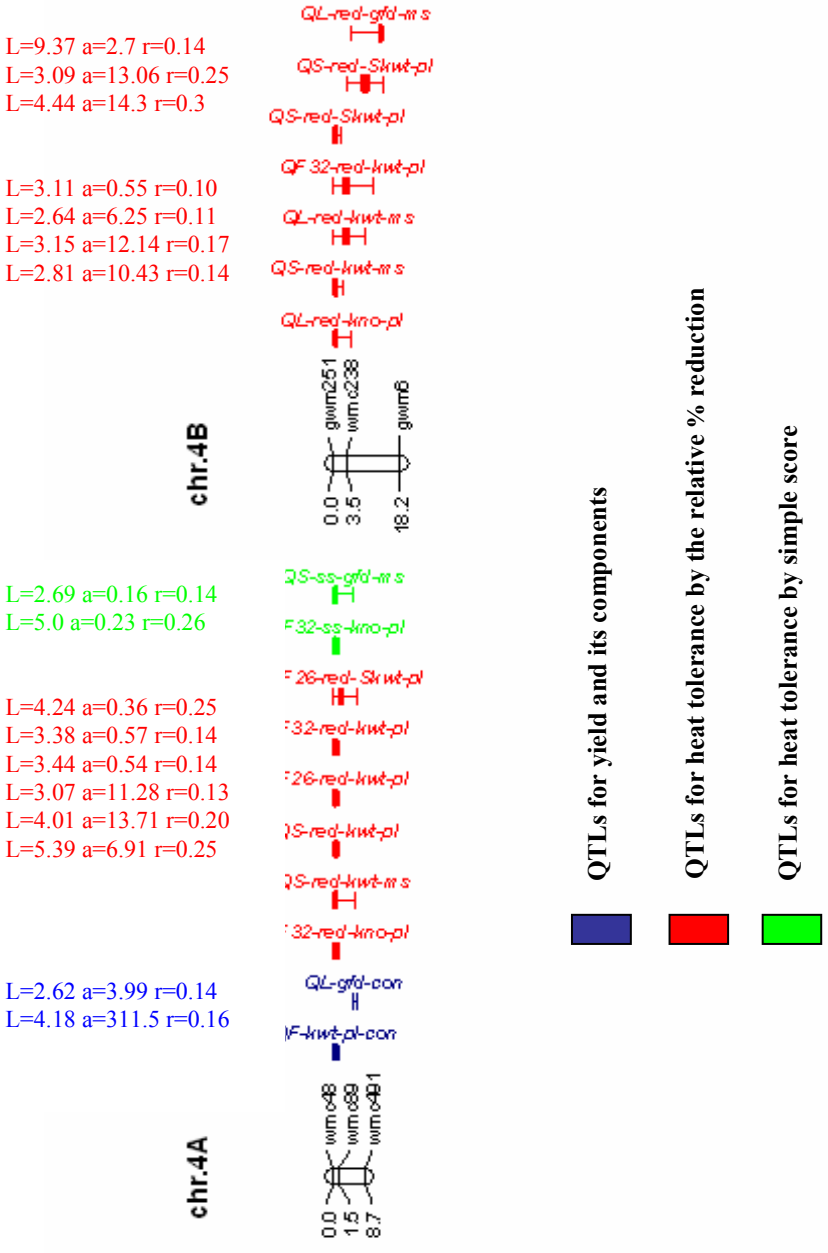

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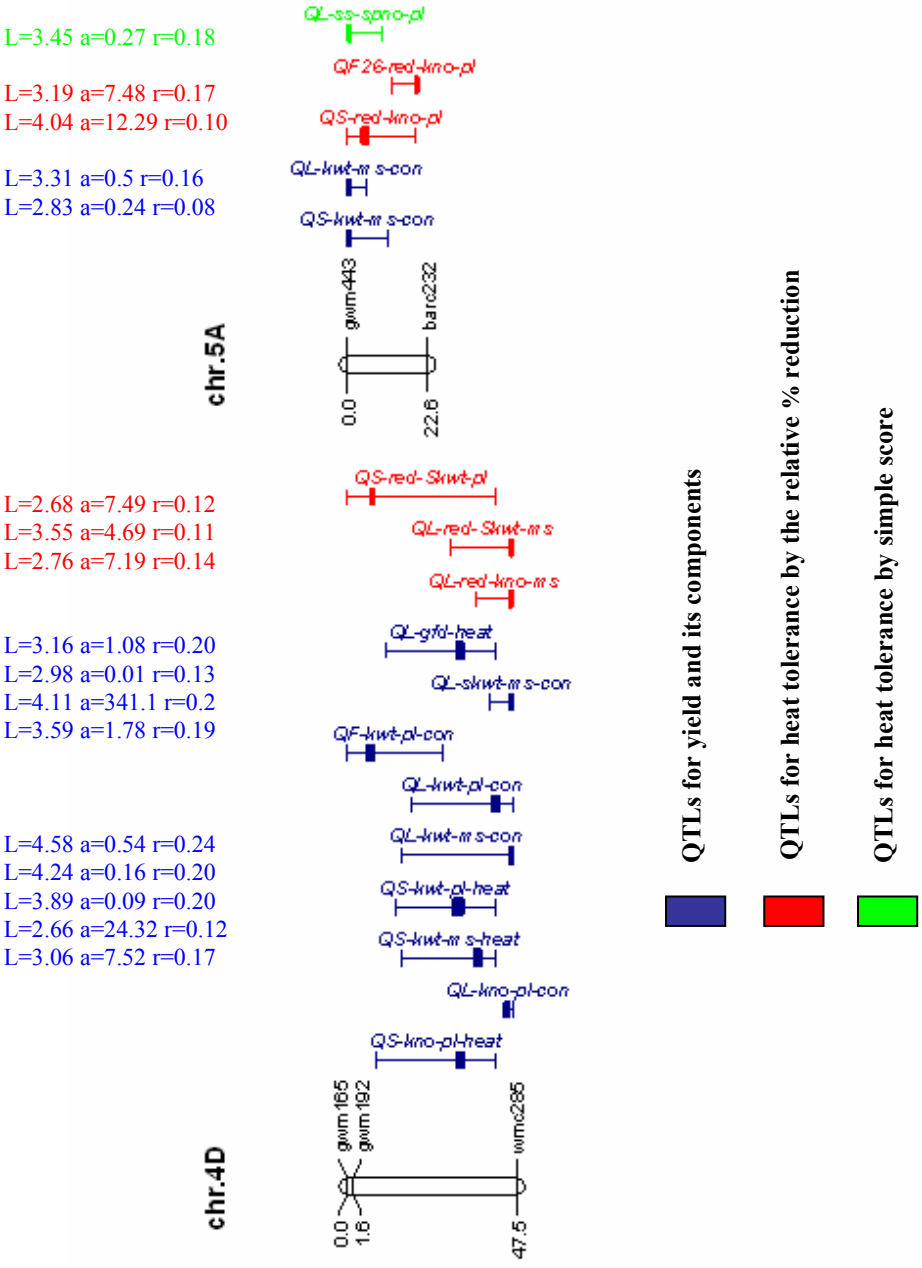

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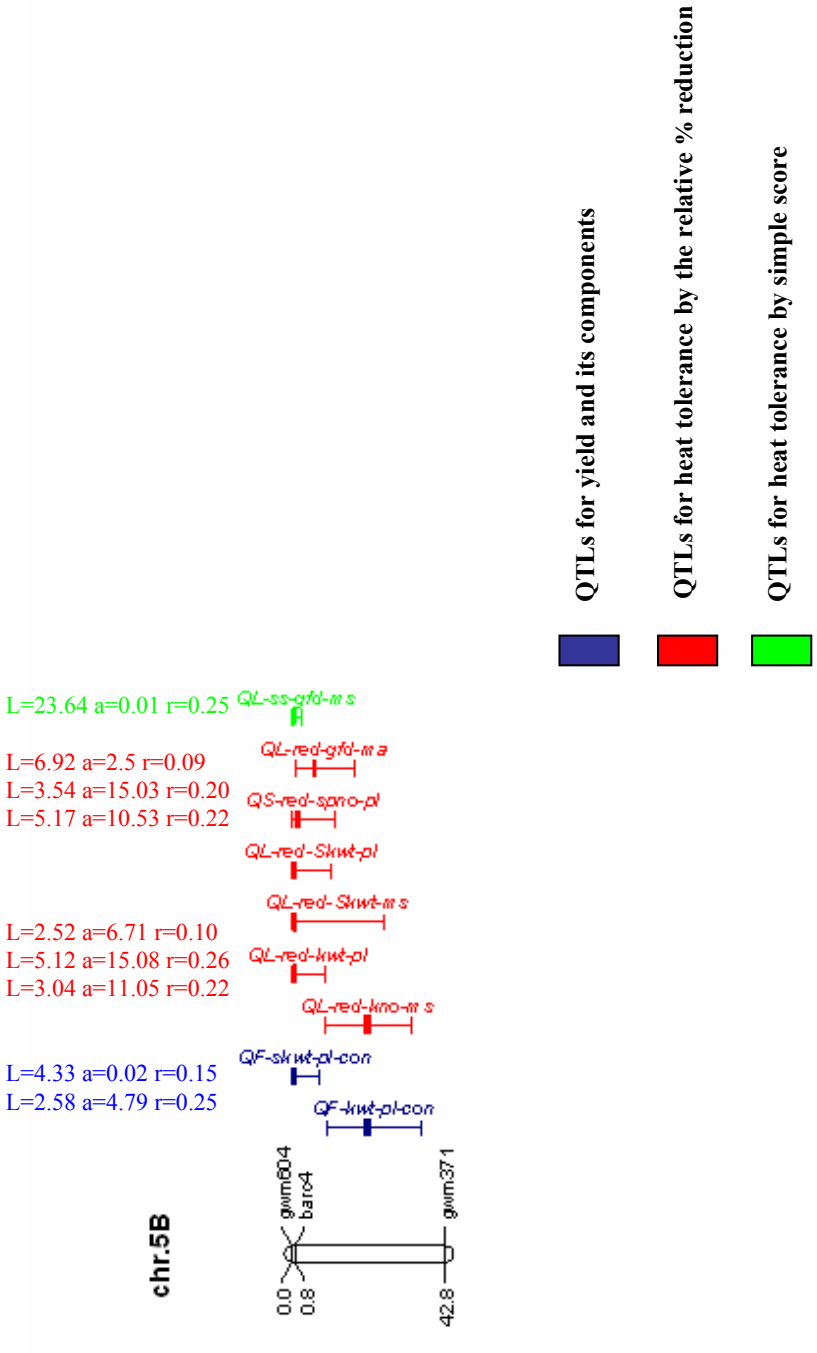

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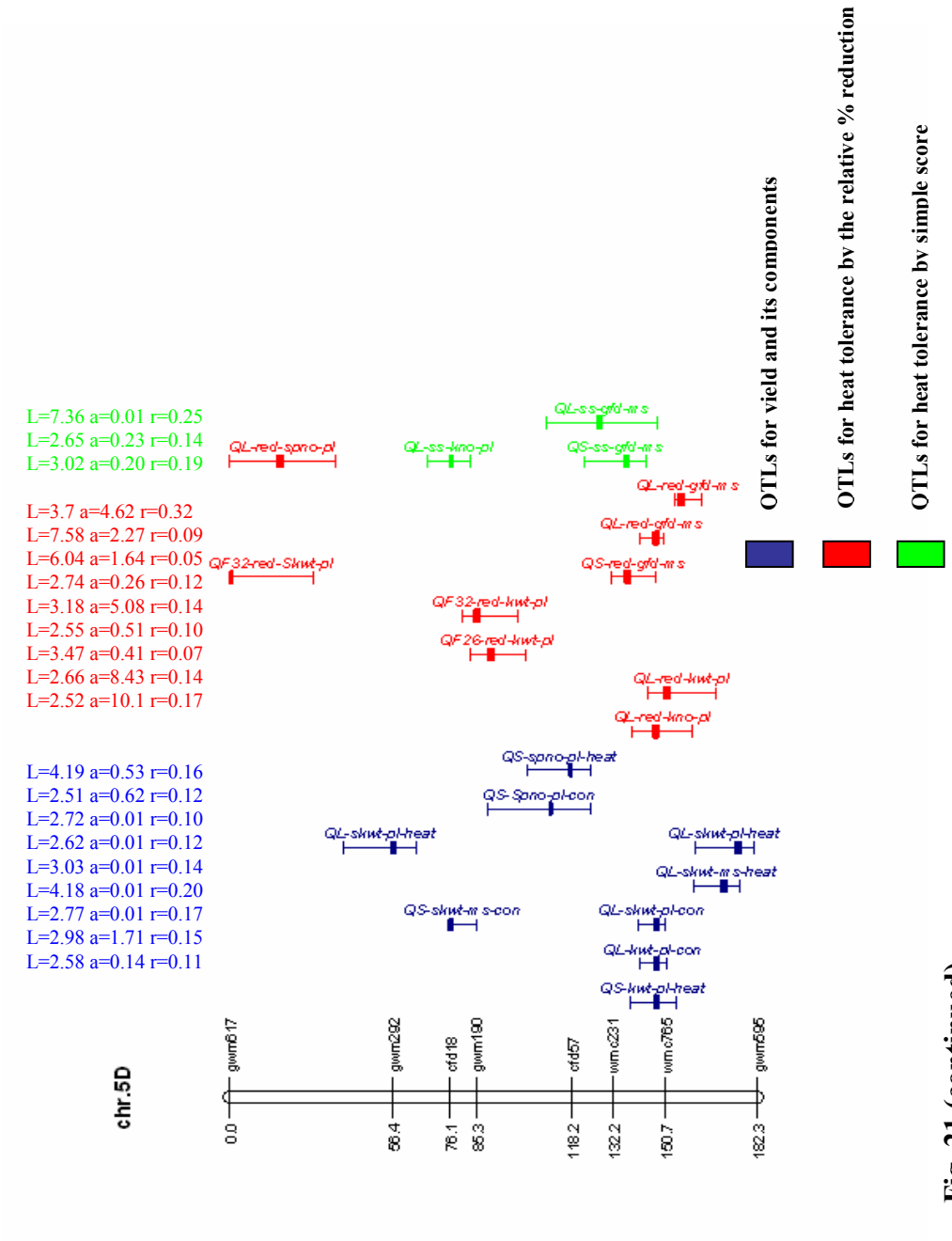

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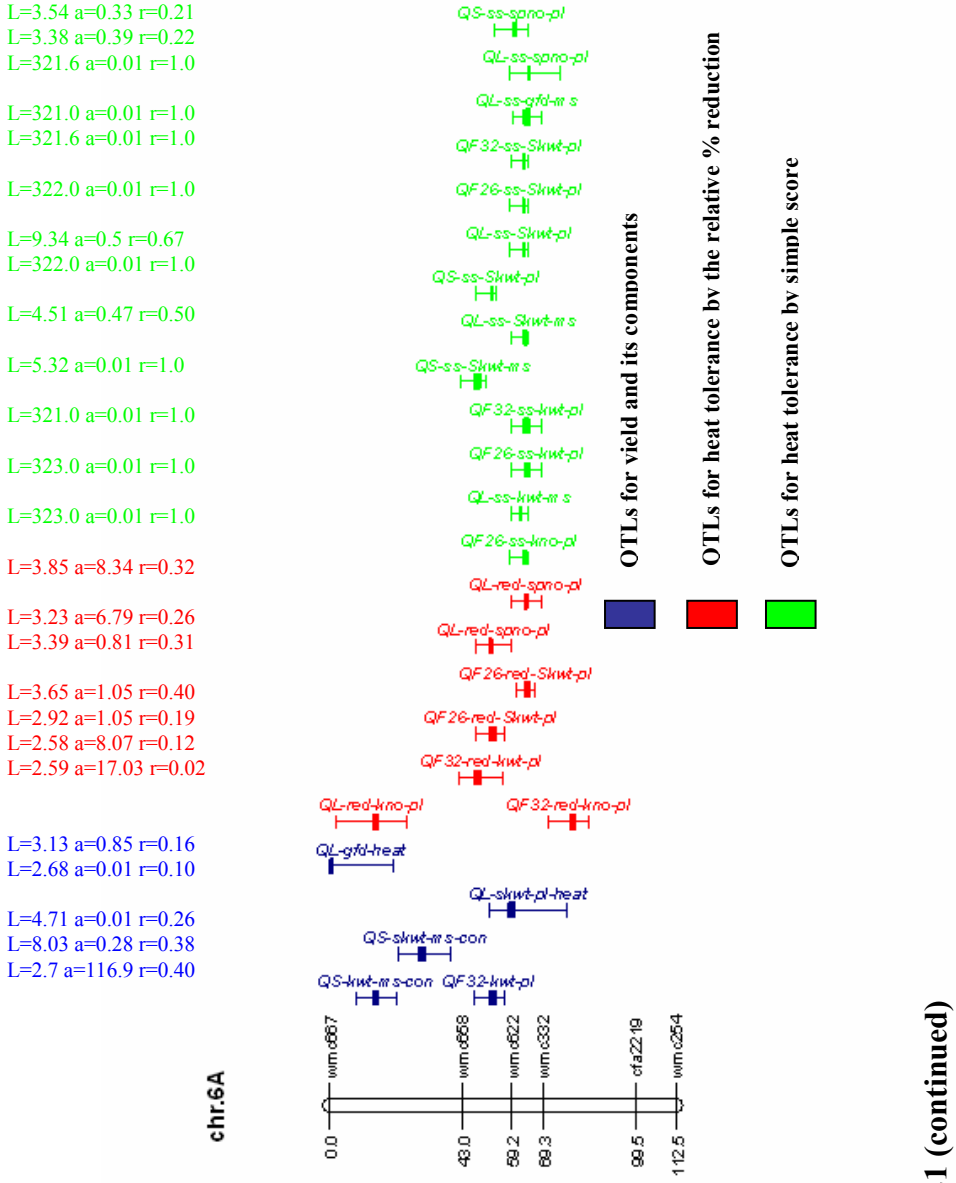

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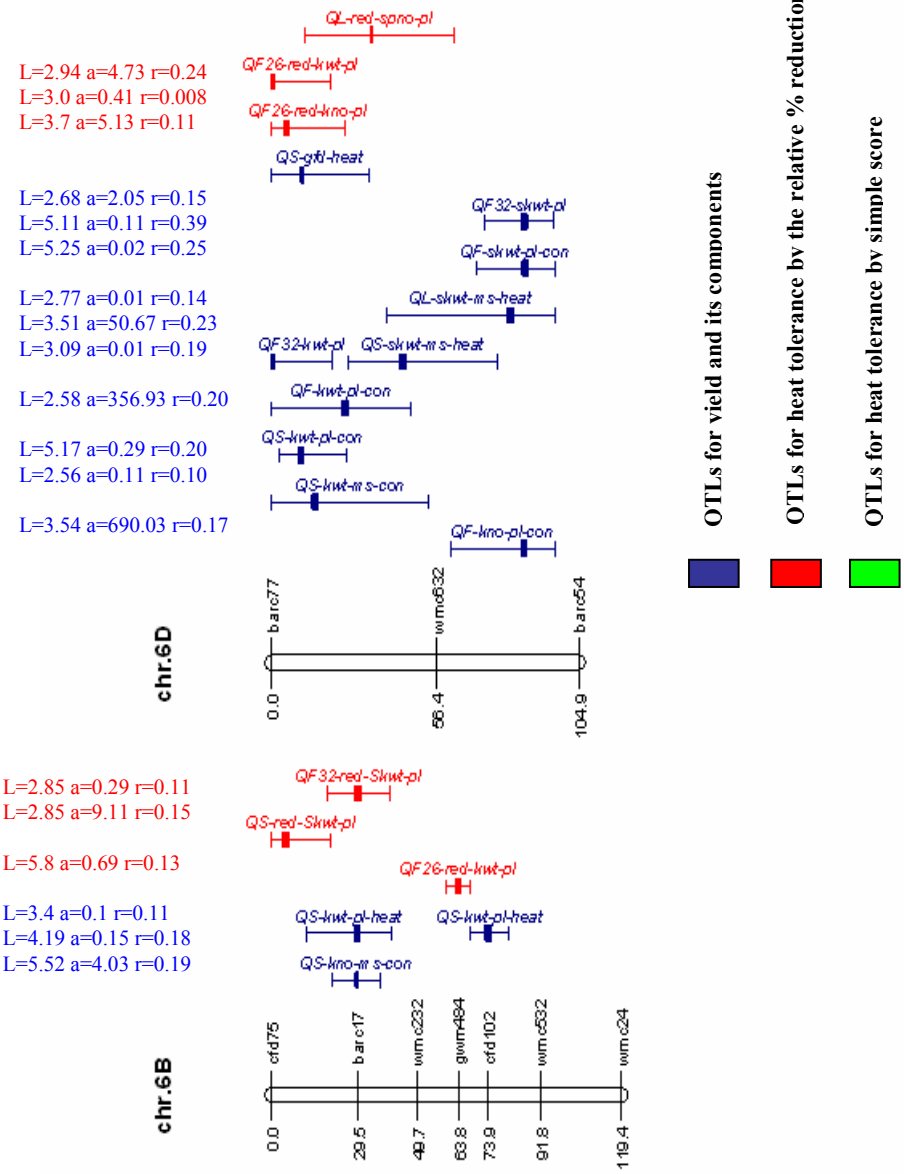

Fig. 21 (continued)

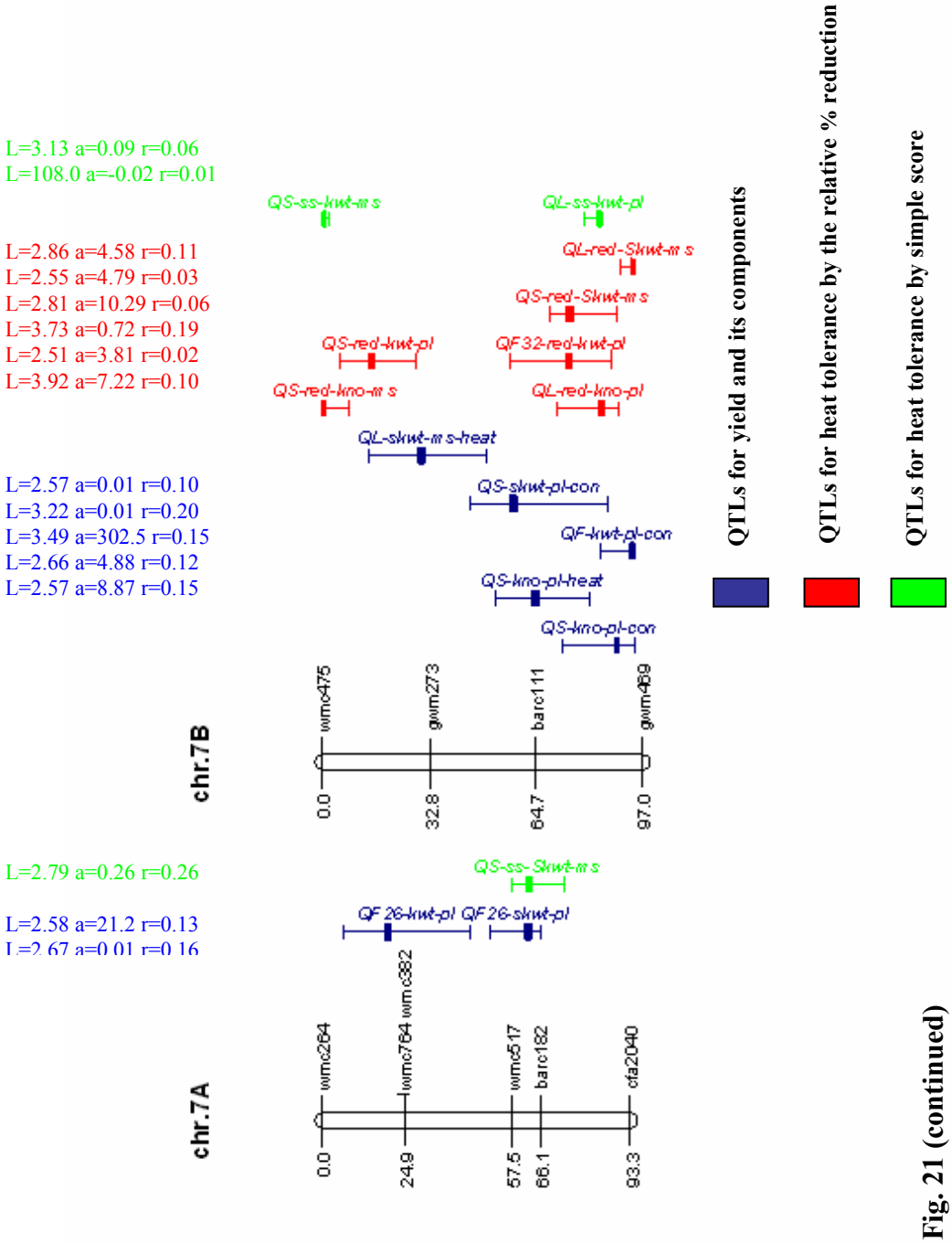

Fig. 21 (continued)

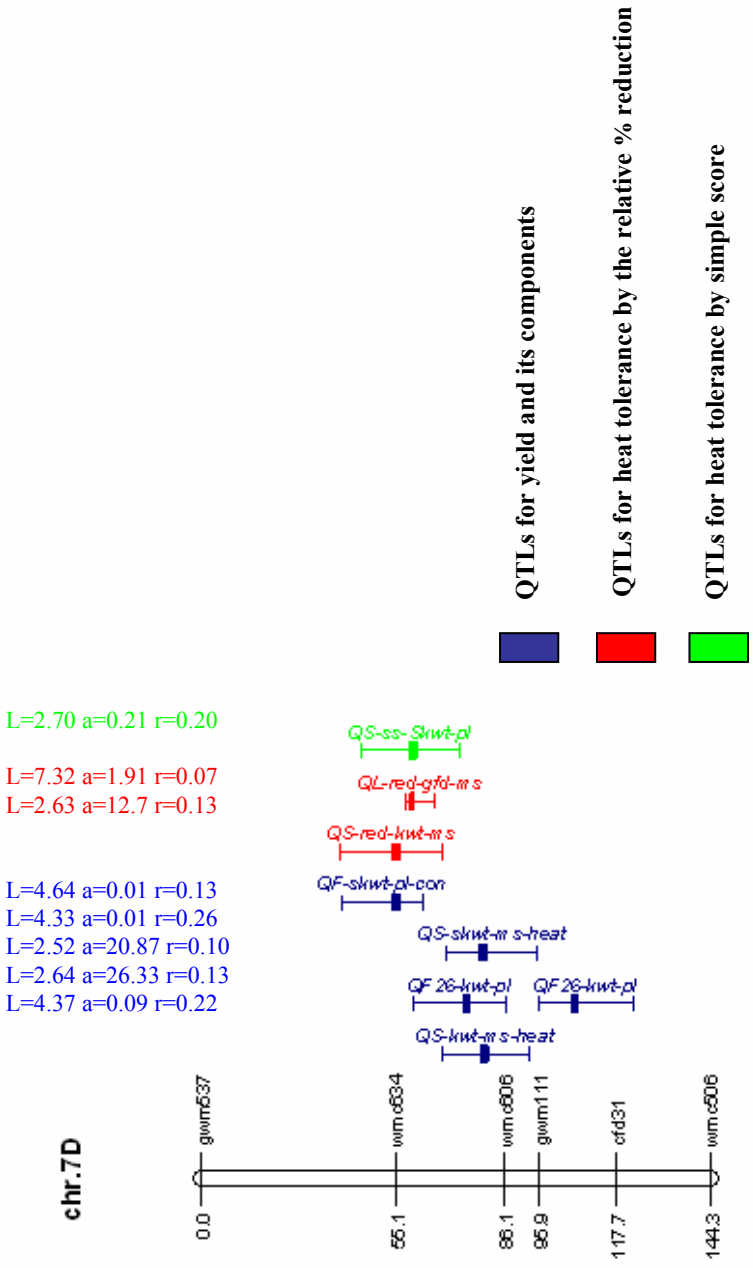

Fig. 21 (continued)

REFERENCES

Araus, J.L., H.R. Brown, A. Febrerd, J. Bort, and M.D. Serret. 1993. Ear photosynthesis, carbon isotope discrimination & the contribution of respiratory CO_2 to differences in grain mass in durum wheat. Plant Cell & Environment. 16: 237-247.

Assad, M.T., and G.M. Paulsen 2002. Genetic changes in resistance to environmental stresses by U.S. Great Plains wheat cultivar. Euphytica 128: 87-96.

Atak, B., M. Geleta, P.S. Baenziger, L.A. Nelson, Baltenesperger, K.M. Eskridge, and M.J. Shipman, 2002. Seedling rate and genotype effect on agronomic performance and end-use quality of winter wheat, Crop Sci. 42: 827-832.

Benmoussa, M., A. Achouch, and J. Zhu. 2005. QTL analysis for yield compoments in rice (Oryza sativa L.) under different environments. Journal Central European Agriculture. 6: 317-322.

Bezant, J., D. Laurie, N. Pratchett, J. Chojecki, and M. Kearsey. 1997. Mapping QTL controlling yield and yields components in a spring barley (Hordeum Bulgare L.) cross using marker regression. Molecular Breeding. 3: 29-38.

Blair, M., A. Garris, A. Iyer, B. Chapman, S. Kresovich, and S. McCouch, 2003. High resolution genetic mapping and candidate gene identification at the xa5 locus for bacterial blight resistance in rice (Oryza sativa L.) Theor Appl Genet. 107: 62-73.

Blum, A., J. Mayer, and G. Gozlan. 1982. Infrared thermal sensing of plant canopies as a screening technique for dehydration avoidance in wheat. Field Crops Res. 5:137-146.

Blum, A. 1988. Plant breeding for stress environments. CRC Press, Inc., Boca Raton, FL.

Blum, A., N. Klueva, and H. T. Nguyen. 2001. Wheat cellular thermo tolerance is related to yield under heat stress. Euphytica 117: 117-123.

Brancourt-Hulmel, M., G. Doussinault, C. Lecomte, P. Bérard, B. Le Buanec, and M. Trottet. 2003. Genetic improvement of agronomic traits of winter wheat cultivars released in France from 1946 to 1992. Crop Sci. 43: 37-45.

Cakir, M., S. Gupta, G.J. Platz, G.A. Ablett, R. Loughman, L.C. Emebiri, D. Poulsen, C.D. Li, R.C.M. Lance, N.W. Galwey, M.G.K. Jones, and R. Appels, 2003. Mapping and validation of the genes for resistance to Pyrenophora teres f. teres in barley (Hordeum vulgare L.) Aust J Agri Res. 54:1369-1377.

Campbell, K.G., C.J. Bergman, D.G., Gaulberto, J. A. Anderson, M. J. Gorpix, G. Hareland, R.G. Gulcher, M. E. Sorrells, and P. L. Finney. 1999. Quantitative trait loci associated with kernel traits I a soft x hard wheat cross. Crop Sci. 39; 1184-1195.

Campbell, B T., P.S. Baenziger, K.S. Gill, K.M. Eskridge, H. Budak, M. Erayman, and Y. Yen. 2003. Identification of QTLs and environmental interactions associated with agronomic traits on chromosome 3A of wheat. Crop Sci. 43:1493-1505.

Cardon, L.R., and J.I. Bell. 2001. Association study designs for complex disease, Nat. Rev. Genet. 2: 91-99.

Chojeck, A.J.S., M.W. Bayliss, and M.D. Gale. 1986. Cell production and accumulation in wheat endosperm, and their association with grain weight. Ann. Bot. 58: 809-817.

Chowdhury, S.I., and I.F. Wardlaw. 1978. The effect of temperature on kernel development in cereals. Aust. J. Agri. Res. 29: 205-223.

Chunwongse, J., S. Doganlar, C. Crossman, J. Jiang, and S.D. Tanksley. 1997. Hihg-resolution genetic map the Lv resistance locus in tomato. Theor Appl Genet. 95: 220-223.

Collard, B.C.Y., E.C.K. Pang, and P.W.J. Taylor, 2003. Selection of wild Cicer accessions for the generation of mapping populations segregation for resistance to ascochyta blight. Euphytica 130:1-9.

Collins, H.M., J.F. Panozzo, S.J. Logue, S.P. Jefferies, and A.R. Barr. 2003. Mapping and validation of chromosome regions associated with high malt extra in barley (Hordeum vulgare L.). Aust J. Agric Res. 54: 1223-1240.

CIMMYT.1995. CIMMYT/ NARS Consultancy on MEI bread wheat breeding. wheat Special Report No.38. CIMMYT, Mexico, D. F.

Falconer, D.S., and T.F.C. Mackay. 1996. Introduction to quantitative genetics. 4th ed. Longman Group Ltd., London.

Feuillet, C., M. Messmer, G. Schachermayr, B. Keller. 1995. Genetic, and physical characterization of the Lrl leaf rust locus in wheat (Triticum aestivumL.). Mol Gen Genet. 248: 553-562.

Feuillet, C., G. Schachermayr, and B. Keller. 1997. Molecular cloning of a new receptor-like kinase gene encoded at the lr10 disease resistance locus of wheat. Plant J. 11: 45-52.

Fisher, R. A. 1993. Irrigated spring wheat and timing and amount of nitrogen fertilizer. II. Physiology of grain yield response. Field Crops Research, 33: 57-80.

Fokar, M., H.T. Nguyen, and A. Blum. 1998. Heat tolerance in spring wheat. I. Estimating cellular thermotolerance and its heritability. Euphytica 104: 1-8.

Frova, C., and M. Sari-Gorla. 1993. Quantitative expression of maize HSPs: genetic, dissection and association with thermo tolerance. Theor. Appl. Genet. 86: 213-220.

Frova, C. 1996. Genetic dissection of thermotolerance in maize. In: S. Grillo and A. Leone (Eds.) Physical stresses in plants: genes and their products for tolerance, Springer-Verlag, Berlin, pp.31-38.

Fuse, T., K. Iba, H. Satoh, and M. Nishimura. 1993. Characterization of rice mutant having an increased susceptibility to light stress and high temperature. Physiol. Plant. 89: 799-804.

Gandhi, S.M., A.K. Sanghi, K.S. Nathawat, and M. P. Bhatnagar 1963. Genotype variability and correlation coefficient relating to grain yield and a few other quantitative characters in Indian wheats. Indian J Genet. 24: 1-8.

Gebber, T., and H. Schnyder. 1999. Pre-anthesis reserve utilization for protein and carbohydrate synthesis in grains of wheat. Plant Physiol. 121: 871-878.

Gibson, L.R., and G.M. Paulsen. 1999. Yield components of wheat grown under high temperature stress during reproductive growth. Crop Sci. 39: 1841-1846.

Giura, A., and N.N. Saulescu. 1996. Chromosomal location of genes controlling grain size in a large grained selection of wheat (Triticum aestivum L.). Euphytica. 89: 77-80.

Gusta, L.V., and T. H. H. Chen. 1987. The physiology of water and temperature stress. p.115-150. *In* R.G. Heyne (ed.) Wheat and wheat improvement. Amer Soc Agron, Madison, WI.

Gutierrez-Rodriguez, M., M. P. Reynolds, and A. Larque-Saavedra. 2000. Photosynthesis of wheat in a warm, irrigate environment II: traits associated with genetic grains in yield. Field Crops Research. 66: 51-62.

Hays, P.M., B.H. Liu, S.J. Knapp, F. Chen, B. Jones, T. Blake, J. Frankowiak, D. Rasmusson, M. Sorrells, S.E. Ullrich, D.Wesenberg, and A. Kleinhofs.1993. Quantitative trait locus effects and environmental interaction in a sample of North American barley germ plasm. Theor Appl Genet. 87:392-401.

Ibrahim, A.M.H., and Quick J.S. 2001. Heritability of heat tolerance in winter and spring wheat. Crop Sci. 41:1401-1405.

Jenner, C.F., 1991b. Effects of exposure of wheat ears to high temperature on dry matter accumulation and carbohydrate metabolism in the grain of two cultivars. II. Carry-over effects. Aust. J. Plant Physiol. 18: 179-190.

Johnson, R.R., and D.N. Moss. 1976. Effect of water stress on 14 CO2 fixation and translocation in wheat during grain filling. Crop Sci. 16: 697-701.

Johnson, R.C., and E.T. Kanemasu. 1983. Yield and development of winter wheat at elevated temperatures. Agron. J. 75: 561-565.

Jones, H.G., 1977. Aspects of water relations of spring wheat (Triticum aestivum L.) in response to induced drought. J. Agric. Sci. 88:267-282.

Jung, G., P.W. Skroch, J. Nienhuis, D.P. Coyne, E. Arnaud-Santana, H.M. Ariyarathne, and J.M. Marita, 1999. Confirmation of QTL associated with common bacterial blight resistance in four different genetic backgrounds in common bean. Crop Sci. 39: 1448-1455.

Kato, K., H. Miura, and S. Sawada. 2000. Mapping QTLs controlling grain yield and its components on chromosome 5A of wheat. Theor Appl Genet. 101: 1114-1121.

Kaul, R., and W.L. Crowle. 1974. An index derived from photosynthetic parameters for predicting grain yields of drought-stressed wheat cultivars. Z. Pflanzensucht. 71:42-51.

Kearsey, R.C., and H.S.Pooni. 1996. The genetical analysis of quantitative traits. Chapman and Hall, London.

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. Breeding. 20: 401-413.

Kosambi, D.D. 1944. The estimation of map distances from recombination values, Annu. Eugen. 12: 172-175.

Korzun, V., M. Roder, A. J., Worland, and A. Borner. 1997. Intrachromosomal mapping of genes for dwarfing (Rht12) and vernalization response (Vrn1) in wheat by using RFLP and microsatellite markers. Plant Breed. 116: 227-232.

Kumar, N., P.L. Kulwal, A. Guar, A.K. Tyagi., J.P. Khurana, P. Khurana, H.S. Balyan, and P.K. Gupta 2006. QTL analysis for grain for grain weight in common wheat. Euphytica. 151: 135-144.

Langridge, P., E. Lagudah, T. Holton, R. Appels, P. Sharp, and K. Chalmers, 2001.Trend in genetic and genome analyses in wheat: a review. Aust J Agric Res. 52:1043-1077.

Leegood, R.C., and G. Edwards. 1996. Carbon metabolism and photorespiration: temperature dependence in relation to other environmental factors. In Baker NR (ed) Photosynthesis and the environment. Kluwer Academic Publishers, Dordrecht., pp 191-221.

Li, L., S. Lu, D. O'Halloran , D. Garvin, and J. Vrebalo. 2003. High resolution genetic and physical mapping of the cauliflower high beta-carotene gene Or (Orange). Mol Genet Genome. 270: 132-138.

Li, S.J. Jia, X. Wei, X. Zhang, L. Li, H. Chen, Y. Fan, H. Sun, X. Zhao, T. Lei, Y. Xu, F. Jiang, H. Wang, and L. Li. 2007. An intervarietal genetic map and QTL analysis for yield traits in wheat. Mol Breeding. 20: 167-178.

Li, Z., L. Jakkula, R.S. Hussey, J.P. Tamulonis, and H.R. Boerma 2001. SSR mapping and confirmation of the QTLs from PI96354 conditioning soybean resistance to southern root-knot nematode. Theor Appl Genet. 103: 1167-1173.

Ma, Z., D. Zhao, C. Zhang, Z. Zhang, S. Xue, F Lin, Z. Kong, D. Tian, and Q. Luo. 2007. Molecular genetic analysis of five spike- related traits in wheat using RIL and immortalized F2 populations. Mol Gen Genomics. 277: 31-42.

Maestri, E., N. Klueva, C. Perrotta, M. Gulli, H. T. Nguyen, and N. Marmiroli. 2002. Molecular genetics of heat tolerance and heat shock protein in cereals. Plant Molecular Biology 48: 667-681.

Marza, F., G.H. Bai, B.F., Carver, and W.C. Zhou. 2006. Quantative trait loci for yield and related traits in the wheat population Ning7840 x Clark. Theor Appl Genet. 112: 688-698.

Malik, M.K, J.P. Slovin, C.H. Hwang, and J.L. Zimmerman. 1999. Modified expression of a carrot small heat shock protein gene, Hsp17.7, results in increased or decreased thermotolerance. Plant J. 20:89–99.

Mesfin, A., R.C. Frohberg, and J.A. Anderson. 1999. RFLP markers associated with high grain protein from Triticum turgidum L. var dicoccoides introgressed into hard red spring wheat. Crop Sci. 39:508-513.

Midmore, D.J., P.M. Cartwright and R.A. Fischer. 1984. Wheat in tropical environments. II. Crop growth and grain yield. Field Crops Res. 8: 207-227.

Moffatt, L.M., R.G. Sears, T.S. Cox, and G.M. Paulsen.1990. Wheat high temperature tolerance during reproductive growth: II. Genetic analysis of chlorophyll fluorescence. Crop Sci. 30:886-889.

Mohan, M., S. Nair, A. Bhagwat, T.G. Krishna, M. Yano, C.R. Bhatia, and T. Sasaki, 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. Mol. Breed 3: 87-103.

Naik, S., K. S. Gill, V. S. Rao, V. S. Gupta, S. A. Tamhanker, S. Pujar, B. S. Gill, P. K. Ranjekar. 1998. Identification of a STS marker linked to the Aegilops speltoides-derived leaf rust resistance gene Lr28 in wheat. Theor Appl Genet. 97: 535-547.

Pastenes, C., and P. Horton. 1996. Effect of high temperature on photosynthesis in beans. I. Oxygen evolution and Chl fluorescence. Plant Physio. 112: 1245-1251.

Paulsen, G.M. 1994. High temperature responses of crop plants. ASA, CSSA, SSSA. Physiology and Determination of Crop Yield 14A: 365-389.

Poehlman, J.M., and D.A. Sleper. 1995. Breeding field crops. 4th ed. Iowa St. Univ. Press, Ames.

Prasad, M., R.K. Varshney, A. Kumar, H.S. Balyan, P.C. Sharma, K.J. Edwards, H. Singh, H.S. Dhaliwal, J.K. Roy, and P.K. Gupta. 1999. A microsatellite marker associated with a QTLs for grain protein content on chromosome arm 2DL of bread wheat. Theor Appl Genet. 99: 107-112.

Preczewski, P.J., S.A. Heckathorn, C.A. Downs, and J.S. Coleman. 2000. Photosynthetic thermotolerance is quantitatively and positively correlated with production of specific heat-shock proteins among nine genotypes of Lycoperisicon (Tomato). Photosynthetica. 38 (1): 127-134.

Qi, L., M. Cao, P. Chen, W. Li, and D. Liu. 1996. Identification, mapping and application of polymorphic DNA associated with the resistance gene Pm21 of wheat. Genome. 39: 191-197.

Rami, J.F., P. Dufour, G. Trouche, G. Fliedel, C. Mestres, F. Davrieux, P. Blanchard, and P. Hamon. 1998. Quantitative trait loci for grain quality, productivity, morphological and agronomical traits in sorghum (Sorghum bicolor L. Moench) Theor Appl Genet. 97: 605-616.

Rasyad, A., and D.A. Van Sanfold. 1992. Genetic and maternal variances and covariances of kernel growth traits in winter wheat. Crop Sci. 32: 1139-1143.

Rebecca, W. Doerge, 2001. Mapping and analysis of quantitative trait loci in experimental populations. Nature review, Genetics, 3:43-52.

Reyna, N., and C.H. Sneller. 2001. Evaluation of marker-assisted introgression of yield QTL alleles into adapted soybean. Crop Sci. 41: 1317-1321.

Reynolds, M.P., R. Trethwan, J. Crossa, M. Vargas, and K.D. Sayre. 2002. Physiological factors associated with genotype by environment interaction in wheat. Field Crops Res. 75: 139-160.

Ristic, Z., G.P., Yang, and B. Martin, and S. Fullerton. 1998. Evidence of association between specific heat –shock protein(s) and the drought and heat tolerance phynotype in maize. J. Plant Physiol. 153: 497-505.

Romagosa, I., F. Han, S.E Ullrich., P. M. Hayes, and D.M. Wesenberg, 1999. Verification of yield QTL through realized molecular marker-assisted selection responses in barley cross. Molecular Breeding. 5: 143-152.

Roy, J.K., M. Prasad, R.K. Varshney, H.S. Balyan, T.K. Blake, H.S. Dhaliwal, H. Singh, K.J. Edwards, and P.K. Gupta. 1998. Identification of a microsatellite on chromosome 6B and a STS on 7D of bread wheat showing association with preharvest sprouting tolerance. Theor Appl Genet. 99: 336-340.

Röder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.H. Tixier, P. Leroy, and M.W. Ganal. 1998. A microsatellite map of wheat. Genetics. 149: 2007-2023.

Saini, H.S., and D. Aspinall. 1983. Effects of heat stress during floral development on the pollen tube growth and ovary anatomy in wheat (Triticum aestivum L.) Aust. J. Plant physiol. 10: 137-144.

SAS Institute. 1996. The SAS system for windows. Release 6. 12. SAS Inst, Cary, NC.

Shanahan, J.F., I.B. Edward, J.S. Quick, and J. R. Fenwick. 1990. Membrane thermostability and heat tolerance of spring wheat. Crop Sci. 30: 247-251.

Singla, S.L., A. Pareek, A.K. Kush, and A. Grover. 1998. Distribution patterns of 104 kDa stress-associated protein in rice. Plant Mol. Biol. 37: 911-919.

Sharp, P. J., S. Johnston, G. Brown, R. A. McIntosh, M. Pallotta, M. Carter, H.S. Bariana, S. Khartkar, E.S. Lagudah, R.P. Singh, M. Khairallah, R. Potter, and M.G.K. Jones, 2001. Validation of molecular markers for wheat breeding. Aust J Agric Res. 52: 1357-1366.

Shipler, l., and A. Blum. 1986. Differential reaction of wheat cultivars to hot environments. Euphytica 35: 483-492.

Slafer, G.A., and F.H. Andrade. 1989. Genetic improvement in bread wheat (Triticum aestivum L.) yield in Argentina. Field Crops Res. 21: 289-296.

Sofield, I., L.T. Evans, M.G., Cook, and I. F., Wardlaw. 1977. Factors influencing the rate and duration of grain filling in wheat. Aust. J. Plant Physiol. 4: 785-797.

Somer, D.J., P. Isaac, and k. Edwards. 2004. A high-density microsatellite consensus map for bread wheat (Triticum aestrivum L.) Theor Appl Genet. 109: 1105-1114.

Sourdille, P., M.R. Perretant, G. Chzarmet, P. Leroy, M.E. Gautier, P. Joudrier, J. C. Nelson, M.E. Sorrells. 1997. Linkage between RFLP markers and gene affecting kernel hardness in wheat (Triticum aestium L. em thell). Theor Appl Genet. 93: 580-586.

Stone, P.J., M.E. Nicolas. 1994. Wheat cultivars vary widely in their responses of grain yield and quality to short period of post-anthesis heat stress. Aust. J. Plant Physiol. 21: 887-900.

Stone, P.J., and M.E. Nicolas. 1995. A survey of the effects of high temperature during grain filling on yield and quality of 75 wheat cultivars. Aust. J. Agric. Res. 46: 475-492.

Tashiro, T., and I.F. Wardlaw. 1989. A comparison of the effect of high temperature on grain development in wheat and rice. Ann. Bot. (London) 64: 59-65.

Tashiro, T., and I.F. Wardlaw. 1990a. The effect of high temperature at different stages of ripening on grain set, grain weight and grain dimensions in the semi-dwarf wheat ‘Bank’. Ann. Bot. (London) 65: 51-61.

Tashiro, T., and I.F. Wardlaw. 1990b.The response to high temperature shock and humidity changes prior to and during the early stages of grain development in wheat. Aust. J. Plant Physiol. 17: 551-561.

Thomas, W.T.B., W. Powell, R. Waugh, K.J. Chalmers, U.M. Barua, P. Jack, V. Lea, B.P. Foster, J.S. Swanston, P.R. Hanson, and R.C.M. Lance. 1995. Detection of quantitative trait loci for agronomic yield, grain and disease characters in spring barley (Hordeum vulgare L.). Theor Appl Genet. 91:1037-1047.

Udall, J.A., E. Souza, J.A. Anderson, and M.E. Sorrells. 1999. Quantitative trait loci for flour viscosity in winter wheat. Crop Sci. 39: 238-242.

Waddington, S.R., M. Osmanzai, M. Yoshida, and J.K. Ransom. 1987. The yield of durum wheats released in Mexico between 1960 and 1984. J. Agric. Sci. Camb. 108: 469-477.

Wan, X.Y., J.M. Wan, J.F. Weng, L. Jiang, J.C. Bi, C.M. Wang, and H.Q. Zhai, 2005. Stability of QTLs for rice grain dimension and endosperm chalkiness characteristics across eight environments. Theor. Appl. Genet. 110:1334-1346.

Wardlaw, I.F., I.A. Dawson, P. Munibi, and R. Fewster. 1989a. The tolerance of wheat to high temperatures during reproductive growth. I. Survey procedures and general response patterns. Aust. J. Agric. Res. 40: 1-13.

Wardlaw, I.F., I.A. Dawson, P. Munibi, and R. Fewster. 1989b. The tolerance of wheat to high temperatures during reproductive growth. I. Reproductive growth. Aust. J. Agri. Res. 40: 15-24.

Yang, J.J., Z. Zhang, Q. Wang, Q. Zhu, and L. Liu. 2001. Water deficient-induced senescence and its relationship to the remobilization of pre-stored carbon in wheat during grain filling. Agron J. 93: 196-206.

Yang, J., R.G. Sears, Gill B.S., and Paulsen G.M., 2002a. Genotypic differences in utilization of assimilate sources during maturation of wheat under chronic heat and heat shock stress. Euphytica. 125: 179-188.

Yang, J., R.G. Sears, Gill B.S., and Paulsen G.M., 2002b. Quantitative and molecular characterization of heat tolerance in hexaploid wheat. Euphytica. 126: 275-288.

Yano, M., and T. Sasaki. 1997. Genetic and molecular dissection of quantitative traits in rice. Plant Mol Biol. 35: 145-153.

Zhong-hu, H., and S. Rajaram, 1994 Differential responses of bread wheat characters to high temperature. Euphytica 72: 197-203.

Zhuang, J.Y., H.X. Lin, J. Lu, H.R. Qian, S. Hittalmani, N. Huang, K.L. Zheng, 1997. Analysis of QTL x environment interaction for yield components and plant height in rice. Theor Appl Genet. 95: 799-808.

APPENDIX

Table XVI. Results of single marker regression analysis for yield and its components trait in the 62 RILs in seven individual environments.

Markers	Traits	chr.	Sig.	Markers	Traits	chr.	Sig.
cfa2129	SknomsHeat	c1a	***	barc148	SknomsCon	c1a	***
wmc419	SknomsCon	c1b	***	wmc336	SknomsHeat	c1d	****
cfd48	SknomsCon	c1d	****	cfd48	SknomsHeat	c1d	***
cfd48	SknomsHeat	c1d	***	barc124	SknomsHeat	c2a	***
gwm95	SknomsCon	c2a	***	gwm372	SknomsCon	c2b	***
wmc418	SknomsHeat	c3a	***	gwm108	LknomsHeat	c3b	***
barc164	SknomsHeat	c3d	***	barc4	LknomsCon	c5b	***
cfd18	SknomsCon	c5d	***	wmc667	SknomsCon	c6a	****
wmc667	SknomsHeat	c6a	****	barc17	SknomsCon	c6b	****
barc17	SknomsHeat	c6b	****	barc54	SknomsCon	c6d	****
barc77	SknomsHeat	c6d	***	wmc606	SknomsCon	c7d	***
cfd31	SknomsCon	c7d	****	cfd31	SknomsHeat	c7d	***
wmc336	SknoplCon	c1d	****	wmc336	SknoplHeat	c1d	***
barc124	F26knopl	c2a	***	barc124	F32knopl	c2a	***
wmc522	Sknoplcon	c2a	***	barc154	SknoplCon	c2b	***
wmc522	F26knopl	c2b	***	wmc418	SknoplHeat	c3a	***
gwm108	LknoplCon	c3b	***	wmc471	SknoplHeat	c3d	***
gwm108	FknoplCon	c3d	***	barc164	SknoplHeat	c3d	***
wmc491	SknoplHeat	c4a	***	gwm165	SknoplHeat	c4d	***
gwm192	SknoplHeat	c4d	****	gwm443	F32knopl	c5a	***
gwm604	FknoplCon	c5b	***	gwm371	SknoplCon	c5b	****
gwm190	SknoplHeat	c5d	***	cfd57	SknopCon	c5d	****
cfd57	SknoplHeat	c5d	****	wmc231	SknoplHeat	c5d	***
wmc765	LknoplCon	c5d	***	wmc667	SknoplCon	c6a	****
wmc667	SknoplHeat	c6a	***	wmc667	F26knopl	c6a	***
barc17	SknoplCon	c6b	****	barc17	SknoplHeat	c6b	****
gwm484	SknoplCon	c6b	***	cfd102	SknoplCon	c6b	****
cfd102	SknoplHeat	c6b	****	barc77	F32knopl	c6d	***
wmc632	FknoplCon	c6d	****	barc111	SknoplHeat	c7b	***
gwm469	SknoplCon	c7b	***	gwm111	F26knopl	c7d	***
cfa2129	LkwrmsHeat	c1a	****	barc148	SkwrmsCon	c1a	***
wmc336	SkwrmsCon	c1d	****	wmc336	SkwtmsHeat	c1d	***
gwm642	LkwrmsHeat	c1d	****	wmc154	SkwrmsCon	c2b	***
wmc418	SkwtmsHeat	c3a	****	wmc471	SkwtmsHeat	c3d	****
barc164	SkwtmsHeat	c3d	***	gwm192	SkwtmsHeat	c4d	***
wmc285	SkwtmsHeat	c4d	***	barc232	SkwtmsHeat	c5a	***
gwm371	SkwrmsCon	c5b	***	gwm371	LkwtmsCon	c5b	***
cfd18	SkwrmsCon	c5d	****	gwm190	SkwrmsCon	c5d	***
cfd57	SkwrmsCon	c5d	****	cfd57	SkwtmsHeat	c5d	***
cfd57	LkwtmsCon	c5d	****	wmc765	LkwtmsCon	c5d	***
wmc667	SkwrmsCon	c6a	****	wmc658	LkwtmsCon	c6a	***

Table XVI (continued)

Markers	Traits	chr.	Sig.	Markers	Traits	chr.	Sig.
barc17	SkwrmsCon	c6b	***	barc17	SkwtmsHeat	c6b	***
cfd102	SkwrmsCon	c6b	***	wmc532	LkwrmsHeat	c6b	****
barc54	SkwrmsCon	c6d	****	barc111	SkwrmsCon	c7b	***
barc111	LkwtmsCon	c7b	***	wmc606	SkwrmsCon	c7d	***
wmc606	SkwtmsHeat	c7d	****	gwm111	SkwtmsHeat	c7d	****
barc148	SkwtplCon	c1a	***	wmc336	SkwtplCon	c1d	****
wmc336	F26kwtpl	c1d	***	barc124	FkwtplCon	c2a	****
barc124	F26kwtpl	c2a	****	barc124	F32kwtpl	c2a	****
wmc154	SkwtplCon	c2b	***	wmc418	SkwtplCon	c3a	***
wmc418	SkwtplHeat	c3a	***	gwm108	LkwtplCon	c3b	***
wmc471	SkwtplHeat	c3d	***	barc164	SkwtplCon	c3d	***
barc164	SkwtplHeat	c3d	***	gwm6	SkwtplCon	c4b	***
gwm165	SkwtplHeat	c4d	***	gwm192	SkwtplHeat	c4d	***
wmc285	SkwtplHeat	c4d	***	gwm371	SkwtplCon	c5b	****
gwm190	SkwtplCon	c5d	***	gwm190	SkwtplHeat	c5d	***
cfd57	SkwtplCon	c5d	****	cfd57	SkwtplHeat	c5d	****
cfd57	LkwtplCon	c5d	***	cfd57	F26kwtpl	c5d	***
wmc231	SkwtplHeat	c5d	***	wmc765	LkwtplCon	c5d	****
wmc667	SkwtplCon	c6a	****	wmc667	F26kwtpl	c6a	****
wmc658	FkwtplCon	c6a	***	barc17	SkwtplCon	c6b	***
barc17	SkwtplHeat	c6b	****	barc17	LkwtplCon	c6b	***
barc17	F26kwtpl	c6b	***	cfd102	SkwtplCon	c6b	****
cfd102	SkwtplHeat	c6b	***	wmc532	LkwtplHeat	c6b	***
barc77	FkwtplHeat32	c6d	****	barc54	SkwtplCon	c6d	***
gwm469	SkwtplCon	c7b	***	wmc606	SkwtplCon	c7d	***
wmc606	SkwtplHeat	c7d	***	gwm111	SkwtplCon	c7d	***
barc148	LskwtmsHeat	c1a	***	cfa128	LskwtmsHeat	c1a	****
wmc419	LskwtmsHeat	c1b	***	wmc336	SskwtmsCon	c1d	****
wmc336	LskwtmsHeat	c1d	****	cfd48	LskwtmsHeat	c1d	****
gwm642	LskwtmsHeat	c1d	****	wmc418	SskwtmsHeat	c3a	***
gwm369	LskwtmsHeat	c3a	***	wmc471	SskwtmsHeat	c3d	***
barc4	LskwtmsHeat	c5b	***	gwm371	SskwtmsCon	c5b	***
cfd18	SskwtmsCon	c5d	***	gwm190	SskwtmsCon	c5d	****
cfd57	SskwtmsCon	c5d	****	wmc622	LskwtmsHeat	c6a	***
barc17	LskwtmsCon	c6b	***	cfd102	SskwtmsCon	c6b	***
wmc532	LskwtmsHeat	c6b	****	barc77	SskwtmsCon	c6d	***
wmc632	SskwtmsHeat	c6d	***	wmc382	SskwtmsHeat	c7a	****
wmc517	SskwtmsHeat	c7a	***	barc111	SskwtmsCon	c7b	****
gwm469	LskwtmsCon	c7b	***				
cfa2129	LskwtplHeat	c1a	***	barc148	SskwtplCon	c1a	***
barc148	LskwtplHeat	c1a	**	wmc419	LskwtplHeat	c1b	***
wmc336	SskwtplCon	c1d	****	wmc336	LskwtplHeat	c1d	***

Table XVI (continued)

Markers	Traits	chr.	Sig.	Markers	Traits	chr.	Sig.
cfd48	LskwtplHeat	c1d	***	gwm642	LskwtplHeat	c1d	***
wmc154	SskwtplCon	c2b	***	wmc418	SskwtplHeat	c3a	***
wmc471	SskwtplCon	c3d	***	wmc471	SskwtplHeat	c3d	***
wmc471	FskwtCon	c3d	***	barc164	SskwtplCon	c3d	***
gwm251	LskwtplHeat	c4b	****	gwm251	LskwtplHeat	c4b	****
cfd18	SskwtplCon	c5d	****	gwm190	SskwtplCon	c5d	****
gwm190	FskwtCon	c5d	***	cfd57	SskwtplCon	c5d	***
wmc765	LskwtplCon	c5d	***	wmc622	LskwtplHeat	c6a	***
cfd102	SskwtplCon	c6b	***	wmc532	LskwtplHeat	c6b	****
wmc632	FskwtCon	c6d	***	barc54	SskwtplCon	c6d	***
barc54	FskwtCon	c6d	****	barc54	F32skwt	c6d	***
barc111	SskwtplCon	c7b	***	wmc606	SskwtplCon	c7d	***
wmc606	SskwtplHeat	c7d	***	gwm111	SskwtplCon	c7d	***
gwm111	SskwtplHeat	c7d	***	cfd31	FskwtCon	c7d	***
wmc522	SspnoCon	c2a	***	wmc154	SspnoHeat	c2b	***
wmc238	SspnoCon	c4b	***	gwm6	SspnoCon	c4b	***
gwm192	SspnoCon	c4d	***	gwm371	SspnoCon	c5b	****
gwm371	SspnoHeat	c5b	***	cfd57	SspnoCon	c5d	****
wmc231	SspnoHeat	c5d	****	barc17	SspnoHeat	c6b	***
cfd102	SspnoCon	c6b	***	barc111	SspnoCon	c7b	****
barc111	SspnoHeat	c7b	****	gw469	SspnoHeat	c7b	****
gwm642	SgfdHeat	c1d	***	barc124	LgfdHeat	c2a	***
wmc471	SgfdHeat	c3d	***	gwm190	SgfdHeat	c5d	***
wmc667	LgfdHeat	c6a	***	cfd102	SgfdHeat	c6b	***
barc77	SgfdHeat	c6d	****	wmc632	SgfdHeat	c6d	***
barc111	SgfdHeat	c7b	***	cfd31	LgfdHeat	c7d	***

, * indicate significance at the 0.1 and 0.01 % levels, respectively.

Table XVII. Results of single marker regression analysis for the relative % reduction of yield and its components traits in the 62 RILs in four individual environments.

Markers	Traits	chr.	Sig.	Markers	Traits	chr.	Sig.
wmc285	Lredknoms	c4d	***				
wmc154	Fred26knopl	c2b	***	barc159	Fred26knopl	c2b	***
barc62	Fred26knopl	c3a	***	wmc418	Fred26knopl	c3a	***
barc164	Fred26knopl	c3d	***	wmc89	Frec32knopl	c4a	***
wmc491	Frec32knopl	c4a	***	barc77	Fred26knopl	c6d	***
cfd31	Fred26knopl	c7d	***				
wmc336	Lredkwtms	c1d	****	wmc418	Lredkwtms	c3a	***
gwm604	Lredkwtms	c5b	***	barc4	Lredkwtms	c5b	***
wmc336	Lredkwtpl	c1d	***	gwm604	Lredkwtpl	c5b	***
wmc336	Lredskwtms	c1d	****	barc124	Lredskwtms	c2a	***
wmc418	Lredskwtms	c3a	****	gwm604	Lredskwtms	c5b	***
barc4	Lredskwtms	c5b	***	gwm371	Lredskwtms	c5b	***
wmc667	Lredskwtms	c6a	***				
wmc336	Lredskwtpl	c1d	****	barc124	Lredskwtpl	c2a	***
gwm429	Fred32skwtpl	c2b	***	wmc418	Lredskwtpl	c3a	****
wmc418	Fred32skwtpl	c3a	***	cfd223	Fred32skwtpl	c3b	***
wmc471	Fred26skwtpl	c3d	****	wmc471	Fred32skwtpl	c3d	***
barc164	Fred26skwtpl	c3d	***	wmc48	Fred26skwtpl	c4a	***
wmc89	Fred26skwtpl	c4a	***	wmc491	Fred26skwtpl	c4a	***
gwm604	Lredskwtpl	c5b	****	barc4	Lredskwtpl	c5b	***
gwm190	Fred32skwtpl	c5d	****	gwm595	Fred26skwtpl	c5d	***
wmc254	Fred32skwtpl	c6a	***	wmc24	Fred26skwtpl	c6b	****
wmc24	Fred32skwtpl	c6b	***	barc54	Fred26skwtpl	c6d	***
gwm273	Fred26skwtpl	c7b	***	cfd31	Fred26skwtpl	c7d	***

, * indicate significance at the 0.1 and 0.01 % levels, respectively.

Table XVIII. Contrasts of yield components per plant and their % reduction after a short-term of 38˚C beginning at 10 DAP in growth chambers and long-term heat stress of 30˚C beginning at 10 DAP until grain maturity in plant growth rooms for markers identified at the level of 0.01% from single marker analysis. A and B indicate alleles from two parental lines '7C' and 'Seri M82', respectively.

			Single Marker Analysis															
			Short-term (mean)				Short-term (mean of % red)				Long-term (mean)				Long-term (mean of % red)			
Markers	Chr.	Alleles	Skwt	Kwt	Kno	GFD	Skwt	Kwt	Kno	GFD	Skwt	Kwt	Kno	GFD	Skwt	Kwt	Kno	GFD
wmc336	c1d	A	0.011	0.38	33	27	20.7	30.6	14.1	12	0.019	4 .07	208	30	47.3	46.3	-3.3	52.1
		B	0.013	0.61	45	30	31.1	45.7	23.8	10	0.017	3.55	210	28	58.7	60.5	7.3	52.4
cfd48	c1d	A	0.012	0.45	36	28	21.9	45.9	34.5	7	0.019	4.00	207	29	52.6	52.8	2.4	52.6
		B	0.012	0.60	46	29	25.1	34.8	15.4	12	0.02	3.58	216	29	50.5	50.0	1.4	53.3
gwm642	c1d	A	0.012	0.48	38	28	24.8	36.7	18.9	11.6	0.019	4.05	211	29	51.2	51.4	2.7	52.9
		B	0.013	0.64	46	29	26.5	34.8	13.0	9.3	0.016	3.33	206	29	55.9	56.0	1.5	52.0
wmc418	c3a	A	0.010	0.39	34	27	27.1	39.6	20.6	14.5	0.019	4.09	213	29	45.9	46.2	-0.48	52.2
		B	0.013	0.63	45	30	23.2	32.9	14.7	8.2	0.017	3.63	206	28	57.7	57.8	4.75	53.4
wmc471	c3d	A	0.010	0.33	32	26	28.1	41.9	23.2	15.6	0.019	3.97	213	29	49.4	51.0	1.36	52.2
		B	0.013	0.61	44	30	24.4	33.9	13.7	8.1	0.018	3.75	206	28	54.0	53.0	2.21	53.2
wmc48	c4a	A	0.012	0.47	36	28	25.3	36.2	17.2	10.9	0.018	3.84	209	29	52.7	52.8	2.4	52.7
		B	0.013	0.63	47	30	27.9	41.8	22.4	13.8	0.018	3.89	209	29	50.9	50.3	1.4	53.0
gwm604	c5b	A	0.012	0.53	41	28	25.3	36.3	18.5	11.7	0.017	3.74	211	28	54.8	55.3	3.1	52.6
		B	0.012	0.45	40	29	25.8	29.6	-2.2	6.3	0.022	4.29	195	30	46.9	48.4	2.2	54.4
gwm371	c5b	A	0.012	0.47	38	28	23.6	32.0	14.1	11.5	0.019	3.85	205	29	50.3	50.8	1.1	52.4
		B	0.013	0.70	49	30	28.9	45.2	23.5	8.9	0.017	3.56	211	27	60.3	62.0	11.3	53.8
cfd18	c5d	A	0.011	0.44	37	28	22.6	32.7	15.4	11.9	0.019	3.98	212	29	50.4	49.0	-0.48	53.3
		B	0.013	0.64	46	30	32.4	43.0	19.8	10.0	0.017	3.58	203	29	56.0	58.5	7.0	51.8

Table XVIII (continued)

			Single Marker Analysis															
			Short-term (mean)				Short-term (mean of % red)				Long-term (mean)				Long-term (mean of % red)			
Markers	Chr.	Alleles	Skwt	Kwt	Kno	GFD	Skwt	Kwt	Kno	GFD	Skwt	Kwt	Kno	GFD	Skwt	Kwt	Kno	GFD
gwm190	c5d	A	0.011	0.42	35	27	22.2	34.6	17.9	13.8	0.018	3.86	211	29	51.1	50.4	0.96	53.0
		B	0.013	0.66	47	31	29.4	38.2	16.3	7.4	0.018	3.80	208	29	54.7	55.9	4.3	52.2
cfd57	c5d	A	0.011	0.41	35	28	23.4	33.7	16.4	12.6	0.018	3.76	205	29	50.2	50.3	-0.14	52.4
		B	0.014	0.73	50	30	28.6	40.4	18.7	8.2	0.018	3.97	217	28	56.8	56.9	6.8	53.1
wmc667	c6a	A	0.013	0.67	50	29	30.1	45.1	22.4	10.4	0.016	3.52	211	27	59.2	60.0	9.1	53.8
		B	0.012	0.46	36	28	22.5	31.6	14.2	11.2	0.019	4.00	210	29	49.6	49.2	-1.2	52.2
barc17	c6b	A	0.011	0.41	34	28	25.6	37.3	18.7	12.7	0.018	3.86	206	29	49.8	49.3	-0.8	52.0
		B	0.014	0.75	52	30	24.7	34.0	14.4	7.4	0.018	3.83	215	28	57.8	58.8	8.5	54.2
cfd102	c6b	A	0.011	0.44	35	28	23.3	35.1	17.4	12.9	0.018	3.83	207	29	51.6	51.9	2.6	53.3
		B	0.013	0.72	51	31	30.4	38.6	16.7	6.6	0.018	3.88	216	28	54.8	53.7	0.3	51.2
wmc532	c6b	A	0.072	0.48	37	28	22.0	32.6	16.8	11.4	0.019	4.07	212	29	50.6	51.0	1.3	52.6
		B	0.012	0.57	44	29	32.8	46.3	22.0	9.3	0.016	3.26	205	28	57.5	57.1	1.6	51.2
wmc24	c6b	A	0.012	0.48	38	28	20.8	30.9	14.4	13.4	0.018	4.00	216	29	53.7	54.7	3.8	53.3
		B	0.012	0.54	42	30	30.6	42.5	20.7	7.4	0.017	3.55	201	29	52.5	52.1	1.1	51.6
barc77	c6d	A	0.011	0.46	38	27	24.9	34.6	14.7	13.1	0.018	3.80	210	29	52.3	53.4	2.4	52.2
		B	0.013	0.67	46	32	26.8	37.3	20.4	4.8	0.019	3.91	207	29	50.0	48.6	-1.6	51.8
barc54	c6d	A	0.011	0.39	34	27	21.3	30.9	14.9	13.6	0.018	3.90	212	29	50.8	50.0	-3.5	51.6
		B	0.013	0.61	44	30	28.2	41.0	21.1	8.3	0.018	3.76	208	29	54.1	55.2	5.9	52.7
barc111	c7b	A	0.012	0.45	36	27	23.2	37.2	20.3	13.1	0.018	3.88	214	29	52.3	51.7	-0.4	52.8
		B	0.013	0.68	49	31	29.5	34.2	11.2	6.8	0.019	3.74	200	28	53.3	55.0	7.9	52.5

Table XIX. Contrasts of yield components per main spike and their % reduction after a short-term of 38˚C beginning at 10 DAP in growth chambers and long-term heat stress of 30˚C beginning at 10 DAP until grain maturity in plant growth rooms for flanking markers identified from composite interval mapping analysis. A and B indicate alleles from two parental lines '7C' and 'Seri M82', respectively.

			Composite Interval Mapping Analysis															
			Short-term (mean)				Short-term (mean of % red)				Long-term (mean)				Long-term (mean of % red)			
Markers	Chr.	Alleles	Skwt	Kwt	Kno	GFD	Skwt	Kwt	Kno	GFD	Skwt	Kwt	Kno	GFD	Skwt	Kwt	Kno	GFD
cfa2129-	c1a	A	0.013	0.37	29	28	28.7	32.3	5.9	11.8	0.023	1.39	61	29	48.6	48.1	0.1	52.8
barc148		B	0.014	0.49	34	29	30.8	32.9	5.6	9.6	0.019	1.12	59	29	53.9	54.9	2.9	52.0
cfa2129-	c1a	A	0.013	0.38	29	28	29.6	33.4	6.2	11.6	0.023	1.38	61	29	49.1	48.9	1.5	52.9
gwm11		B	0.014	0.48	34	29	29.6	32.1	5.6	10.6	0.019	1.16	60	28	53.7	53.9	0.3	52.2
wmc336-	c1d	A	0.012	0.34	27	27	28.1	29.9	4.2	12.5	0.023	1.4	60	29	46.4	45.5	-1.7	52.8
cfd48		B	0.014	0.46	33	29	31.3	38.7	11.2	8.5	0.019	1.23	63	28	55.2	56.7	4.4	51.9
barc124-	c2a	A	0.014	0.45	32	29	32.3	38.0	8.6	11.4	0.021	1.30	62	28	55.1	59.0	55.7	53.0
gwm312		B	0.014	0.41	28	30	25.8	29.7	4.6	7.2	0.024	1.47	61	29	44.9	44.0	44.0	52.6
gwm372-	c2b	A	0.012	0.36	28	28	28.8	30.6	4.2	13.5	0.023	1.36	60	29	48.0	47.3	-1.1	52.7
wmc154		B	0.014	0.45	32	29	31.8	39.8	11.6	8.8	0.020	1.27	62	29	53.3	54.1	3.1	52.1
gwm372-	c2b	A	0.013	0.38	29	28	28.3	5.5	15.0	12.0	0.022	1.38	61	28	48.7	48.7	1.1	52.9
barc159		B	0.013	0.43	32	29	32.1	12.8	25.4	8.8	0.020	1.22	61	29	53.3	53.7	1.5	51.8
wmc418-	c3a	A	0.012	0.35	28	27	31.1	35.3	7.4	13.9	0.022	1.38	61	29	50.3	50.3	1.6	52.5
barc62		B	0.014	0.44	31	29	28.4	31.4	5.7	8.9	0.021	1.29	60	28	52.0	51.2	-0.5	53.0
gwm493-	c3b	A	0.013	0.40	30	28	30.2	34.7	7.2	12.2	0.022	1.35	61	28	52.8	53.4	3.6	52.8
cfd223		B	0.013	0.39	30	29	32.3	33.1	3.9	8.9	0.021	1.25	61	29	52.6	51.2	1.2	52.4
wmc48-	c4a	A	0.013	0.38	29	28	30.3	37.4	10.6	10.2	0.022	1.34	60	29	48.7	48.3	1.0	52.8
wmc89		B	0.014	0.46	32	29	28.2	25.6	0.2	9.1	0.021	1.30	62	28	53.7	55.0	2.6	52.7

Table XIX (continued)

			Composite Interval Mapping Analysis															
			Short-term (mean)				Short-term (mean of % red)				Long-term (mean)				Long-term (mean of % red)			
Markers	Chr.	Alleles	Skwt	Kwt	Kno	GFD	Skwt	Kwt	Kno	GFD	Skwt	Kwt	Kno	GFD	Skwt	Kwt	Kno	GFD
gwm251-	c4b	A	0.013	0.39	29	28	26.0	29.3	6.4	11.6	0.022	1.30	60	29	49.3	50.3	3.0	52.6
wmc238		B	0.014	0.43	31	30	37.5	41.7	5.8	9.7	0.022	1.39	64	29	52.3	50.1	-3.3	52.6
gwm192-	c4d	A	0.012	0.36	29	28	30.7	35.8	8.0	12.7	0.022	1.34	60	29	47.8	47.2	-1.1	52.1
wmc285		B	0.015	0.52	33	30	27.1	28.5	4.2	6.9	0.021	1.26	61	28	56.2	58.4	9.6	54.4
gwm443-	c5a	A	0.013	0.37	29	28	28.9	321	6.8	11.7	0.022	1.36	61	29	49.4	49.3	0.6	52.7
barc232		B	0.015	0.50	34	29	30.7	34.3	5.6	10.3	0.020	1.19	60	28	54.3	55.6	4.3	52.8
gwm604-	c5b	A	0.013	0.41	30	28	30.1	37.1	6.4	11.8	0.021	1.30	61	28	52.1	52.7	2.5	52.6
barc4		B	0.013	0.35	28	31	25.7	28.6	3.7	5.7	0.026	1.47	57	29	41.7	39.8	-2.4	53.5
wmc231-	c5d	A	0.012	0.36	29	28	31.3	35.8	7.0	13.6	0.022	1.33	60	29	48.8	48.5	-1.2	52.2
wmc765		B	0.015	0.51	33	31	26.7	28.0	4.3	6.3	0.021	1.35	63	28	54.2	55.5	7.0	53.7
wmc622-	c6a	A	0.015	0.40	27	32	18.2	28.8	10.2	7.3	0.025	1.50	60	30	43.1	42.3	0.9	53.3
wmc332		B	0.013	0.41	31	29	32.3	35.5	6.5	12.0	0.021	1.30	61	28	52.2	52.4	2.3	52.6
barc17-	c6b	A	0.013	0.37	28	28	29.6	32.3	5.9	12.3	0.022	1.30	60	29	48.9	49.0	0.7	52.4
wmc232		B	0.015	0.48	33	30	28.1	34.8	8.9	8.6	0.022	1.39	64	28	53.7	53.7	2.9	53.5
barc111-	c7b	A	0.013	0.38	29	27	27.7	30.9	5.9	1.8	0.022	1.32	60	29	48.9	49.1	0.3	52.4
gwm469		B	0.014	0.46	31	31	33.5	38.5	8.5	7.1	0.022	1.33	62	28	53.7	53.7	4.3	53.3

Table XX. Contrasts of yield components per plant and their % reduction after a short-term of 38˚C beginning at 10 DAP in growth chambers and long-term heat stress of 30˚C beginning at 10 DAP until grain maturity in plant growth rooms for flanking markers identified from composite interval mapping analysis. A and B indicate alleles from two parental lines '7C' and 'Seri M82', respectively.

			Composite Interval Mapping Analysis											
			Short-term (mean)			Short-term (mean of % red)			Long-term (mean)			Long-term (mean of % red)		
Markers	Chr.	Alleles	Skwt	Kwt	Kno	Skwt	Kwt	Kno	Skwt	Kwt	Kno	Skwt	Kwt	Kno
cfa2129-	c1a	A	0.012	0.46	38	23.1	33.6	16.5	0.019	4.04	212	50.9	50.7	1.2
barc148		B	0.013	0.61	44	29.1	37.8	15.8	0.016	3.30	204	55.7	55.2	0.7
cfa2129-	c1a	A	0.012	0.49	39	24.8	35.1	16.5	0.019	4.01	211	51.3	51.5	3.0
gwm11		B	0.013	0.59	44	26.6	36.8	17.6	0.016	3.37	206	55.8	54.8	1.4
wmc336-	c1d	A	0.011	0.42	34	22.9	32.7	14.8	0.019	4.04	207	48.9	48.2	-0.98
cfd48		B	0.013	0.61	45	28.9	44.8	23.7	0.017	3.56	213	57.4	58.9	5.9
barc124-	c2a	A	0.013	0.62	46	27.9	40.4	20.6	0.017	3.69	213	57.9	59.0	8.1
gwm312		B	0.013	0.54	40	22.4	32.6	14.6	0.017	4.35	212	46.5	44.7	-2.4
gwm372-	c2b	A	0.011	0.45	36	23.1	32.9	15.3	0.019	3.90	209	50.4	49.6	-0.49
wmc154		B	0.013	0.59	44	30.3	45.1	22.6	0.017	3.71	211	55.5	56.5	4.6
gwm372-	c2b	A	0.012	0.51	39	22.4	32.9	15.0	0.019	3.93	212	51.1	51.5	2.8
barc159		B	0.012	0.54	42	32.8	47.7	25.4	0.017	3.67	209	55.0	55.0	1.6
wmc418-	c3a	A	0.011	0.44	35	26.5	37.0	19.5	0.018	3.94	213	49.2	49.6	1.0
barc62		B	0.013	0.59	42	24.1	34.9	15.7	0.018	3.73	206	55.2	55.3	3.6
gwm493-	c3b	A	0.012	0.54	41	24.9	34.4	15.7	0.018	3.87	212	52.8	53.4	3.6
cfd223		B	0.012	0.47	39	28.2	41.7	20.6	0.018	3.76	210	52.6	51.2	1.2
wmc48-	c4a	A	0.012	0.46	36	24.5	32.5	27.6	0.019	3.95	208	51.7	51.5	1.9
wmc89		B	0.012	0.61	46	23.5	44.5	12.9	0.019	3.65	213	53.4	53.9	3.0

Table XX (continued)

			Composite Interval Mapping Analysis											
			Short-term (mean)			Short-term (mean of % red)			Long-term (mean)			Long-term (mean of % red)		
Markers	Chr.	Alleles	Skwt	Kwt	Kno	Skwt	Kwt	Kno	Skwt	Kwt	Kno	Skwt	Kwt	Kno
gwm251-	c4b	A	0.012	0.49	38	21.3	31.8	16.3	0.018	3.70	206	52.3	53.6	4.2
wmc238		B	0.012	0.59	46	34.4	45.5	18.5	0.019	4.07	213	52.3	50.7	1.1
gwm192-	c4d	A	0.011	0.44	36	27.4	39.0	19.0	0.018	3.87	207	49.7	49.5	0.5
wmc285		B	0.014	0.73	50	21.0	29.4	12.9	0.017	3.70	213	58.9	60.4	8.7
gwm443-	c5a	A	0.012	0.47	38	24.3	35.6	17.4	0.018	3.89	211	52.2	52.3	1.6
barc232		B	0.013	0.65	45	26.3	35.4	16.5	0.017	3.50	205	56.0	56.9	4.8
gwm604-	c5b	A	0.012	0.53	41	25.8	37.1	18.7	0.017	3.76	212	54.9	55.3	2.9
barc4		B	0.012	0.46	39	20.5	26.8	3.0	0.022	4.29	191	42.9	42.9	0.5
wmc231-	c5d	A	0.011	0.44	37	29.0	39.9	18.5	0.018	3.80	208	50.5	50.3	-0.5
wmc765		B	0.014	0.72	49	19.4	29.1	12.0	0.018	3.89	217	57.5	59.0	10.3
wmc622-	c6a	A	0.014	0.66	49	15.7	27.4	9.0	0.021	4.43	208	45.2	42.2	-2.4
wmc332		B	0.012	0.53	42	28.0	39.3	19.3	0.017	3.75	212	54.3	54.8	3.6
barc17-	c6b	A	0.012	0.45	36	25.4	36.4	18.2	0.018	3.80	205	50.9	50.7	0.7
wmc232		B	0.013	0.68	49	24.3	35.9	15.8	0.018	3.99	218	55.6	56.4	6.3
barc111-	c7b	A	0.012	0.46	36	23.7	35.4	18.1	0.018	3.85	211	51.6	51.3	-0.3
gwm469		B	0.013	0.67	49	28.6	38.0	15.5	0.019	3.80	205	55.0	56.1	8.1

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