Mapping QTL for chlorophyll fluorescence kinetics parameters at seedling stage as indicators of heat tolerance in wheat

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Abstract High temperature or heat stress is one of the most important abiotic stresses that affect wheat production in almost every part of the world. Parameters of chlorophyll fluorescence kinetics (PCFKs) are the most powerful and reliable characters available to understand the impact of various abiotic stresses on plant physiological processes and heat tolerance. The present research was aimed to identify genomic regions controlling PCFKs at early growth stages of wheat through quantitative trait loci analysis by applying heat stress for different duration of time. A doubled haploid population derived from the cross of two Chinese wheat cultivars Hanxuan 10 and Lumai 14 was exposed to 38 °C for 2, 4, 6 and 8 h of heat stress and PCFKs (initial fluorescence, maximum fluorescence, variable fluorescence and maximum quantum efficiency of photosystem II) were measured. A total of 37 QTLs were identified for the target traits, among which 13 were detected under normal temperature of 25 °C and the remaining 24 under the stressful temperature of 38 °C. Stable or consistently expressed QTLs for initial, maximum and variable fluorescence were detected on chromosomes 1A, 1B, 2B, 4A and 7D. In addition, 24 QTLs were clustered in 9 clusters on chromosomes 1A, 1B, 2B, 3B, 3D, 4A, 5A and 7D. These QTL hot spot regions along with stable QTLs should be targeted for better understanding the genetic basis of chlorophyll fluorescence kinetics parameters in future mapping studies.

Keywords Chlorophyll fluorescence kinetics parameters · Heat tolerance · Maximum quantum efficiency of PS II · QTL · Wheat

Introduction

Heat stress is considered as one of the major factors that limit wheat (Triticum aestivum L.) production in arid, semiarid, tropical, and subtropical regions of the world by affecting wheat during different developmental stages in its life cycle (Rehman et al. 2009; Joshi et al. 2007). In fact almost every crop and especially cereals faces the same situation at some growth stages and are exposed to high temperature (Stone 2001), which results in reduction in yield, as well as grain quality.

Plant responses to high temperature depend on species, variety and developmental stage. Thus, for crop production under high temperatures, it is important to know the developmental stage and plant processes that
are most sensitive to heat stress as well as whether high day or high night temperatures are more injurious (Nahar et al. 2010). The optimum temperature for photosynthetic activity in wheat is about 20 °C (Al-Khatib and Paulsen 1989). High temperatures diminish photosynthetic activities by accelerating senescence and by reducing viable leaf area (Harding et al. 1990). Nowadays, chlorophyll fluorescence is one of the most powerful techniques available to plant breeders and physiologists (Maxwell and Johnson 2000; Sayed 2003) to study the changes in photosynthesis in response to stresses. The ratio of variable fluorescence ($F_v$) to maximum fluorescence ($F_m$) plays a vital role in photosynthesis and is an estimate of PS II maximum efficiency under abiotic stress conditions (Rachmilevitch et al. 2006) and can be used as a screening tool for stress tolerance (Prasad et al. 2007). A low $F_v/F_m$ indicates low photosynthetic efficiency, thus genotypes with higher $F_v/F_m$ values under stress conditions may be more tolerant to stress (Kumar et al. 2012).

It is predicted that global climate change associated with increases in temperature will further decrease yield and quality (Nelson et al. 2009; Dixon et al. 2009; Caffe-Treml et al. 2011). Therefore breeding for high temperature tolerance is a major objective of wheat breeding programs around the world, so it is important to incorporate heat tolerance into the current wheat cultivars. However breeding for heat tolerance is still in its early stages and needs more attention in future (Ortiz et al. 2008; Ashraf 2010).

Heat tolerance is a quantitative trait controlled by a number of genes/quantitative trait loci (QTLs) (Blum 1988). QTLs for heat tolerance in wheat were reported using different traits like grain filling duration, canopy temperature depression and yield (Yang et al. 2002; Mason et al. 2010; Pinto et al. 2010), carbon isotope discrimination (Rebetzke et al. 2008) and senescence related traits (Vijayalakshmi et al. 2010). Our research studies were directed to identify QTLs for PCFKs ($F_o$, $F_m$, $F_v$, $F_v/F_m$) for heat tolerance using a wheat doubled haploid population under controlled environmental conditions at seedling stage.

Materials and methods

Plant materials

A doubled haploid (DH) wheat population consisting of 150 lines derived from the cross of two elite Chinese wheat cultivars, Hanxuan 10 and Lumai 14, was used for QTL mapping of heat tolerance traits (Jing et al. 1999). The female parent Hanxuan 10 is a drought tolerant cultivar developed and released by Shanxi Academy of Agricultural Sciences in 1966. The male parent Lumai 14 is a high yielding cultivar adapted to abundant water and fertile conditions, developed and released by Yantai Institute of Agricultural Sciences, Shandong Province, in 1986.

Growth media and treatments

The experiment was conducted in the environmentally controlled growth chambers (GZP-300B, Nanjing Hancon Instrument Factory, China) at the Institute of Crop Science, Chinese Academy of Agriculture Sciences. The growth media (soil mixture) used in the experiment contained adequate amount of nutrients. The mixture contains soil, peat and sand in the ratio of 2:1:1. Fifty seeds of parents and their DH lines each were sown in individual germination boxes (12 cm × 12 cm × 5 cm), respectively. The plants were germinated at optimum temperature of 25/20 °C (day/night). Eight days after emergence the seedlings were thinned to 10 seedlings per germination box to ensure proper growth. Seedlings were regularly watered throughout the growing period. After 15 days, heat stress of 38 °C was applied for 2, 4, 6 and 8 h. The experiment was carried out with three replications.

Physiological characterization

A portable fluorometer (OS-30p, Opti-Sciences, Inc., Hudson, USA) was used to measure the parameters of chlorophyll fluorescence kinetics (PCFKs), including initial fluorescence ($F_o$), maximum fluorescence ($F_m$), variable fluorescence ($F_v$) and maximum quantum efficiency of photosystem II ($F_v/F_m$). Data was recorded on five randomly selected plants in every replication from parents and their DH lines, 15 days after planting. Measurements were made on the abaxial surface of first leaf under optimum temperature of 25 °C and then under stressful temperature of 38 °C at interval of 2, 4, 6 and 8 h. Before taking measurement the plants were first acclimated to dark for 1 h. The mean of three replications for each genotype was used for QTL analysis.
Statistical Analysis and QTL detection

All the statistical analyses were computed by SPSS version 17 statistical package. Association between different PCFKs under different temperatures and stress intensity durations was calculated using the same statistical software. The available genetic map established from the 150 DH lines (Hao et al. 2003; Zhou et al. 2005) was used for QTL detection. The genetic map consists of 395 marker loci (132 AFLP and 263 SSR). The map covered 3,904 cM with an average distance of 9.9 cM between adjacent markers. The QTLs were detected by the software QTL Ici mapping version 3.2 developed and released by the Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China (http://www.isbreeding.net). To identify an appropriate threshold likelihood of odd (LOD) score for declaring a significant QTL, permutation test was conducted for 1,000 times, however at this criterion relatively very few significant QTLs were identified. So LOD score of 2.0 was used in the study and some minor QTLs having LOD score from 2.0 to 3.0 were also reported. QTLs were named according to the rule “QTL + trait + research department + chromosome” (Zhou et al. 2005). Broad sense heritability estimates for each trait under different temperature regimes were calculated by using QTL Ici mapping software version 4.01 (http://www.isbreeding.net).

Results

Phenotypic analysis of DH population

The phenotypic values for the PCFKs under different temperatures and stress duration periods for parents and their DH lines are summarized in Table 1. The mean phenotypic values for different traits for Lumai 14 in most of the cases were higher than Hanxuan 10. In both parents under normal temperature condition the value of \( F_o \) was lower but with the increase in intensity and duration of stress the value was increased. The phenotypic values for \( F_m \) and \( F_v \) increased in both parents when temperature stress was applied for 2 h but at the end of 8 h stress the values for both of these traits were reduced. In DH lines increase in intensity and duration of temperature stress was associated with reduction in both \( F_m \) and \( F_v \). The maximum quantum efficiency of PS II i.e. \( F_v/F_m \) exhibited a decreasing trend in parents and their DH lines. They had maximum value for this trait under 25 °C but with the increase in temperature intensity and duration a gradual decrease was observed for this trait. Transgressive segregations were observed in the population under different temperature and stress duration periods where some of the lines have greater value than that of the higher parent and less than that of the lower parent. It showed that the favourable alleles controlling the target traits had been widely separated in the population thus indicating that the phenotypic data is appropriate for QTL analysis.

Association between different parameters of chlorophyll fluorescence kinetics

Association between different PCFKs under different temperature and stress intensity duration was computed by SPSS version 17 statistical package. Highly significant and positive associations were observed between \( F_o \), \( F_m \) and \( F_v \) under all temperature regimes and stress intensities (Table 2). In addition \( F_m \) and \( F_v \) further showed positive relationship with \( F_v/F_m \), but the correlation coefficients between \( F_v \) and \( F_v/F_m \) are large than that between \( F_m \) and \( F_v/F_m \). The correlation between \( F_o \) and \( F_v/F_m \) was highly significant but in the opposite direction in almost every condition, however, under the temperature regime of 38 °C (6 h stress) the association was weak and non significant (−0.123). Overall maximum, positive and highly significant correlations were observed between \( F_m \) and \( F_v \) (0.984**, 0.983**, 0.983**, 0.987**, 0.927**) under different studied conditions.

Additive and epistatic QTLs for chlorophyll fluorescence kinetics parameters

A total of 37 additive QTLs, including 13 QTLs detected under the normal temperature regime of 25 °C and 24 under the stressful temperature of 38 °C, were identified for \( F_o, F_m, F_v \) and \( F_v/F_m \) at seedling stage in wheat (Table 3; Fig. 1). Among the 24 QTLs detected under 38 °C, 5 were detected when temperature stress was applied for 2 h, 8 were mapped when stress was applied for 4 and 8 h and 3 were identified when stress was applied for 6 h. These QTLs were located on 13 linkage groups 1A, 1B, 2A, 2B, 3B, 3D, 4A, 5A, 5B, 6A, 7A, 7B and 7D. The variance
explained by these QTLs ranged from 5.6 % to 14.9 %. Majority of QTLs were located on chromosomes 1A (8) followed by 4 each on chromosome 3B and 4A.

In addition to the above additive QTLs 16 epistatic QTL pairs were detected for the target PCFKs under two temperature regimes. Among them four pairs were found under temperature regime of 25 °C and the remaining 12 under stressful temperature of 38 °C (Table 4). Maximum number of epistatic pairs were detected for \( F_o/F_m \) (6) followed by \( F_v \) (5) where as minimum were detected for \( F_o \) (2). The overall phenotypic variance explained by these epistatic pairs ranged from 12.6 to 27.2 %.

Initial fluorescence

Nine additive QTLs affecting \( F_o \) were mapped on linkage groups 1B (3), 2B (2), 5B (1), 7D (2) and 7B (1). Among them \( QF_o.cgb-2B \) (Xgwm319–Xwmc441) was detected under 25 °C and the remaining eight were identified under 38 °C. The total phenotypic variance explained of the nine additive effect loci is 65.2 %. The QTL detected under normal temperature regime is a major locus having LOD score of 2.93 and explained phenotypic variance of 10.8 % which is higher than that of the other eight loci. Both parents contributed favourable alleles to the population with four coming from male parent Lumai 14 and five transmitted from the female parent Hanxuan 10. Three QTLs, \( QF_o.cgb-2B \) (Xgwm319–Xwmc441), \( QF_o.cgb-1B \) (P3616.3–P3477.1) and \( QF_o.cgb-7D \) (Xgwm44–Xgwm121) can be regarded as consistently expressed or stable QTLs as all of them were identified in more than one stress duration treatments (Table 5). \( QF_o.cgb-2B \) was observed when stress was applied for 2 h and again identified when stress was applied for 8 h. In the same way \( QF_o.cgb-1B \) and \( QF_o.cgb-7D \) were detected two times under 38 °C when stress was applied for different durations.

Table 1 Phenotypic values of wheat doubled haploid lines and their parents for parameters of chlorophyll fluorescence kinetics

| Trait | Temp. (°C) | StP. | Hanxuan 10 Mean ± SD | Lumai 14 Mean ± SD | DH population Mean ± SD | Min | Max | CV (%) | Kurt | Skew | \( h^2 \) |
|-------|------------|------|----------------------|------------------|-----------------------|-----|-----|--------|------|------|--------|
| \( F_o \) | 25 | 0 | 37.00 ± 3.61 | 37.33 ± 2.89 | 36.71 ± 3.77 | 28.22 | 46.44 | 10.26 | −0.40 | −0.11 | 0.66 |
| | | 38 | 4.00 ± 2.65 | 35.13 ± 3.44 | 27.22 | 46.11 | 9.74 | −0.02 | 0.15 | 0.69 |
| \( F_m \) | 25 | 0 | 148.33 ± 16.01 | 156.33 ± 9.82 | 154.17 ± 16.19 | 113.00 | 195.22 | 10.50 | −0.06 | −0.46 | 0.76 |
| | | 38 | 167.33 ± 3.79 | 161.33 ± 12.50 | 136.03 ± 13.27 | 106.56 | 172.67 | 9.75 | −0.01 | 0.19 | 0.70 |
| \( F_v \) | 25 | 0 | 111.33 ± 12.50 | 119.00 ± 6.93 | 117.46 ± 13.49 | 85.00 | 155.00 | 11.49 | −0.01 | −0.27 | 0.78 |
| | | 38 | 124.33 ± 2.89 | 122.00 ± 9.54 | 100.90 ± 10.68 | 78.67 | 129.44 | 10.59 | −0.17 | 0.16 | 0.70 |
| \( F_{v/F_m} \) | 25 | 0 | 0.751 ± 0.001 | 0.760 ± 0.002 | 0.761 ± 0.02 | 0.719 | 0.806 | 2.14 | −0.16 | 0.50 | 0.73 |
| | | 38 | 0.742 ± 0.002 | 0.753 ± 0.006 | 0.741 ± 0.02 | 0.686 | 0.775 | 2.17 | 0.91 | −0.47 | 0.71 |

Temp temperature, StP stress period, h hours, SD standard deviation, DH doubled haploid, Min minimum, Max maximum, CV coefficient of variation, Kurt Kurtosis, Skew Skewness, \( h^2 \) heritability, \( F_o \) initial fluorescence, \( F_m \) maximum fluorescence, \( F_v \) variable fluorescence, \( F_{v/F_m} \) maximum quantum efficiency of PS II.
Two pairs of epistatic QTLs associated with Fo located on chromosomes 5B-6A and 2D-4B were detected when a stressful temperature of 38°C was applied for 2 h and 8 h respectively. One of the pair has negative effect (i.e. recombinant type effect was higher than the parent type effect) and another has positive effect (i.e. parent type effect was higher than the recombinant type effect) and they explained phenotypic variance of 15.0 and 27.2% respectively.

Maximum fluorescence

Four additive QTLs controlling Fm were mapped on chromosome 3B (2) and 5A under 25°C. These QTLs together contribute about 26.2% of phenotypic variance whereas the individual contribution of these QTLs ranged from 5.7 to 7.6%. Three of them have negative effects with favourable alleles coming from Lumai 14 while one has positive effect with favourable alleles donated by Hanxuan 10. Under the stressful temperature of 38°C six additive QTLs associated with Fm were identified on linkage groups 1A (3), 4A (2) and 7A (1). Lumai 14 alleles increase this trait at chromosome 7A that can explain 9.5% of phenotypic variance while for the remaining QTLs the favourable alleles were donated by Hanxuan 10. QFm.cgb-4A (Xwmc89–Xwmc420) can be considered as a stable or consistently expressed QTL as it was expressed when stress was applied for 4 and 8 h (Table 5). This QTL is a major locus as it has LOD score of >4 coupled with phenotypic variance explained of >11%.

Three pairs of epistatic QTLs for maximum fluorescence were identified under two temperature regimes. Two pairs were detected under the stressful temperature of 38°C (stress duration 2 and 4 h, respectively) on chromosomes 2B-4A and 4A-4A. Only a single pair was mapped on chromosomes 2B-5A under normal temperature regime of 25°C. All the three pairs have positive effect and together explained phenotypic variance of 42.2%.

Variable fluorescence

Under 25°C five additive QTLs linked with Fv were mapped on chromosomes 3B (2), 3D (1) and 5A (2) explaining phenotypic variance ranging from 5.8 to 7.5%. Favourable alleles for QFv.cgb-3B (P3622.4–P2076) came from Hanxuan 10 while for the remaining four QTLs the favourable alleles were contributed by Lumai 14.

The variable fluorescence was further linked to five QTLs under stressful temperature of 38°C which were mapped on chromosomes 1A (4) and 4A (1). All of these QTLs have positive effect which means that the donor of the favourable allele for the trait was Hanxuan 10. These QTLs collectively explained 43.7% of phenotypic variance, whereas the individual contribution of these QTLs ranged from 6.5 to 14.9%. One stable QTL, QFv.cgb-1A (Xgwm164–Xwmc183) was detected for variable fluorescence as it was identified twice when the stress was applied for 2 h and again when stress was imposed for 8 h (Table 5). Another QTL QFv.cgb-4A (Xwmc89–Xwmc420) is a major QTL as it has LOD score of 5.48 and explained phenotypic variance of 14.9% which is higher than the remaining loci detected either under normal or stress conditions. No QTL was found for variable fluorescence when high temperature stress of 38°C was applied for 6 h.

There are five pairs of interaction QTLs affecting variable fluorescence mapped on chromosomes 2B-5A, 6B-6B, 1B-2D, 3B-3B and 3D-3D. The first two

### Table 2: Association between parameters of chlorophyll fluorescence kinetics

| Trait | Temp. (°C) | Stress period | Fm  | Fv  | Fm/Fv |
|-------|------------|---------------|-----|-----|-------|
| Fo    | 25         | 0             | 0.767** | 0.640** | -0.287** |
|       | 38         | 2 h           | 0.820** | 0.701** | -0.284** |
|       | 38         | 4 h           | 0.820** | 0.702** | -0.230** |
|       | 38         | 6 h           | 0.868** | 0.775** | -0.123 |
|       | 38         | 8 h           | 0.811** | 0.616** | -0.330** |
| Fm    | 25         | 0             | 0.984** | 0.392** |
|       | 38         | 2 h           | 0.983** | 0.312** |
|       | 38         | 4 h           | 0.983** | 0.365** |
|       | 38         | 6 h           | 0.987** | 0.382** |
|       | 38         | 8 h           | 0.927** | 0.281** |
| Fv    | 25         | 0             | 0.549** |
|       | 38         | 2 h           | 0.478** |
|       | 38         | 4 h           | 0.527** |
|       | 38         | 6 h           | 0.526** |
|       | 38         | 8 h           | 0.479** |

*Fo*: initial fluorescence, *Fm*: maximum fluorescence, *Fv*: variable fluorescence, *Temp*: temperature, *Fm/Fv*: maximum quantum efficiency of PS II

** Significant at *P* = 0.01 level
| Trait | Temp. | QTL          | Marker interval          | Position (cM) | LOD   | PVE (%) | Additive |
|-------|-------|--------------|--------------------------|---------------|-------|---------|----------|
| $F_o$ | NT    | $QF_c$-cgb-5B | Xgwm234–Xwmc363          | 17            | 2.93  | 10.8    | -1.24    |
|       | ST-2 h| $QF_c$-cgb-2B | Xgwm319–Xwmc441          | 184           | 2.19  | 6.6     | 0.93     |
|       | ST-4 h| $QF_c$-cgb-1B | P3616.3–P3477.1          | 123           | 2.38  | 6.5     | 0.83     |
|       | ST-6 h| $QF_c$-cgb-7D | Xgwm44–Xgwm121           | 132           | 2.71  | 8.0     | -0.91    |
|       | ST-8 h| $QF_c$-cgb-9B | Xgwm319–Xwmc441          | 184           | 2.64  | 7.9     | 1.06     |
|       | ST-8 h| $QF_c$-cgb-2B | Xgwm411–Xwmc302          | 71            | 2.01  | 5.6     | -0.86    |
| $F_m$ | NT    | $QF_m$-cgb-3B | P3622.4–P2076            | 102           | 2.11  | 5.9     | 3.92     |
|       | NT    | $QF_m$-cgb-3B | Xgwm299–Xcwm539.1        | 244           | 2.60  | 7.1     | -4.54    |
|       | NT    | $QF_m$-cgb-3D | Xgdm72–Xgwm341           | 58            | 2.71  | 7.6     | -4.44    |
|       | ST-2 h| $QF_m$-cgb-5A | P2470–Xgwm154            | 25            | 2.04  | 5.7     | -3.86    |
|       | ST-4 h| $QF_m$-cgb-1A | Xgwm164–Xwmc183          | 74            | 2.18  | 6.6     | 3.56     |
|       | ST-4 h| $QF_m$-cgb-1A | Xwmc120–Xgwm135          | 79            | 2.07  | 5.7     | 3.06     |
|       | ST-4 h| $QF_m$-cgb-1A | Xwmc59–Xwmc254           | 175           | 2.43  | 6.5     | 3.17     |
|       | ST-4 h| $QF_m$-cgb-1A | Xwmc89–Xwmc420           | 0             | 4.41  | 11.9    | 4.27     |
|       | ST-6 h| $QF_m$-cgb-7A | P3156.3–Xwmc83           | 200           | 2.31  | 9.5     | -4.27    |
|       | ST-8 h| $QF_m$-cgb-4A | Xwmc89–Xwmc420           | 0             | 2.13  | 6.3     | 2.99     |
| $F_v$ | NT    | $QF_v$-cgb-3B | P3622.4–P2076            | 103           | 2.04  | 5.8     | 3.25     |
|       | NT    | $QF_v$-cgb-3B | Xgwm299–Xcwm539.1        | 244           | 2.66  | 7.3     | -3.83    |
|       | NT    | $QF_v$-cgb-3D | Xgdm72–Xgwm341           | 58            | 2.68  | 7.5     | -3.68    |
|       | NT    | $QF_v$-cgb-5A | Xgwm156–Xwmc415          | 3             | 2.15  | 6.3     | -3.41    |
|       | NT    | $QF_v$-cgb-5A | P2470–Xgwm154            | 24            | 2.19  | 6.1     | -3.35    |
|       | ST-2 h| $QF_v$-cgb-1A | Xgwm164–Xwmc183          | 74            | 2.51  | 7.6     | 3.07     |
|       | ST-4 h| $QF_v$-cgb-1A | Xwmc120–Xgwm135          | 79            | 2.73  | 7.8     | 2.88     |
|       | ST-4 h| $QF_v$-cgb-4A | Xwmc89–Xwmc420           | 0             | 5.48  | 14.9    | 3.84     |
|       | ST-8 h| $QF_v$-cgb-1A | P2478.3–Xwmc337          | 69            | 2.31  | 7.0     | 2.81     |
|       | ST-8 h| $QF_v$-cgb-1A | Xwmc164–Xwmc183          | 74            | 2.15  | 6.5     | 2.70     |
| $F_o/F_m$ | NT    | $QF_o/F_m$-cgb-5B | P5140.2–P4138         | 91            | 4.19  | 11.4    | 0.01     |
|       | NT    | $QF_o/F_m$-cgb-7A | Xcwm462.2–Xgwm635.2      | 37            | 2.32  | 7.3     | 0.01     |
|       | NT    | $QF_o/F_m$-cgb-7B | Xgwm302–P3461.1          | 116           | 3.63  | 9.3     | -0.01    |
|       | ST-2 h| $QF_o/F_m$-cgb-4A | Xwmc89–Xwmc420          | 2             | 2.05  | 6.8     | 0.01     |
|       | ST-2 h| $QF_o/F_m$-cgb-6A | Xcwm487–P3465.4         | 86            | 2.29  | 8.3     | -0.01    |
|       | ST-4 h| $QF_o/F_m$-cgb-6A | Xcwm120–Xgwm135         | 76            | 2.43  | 7.5     | 0.01     |
|       | ST-8 h| $QF_o/F_m$-cgb-2A | P5858.2–Xgwm328         | 7             | 2.06  | 8.5     | 0.01     |

*Temp* temperature, *QTL* quantitative trait locus, *LOD* log of odds, *PVE* phenotypic variance explained by additive QTLs, *NT* normal temperature, *ST* stress temperature, 2 h 2 h stress period, 4 h 4 h stress period, 6 h 6 h stress period, 8 h 8 h stress period, *$F_o$* initial fluorescence, *$F_m$* maximum fluorescence, *$F_v$* variable fluorescence, *$F_o/F_m$* maximum quantum efficiency of PS II

*a* Position of QTL on chromosome

*b* A positive value indicates that the positive alleles are from Hanxuan 10, negative values mean the positive alleles came from Lumai 14
interaction QTL pairs were identified under normal temperature conditions while the remaining three were observed in the temperature regime of 38 °C. Four of the interaction pairs have negative effect while one has positive effect. The interaction pairs with negative effect explained phenotypic variance ranging from 12.6 to 21.4 % while the single pair with positive effect explained phenotypic variance of 15.0 %.

Maximum quantum efficiency of PS II

Eight additive QTLs for maximum quantum efficiency of PS II were mapped under two temperature regimes. Under 25 °C three additive QTLs were detected on chromosomes 5B, 7A and 7B, respectively, explaining phenotypic variance ranging from 7.3 to 11.4 %. The QTLs located on chromosome 5B and 7A had positive effect with favourable alleles coming from Hanxuan 10 while the QTL mapped on chromosome 7B had negative effect and increasing effect for the trait was donated by Lumai 14. Furthermore the QTL mapped on chromosome 5B (P5140.2–P4138) was a major locus as it had LOD value of 4.19 and explained phenotypic variance of 11.4 %. Under the stressful temperature regime of 38 °C five additive QTLs were mapped on chromosome 4A (1), 6A (2), 1A (1) and 2A (1) explaining phenotypic variance ranging from 6.8 to 8.5 %. Three of the QTLs had positive effect with favourable alleles donated by Hanxuan 10 while two of them had negative effect with favourable alleles transmitted by Lumai 14. No consistent or stable QTL was identified for this trait as well as no QTL was detected when stress was applied for 6 h.

Six pairs of epistatic QTLs for $F_v/F_m$ were detected under two temperature regimes. Only one pair located on chromosomes 1B-1B having negative effect and explained phenotypic variance of 24.7 % was identified under 25 °C. All the remaining pairs were detected under temperature regime of 38 °C and mapped on chromosomes 3D-2A, 6B-6B, 6B-3A and 1A-3A. Two interaction pairs were detected each when stress was applied for 2 and 4 h and one was identified when stress was applied for 8 h. No interaction pair was identified when stress was applied for 6 h. Three of the interaction pairs detected under stressful temperature have positive effect and two have negative effect explaining phenotypic variance ranging from 16.3 to 23.0 %.

Discussion

Wheat is one of the most important cereal crops of the world which can be grown under a wide range of climates. It is best adapted to temperate regions however it is also cultivated in tropical countries where its yield is drastically affected by high temperature (Mathur et al. 2011). High temperature drastically affects the photosynthetic efficiency of the crops resulting in reduced grain yield (Ashraf 2004; Raza et al. 2006; Arfan et al. 2007). In majority of studies crop yields have been directly correlated with photosynthetic efficiency (Georgieva et al. 2000).

Photosynthesis is one of the most heat sensitive processes of the plants (Percival 2005). The enzymatic dependent mechanism of photosynthesis is damaged within a few minutes of exposure to high temperature before any visible symptoms appeared. High temperature impairs many processes involved in photosynthesis. Chlorophyll fluorescence is a rapid, inexpensive, powerful and non destructive criterion to study the photochemical and non photochemical processes in plants in response to any stress. During the past few years it has been used to reveal responses of plants and photosynthesizing organisms to extreme environmental conditions. Among them, some of the important studies were focused to study the effects of excessive light (Favaretto et al. 2011), ozone (Bussotti et al. 2011), low/high temperature, water stress (Percival 2005; Mathur et al. 2011), salt stress, environmental pollution and herbicides (Percival 2005).

The quantum efficiency of Photosystem II, $F_v/F_m$, plays a vital role in photosynthesis, and can be used as screening criteria for stress tolerance (Blum and Ebercon 1981; Prasad et al. 2007). A low $F_v/F_m$ ratio indicates low photosynthetic efficiency, thus, genotypes with higher $F_v/F_m$ values under stress conditions may be referred to as tolerant to stress while those with low $F_v/F_m$ will be declared as susceptible. The role of $F_v/F_m$ in relation to grain yield under stressed conditions is well recognized in the selection of heat tolerant wheat plants (Blum 1988; Blum et al. 1989; Krause and Weis 1991). In the present studies Lumai 14 exhibited higher values for $F_v/F_m$ due to its dark green
Fig. 1  QTLs for parameters of chlorophyll fluorescence kinetics in the Hanxuan 10 × Lumai 14 population. Markers are given on the right side of each chromosome. The distance in centiMorgan (cM) is given on the left side of the chromosome.
leaves (stay green trait) suggesting that it has higher photosynthetic efficiency and can maintain photosynthesis even in the presence of stress. Quite similar findings were reported by Li et al. (2012) while studying the same DH population. They further observed that Lumai 14 has slow rate of senescence as compared to Hanxuan 10. The prolonged leaf duration has been positively correlated with resistance to high temperature (Thomas and Howarth 2000; Vijayalakshmi et al. 2010).

In the present study the value for $F_o$ increased with the increase in the intensity and duration of the stress together with a gradual decrease in the value of $F_v/F_m$. This decrease shows that photochemical yield of PS II has been affected. Increase in $F_o$ may be due to physical separation of the PS II reaction centers from...
| Trait | Temp. | QTL     | Marker interval | Position\(^a\) (cM) | QTL     | Marker interval | Position\(^a\) (cM) | LOD   | PVE (%) | AA\(^b\) |
|-------|-------|---------|-----------------|----------------------|---------|-----------------|----------------------|-------|---------|---------|
| \(F_o\) | ST-2 h | \(QF_o.cgb-5B\) | \(Xwmc234-Xwmc363\) | 5        | \(QF_o.cgb-6A\) | \(P1832-Xgwm617\) | 75    | 5.16    | 15.0   | −1.37  |
|       | ST-8 h | \(QF_o.cgb-2D\) | \(Xwmc453.1-Xwmc18\) | 20       | \(QF_o.cgb-4B\) | \(Xwmc47-P3459.1\) | 130   | 5.80    | 27.2   | 1.83   |
| \(F_m\) | NT    | \(QF_m.cgb-2B\) | \(P3601-P3615.1\) | 0        | \(QF_m.cgb-5A\) | \(Xgwm410-Xwmc340\) | 125   | 5.49    | 13.7   | 6.30   |
|       | ST-2 h | \(QF_m.cgb-2B\) | \(P6411.2-P3470.5\) | 70       | \(QF_m.cgb-4A\) | \(Xgwm601-Xgwm610\) | 15    | 5.42    | 15.4   | 5.24   |
|       | ST-4 h | \(QF_m.cgb-4A\) | \(P3613.2-P8922\) | 100      | \(QF_m.cgb-4A\) | \(P6431.1-Xgwm160\) | 175   | 5.17    | 13.2   | 5.35   |
| \(F_v\) | NT    | \(QF_v.cgb-2B\) | \(P3601-P3615.1\) | 0        | \(QF_v.cgb-5A\) | \(Xgwm410-Xwmc340\) | 125   | 5.75    | 15.0   | 5.43   |
|       | NT    | \(QF_v.cgb-6B\) | \(Xcwm29-P3476.2\) | 80       | \(QF_v.cgb-6B\) | \(Xwmc182-Xgwm644.1\) | 155   | 5.66    | 13.4   | −5.83  |
|       | ST-2 h | \(QF_v.cgb-1B\) | \(P3470.4-P4133\) | 0        | \(QF_v.cgb-2D\) | \(Xgwm296.1-Xgwm296.2\) | 0    | 6.02    | 12.6   | −3.82  |
|       | ST-2 h | \(QF_v.cgb-3B\) | \(P2478.1-Xwmc505.1\) | 0        | \(QF_v.cgb-3B\) | \(Xgwm644.2-Xwmc3\) | 70    | 5.29    | 12.6   | −4.07  |
|       | ST-6 h | \(QF_v.cgb-3D\) | \(Xwmc437-Xwmc529.1\) | 15       | \(QF_v.cgb-3D\) | \(Xgwm341-Xgwm456\) | 70    | 5.42    | 21.4   | −5.13  |
| \(F_v/F_m\) | NT | \(QF_v/F_m.cgb-1B\) | \(Xwmc156-P3446.1\) | 45       | \(QF_v/F_m.cgb-1B\) | \(P3616.1-P3616.3\) | 100   | 8.05    | 24.7   | −0.01  |
|       | ST-2 h | \(QF_v/F_m.cgb-3D\) | \(Xwmc437-Xwmc529.1\) | 35       | \(QF_v/F_m.cgb-2A\) | \(Xwmc524-Xgwm595\) | 5    | 5.10    | 16.3   | −0.01  |
|       | ST-2 h | \(QF_v/F_m.cgb-6B\) | \(EST138.1-Xgwm219\) | 95       | \(QF_v/F_m.cgb-6B\) | \(P1142.1-P1816.1\) | 235   | 5.20    | 18.5   | 0.01   |
|       | ST-4 h | \(QF_v/F_m.cgb-6B\) | \(Xgwm219-Xwmc341\) | 100      | \(QF_v/F_m.cgb-6B\) | \(P1142.1-P1816.1\) | 240   | 5.84    | 23.0   | 0.01   |
|       | ST-8 h | \(QF_v/F_m.cgb-6B\) | \(P1142.1-P1816.1\) | 240      | \(QF_v/F_m.cgb-3A\) | \(P3465.2-Xcwm48.2\) | 10    | 5.55    | 20.0   | 0.01   |
|       | ST-8 h | \(QF_v/F_m.cgb-1A\) | \(Xwmc93-Xwmc304\) | 115      | \(QF_v/F_m.cgb-3A\) | \(P3616.2-Xgdm33\) | 10    | 5.24    | 20.6   | −0.01  |

\(^a\) Position of QTL on chromosome

\(^b\) The epistatic effect at its direction: positive values mean the parent type effect is greater than the recombinant type effect, negative values mean the parent type effect is less than the recombinant type effect

Temp temperature, QTL quantitative trait locus, LOD log of odds, PVE phenotypic variance explained by epistatic QTL, NT normal temperature, ST stress temperature, h hours, \(F_o\) initial fluorescence, \(F_m\) maximum fluorescence, \(F_v\) variable fluorescence, \(F_v/F_m\) maximum quantum efficiency of PS II
associated pigment antennae, resulting in blocked energy transfer of the PS II traps. $F_o$ can be used as an indicator for irreversible damage in PS II, associated with light harvesting complex II dissociation and blocking of electron transference on the reductant side of PS II (Costa et al. 2002).

The increase in $F_o$ coupled with decrease in $F_v/F_m$ may suggest the occurrence of chronic photoinhibition due to photoinactivation of PS II centers, possibly due to D1 protein damage (Oxborough 2004; Baker and Rosengvist 2004). The value of $F_m$ also declined with the rise in temperature, indicating that elevated temperature caused damage to the donor side of PS II (Mathur et al. 2011). The $F_v$ also behaves in similar manner and its value was decreased with the increase in temperature and has minimum value at 38 °C when the stress was applied for 8 h. Yang et al. (1996) reported similar findings, they stated that environmental stresses may reduce $F_v$ value via inhibition of PS II photooxidation. Since $F_v$ indicates full reduction of electron receptor it can be concluded that heat stress has disturbed electron transfer to PS I.

Association between $F_o$, $F_m$ and $F_v$ were positive and highly significant under all temperature regimes. In addition $F_m$ and $F_v$ further showed positive relationship with $F_o/F_m$. The correlation between $F_o$ and $F_v$ was highly significant but in the opposite direction in almost every condition, however, under the temperature regime of 38 °C and with a stress period of 6 h the association between the two traits was negative, weak and non significant. Paknejad et al. (2007) also observed positive correlation between $F_o$ and $F_m$, $F_m$ and $F_v$, $F_m$ and $F_o/F_m$ and $F_v/F_m$. He further reported negative and non significant correlation between $F_o$ and $F_v/F_m$.

All the PCFKs showed transgressive segregation. The existence of some of the DH lines with higher and lower values compared to parents indicated polygenic inheritance with partial gene association (Kearsey and Pooni 1998). According to Poehlman and Sleper (1995) continuous variation and transgressive segregation are the two obvious characters of multiple genes inheritance.

QTLs showed low consistency across temperature regimes and stress periods because different temperature regions may induce or inhibit different gene expression. Different QTLs observed under varying temperatures and stress periods reflects that the phenotypic variances for chlorophyll fluorescence kinetics were controlled by different genetic factors. In our present research a total of 37 additive QTLs for chlorophyll fluorescence kinetics parameters were observed across two temperature regimes (25 °C and 38 °C) with varying stress period intensities (2, 4, 6 and 8 h). Among them only 13 QTLs were detected under 25 °C while the remaining 24 QTLs were mapped when a stressful temperature of 38 °C was

### Table 5 Consistently expressed QTLs for parameters of chlorophyll fluorescence kinetics

| Trait | Temp. | QTL | Marker interval | Position* (cM) | LOD | PVE (%) | Additive* |
|-------|-------|-----|----------------|----------------|-----|---------|-----------|
| $F_o$ | ST-2 h | $QF_o$| $xcgb-2B$ | $xgwm319-Xwm441$ | 184 | 2.19 | 6.6 | 0.93 |
|       | ST-4 h | $QF_o$| $xcgb-1B$ | $P3616.3-P3477.1$ | 123 | 2.38 | 6.5 | 0.83 |
|       | ST-8 h | $QF_o$| $xcgb-1B$ | $P3616.3-P3477.1$ | 123 | 2.37 | 6.5 | 0.93 |
|       | ST-4 h | $QF_o$| $xcgb-7D$ | $xgwm44-Xgwm121$ | 132 | 2.71 | 8.0 | −0.91 |
|       | ST-6 h | $QF_o$| $xcgb-7D$ | $xgwm44-Xgwm121$ | 132 | 2.13 | 5.9 | −0.84 |
| $F_m$ | ST-4 h | $QF_m$| $xcgb-4A$ | $Xwmc89-Xwmc420$ | 0 | 4.41 | 11.9 | 4.27 |
|       | ST-8 h | $QF_m$| $xcgb-4A$ | $Xwmc89-Xwmc420$ | 0 | 2.13 | 6.3 | 2.99 |
| $F_v$ | ST-2 h | $QF_v$| $xcgb-1A$ | $xgwm164-Xwmc183$ | 74 | 2.51 | 7.6 | 3.07 |
|       | ST-8 h | $QF_v$| $xcgb-1A$ | $xgwm164-Xwmc183$ | 74 | 2.15 | 6.5 | 2.70 |

*Temp* temperature, *LOD* log of odds, *PVE* phenotypic variance explained by additive QTL, *QTL* quantitative trait locus, *ST* stress temperature, 2 h 2 h, 4 h 4 h, 6 h 6 h, 8 h 8 h, $F_o$ initial fluorescence, $F_m$ maximum fluorescence, $F_v$ variable fluorescence

*Position of QTL on chromosome

*Additive value indicates that the positive alleles are from Hanxuan 10, negative values mean the positive alleles came from Lumai 14
applied for a period ranging from 2 to 8 h. So we can conclude that almost double numbers of QTLs were identified under stressed conditions as compared to normal temperature. It may be due to the reason that environmental stresses can induce the expression of genes under stressful conditions originally keeping silent under non-stressed conditions to alleviate plant damage to environmental conditions. Therefore most of the QTLs in wheat during the seedling stage especially for the important chlorophyll fluorescence kinetics parameter $F_v/F_m$ were specific to the corresponding temperature regime. Quite similar findings have been reported by previous researchers while working with different crops (Herve et al. 2001; Yang et al. 2007). Salekdeh et al. (2002) reported that several leaf proteins in rice increased significantly upon water stress and contrastingly declined on re-watering. Rabani et al. (2003) while working on drought resistance in rice observed that 62 genes were induced by drought. Kathiresan et al. (2006) found that gene expression is induced by various stresses. So it can be concluded that QTLs detected under the target environment represent the expression of genes associated with the target stress.

There were three QTLs for $F_o$ i.e. $QF_o.cgb-2B$ (Xgwm319–xwmc441), $QF_o.cgb-1B$ (P3616.3–P3477.1) and $QF_o.cgb-7D$ (Xgwm44–Xgwm121), one each for $F_m$ $QF_m.cgb-4A$ (Xwmc89–Xwmc420) and $F_v$ (Xgwm164–Xwmc183) which were consistently expressed when the stress was applied for different periods or intervals i.e. their expression was consistent irrespective of the duration of the stress. These consistently expressed/major/stable QTLs should be given more importance in genetic improvement for the chlorophyll fluorescence kinetics parameters. It can be further observed that 6 QTLs were clustered on linkage group 1A in the interval of Xgwm164–Xgwm135 and 4 on chromosome 4A (Xwmc89–Xwmc420) affecting $F_m$, $F_v$ and $F_v/F_m$ in different stress period intervals. In addition to these, clusters containing two QTLs for the target traits were observed on linkage group 1B, 2B, 3B, 3D, 5A and 7D. These QTLs should be given more attention for studying chlorophyll fluorescence kinetics in future mapping studies.

A number of studies in the past have been carried out to identify QTLs for different traits related to stress tolerance in wheat; however most of them have been carried out in the field under natural conditions and mostly in grain filling stage. Little attention has been given to the emergence or seedling stage which is one of the most sensitive stages to any abiotic stress and determines successful crop establishment. Our studies were directed to map QTLs for PCFKs at seedling stage in the lab under controlled conditions in order to reduce the experimental error. PCFKs were studied in the present studies because they requires less time, economical, non-destructive, reliable, large population can be screened, and previously being used by researches for screening against abiotic stress.

Interestingly a number of common QTLs for chlorophyll fluorescence kinetics were observed in the present studies which were reported by some other researchers. Yang et al. (2007) while studying QTLs for chlorophyll fluorescence kinetics under drought stress at the grain filling stage reported QTL on chromosome 3B flanked by marker interval (P3622.4–P2076) for $F_v/F_m$. In our studies the same cluster region was associated with $F_m$ and $F_v$. Recently another researcher (Liu et al. 2013) who studied the same DH population identified a QTL for seminal root number in the region flanked by marker interval (P2470–Xgwm154) which in the present studies was associated with variable fluorescence. In the same way a genomic region on chromosome 1A (Xwmc120–Xgwm135) which in our study controls $F_m$, $F_v$ and $F_v/F_m$ was earlier reported by Liu et al. (2013) to be associated with total root length while studying root traits at the seedling stage in wheat using the same DH population.

**Conclusion**

All the identified QTLs for chlorophyll fluorescence kinetics parameters were environment specific. A number of QTLs were mapped on linkage groups 1A, 1B, 2B, 4A and 7D which were active in more than one period (i.e. different stress intervals). QTLs for chlorophyll fluorescence kinetics were clustered in different groups in some specific genomic regions. These cluster regions should be given due attention in the future mapping studies and can be used for improvement of multiple PCFKs at the same time. No QTL for $F_v/F_m$ was detected when stress was applied for 6 h. This indicated that compared to other traits, $F_v/F_m$ could be more sensitive to environmental conditions. The present QTL mapping studies for
chlorophyll fluorescence kinetics provides information of genomic regions which may be helpful for marker assisted selection for heat tolerance improvement at the seedling stage. However further studies are needed to validate the presence of putative QTLs in different breeding populations and to identify additional useful loci for improvement of heat tolerance in wheat.

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