Planta (2010) 231:875–885
DOI 10.1007/s00425-009-1094-0

ORIGINAL ARTICLE

# Mapping quantitative trait loci associated with chlorophyll *a* fluorescence parameters in soybean (*Glycine max* (L.) Merr.)

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Received: 22 September 2009 / Accepted: 22 December 2009 / Published online: 7 January 2010


**Abstract** Chlorophyll *a* fluorescence parameters can provide qualitative and quantitative information about photosynthetic processes in chloroplasts. JIP-test and modulated fluorescence (MF) parameters are commonly used chlorophyll *a* fluorescence parameters. This study was conducted to identify quantitative trait loci (QTLs) associated with JIP-test parameters, MF parameters, and photosynthetic rate ($P_N$), and to examine the relationships among them in soybean (*Glycine max* (L.) Merr.). Pot and field experiments were performed to evaluate 184 recombinant inbred lines (RILs) for five JIP-test parameters (ABS/RC, $TR_o$/ABS, $ET_o$/$TR_o$, $RE_o$/$ET_o$, and $PI_{ABS}$), four MF parameters (Fv/Fm, Fv′/Fm′, ΦPSII, and qP), and $P_N$. Significant correlations were commonly observed among JIP-test parameters, MF parameters, and $P_N$. QTL mapping analysis identified 13, 9, and 4 QTLs for JIP-test parameters, MF parameters, and $P_N$, respectively, of which 13 were stable. Four major genomic regions were detected: LG A2 (19.81 cM) for JIP-test parameters, LG C1 (94.31 and 97.61 cM) for $P_N$ and MF parameters, LG M (100.51 cM) for JIP-test and MF parameters, and LG O (30.61–49.91 cM) for $P_N$, JIP-test, and MF parameters. These results indicate that chlorophyll fluorescence parameters, especially ΦPSII and qP, could play an important role in regulating $P_N$, and that JIP-test and MF parameters could be controlled by the same or different genes. The QTLs identified in this study will help in the understanding of the genetic basis of photosynthetic processes in plants. They will also contribute to the development of marker-assisted selection breeding programs for photosynthetic capacity in soybean.

**Keywords** Chlorophyll *a* fluorescence parameters · JIP-test · Photosynthesis · Quantitative trait loci (QTLs) · Recombinant inbred line (RIL) · Soybean (*Glycine max* (L.) Merr.)

Z. Yin and F. Meng contributed equally to this work.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00425-009-1094-0) contains supplementary material, which is available to authorized users.

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## Abbreviations

| | |
|---|---|
| ABS/RC | Light energy absorbed by RC |
| $ET_o$/$TR_o$ | Probability that a trapped exciton moves an electron into the electron transport chain beyond $QA^-$ |
| Fv/Fm | Maximum quantum yield of PSII primary photochemistry in the dark-adapted state |
| Fv′/Fm′ | Maximum quantum yield of PSII primary photochemistry in the light-adapted state |
| JIP-test | Procedure for quantification of OJIP transients |
| LG | Linkage group |
| MF | Modulated fluorescence |

| | |
|---|---|
| NJRIKY | RIL population derived from a cross between soybean varieties Kefeng No.1 and Nannong1138-2 |
| OJIP | Fast chlorophyll *a* fluorescence |
| PFD | Photon flux density |
| $PI_{ABS}$ | Performance index on absorption basis |
| $P_N$ | Photosynthetic rate |
| ΦPSII | Actual quantum yield of PSII primary photochemistry in the light-adapted state |
| qP | Photochemical quenching coefficient |
| QTL | Quantitative trait locus |
| RC | Reaction center of PSII |
| $RE_o/ET_o$ | Probability that an electron beyond $QA^-$ reduces an end acceptor at the PSI electron acceptor site |
| RIL | Recombinant inbred line |
| $TR_o/ABS$ | Flux ratio of trapping per absorption |

## Introduction

Chlorophyll fluorescence analysis has become one of the most powerful techniques in photosynthesis research. Since Kautsky and co-workers first observed changes in the yield of chlorophyll fluorescence, changes in PSII performance of the photosynthetic apparatus have been widely studied by the chlorophyll fluorescence technique. This has mainly been done using fluorometers working on the principle of pulse amplitude modulation (PAM) of the chlorophyll fluorescence emission (Schreiber et al. 1986; Baker and Rosenqvist 2004). Using the PAM-fluorescence technique, many modulated fluorescence (MF) parameters have been derived. These include maximum quantum yield of PSII primary photochemistry in the dark-adapted state (Fv/Fm) and the light-adapted state (Fv′/Fm′), actual quantum yield of PSII primary photochemistry in the light-adapted state (ΦPSII) (Paillotin 1976; Genty et al. 1989), and photochemical quenching coefficient (qP) (Bilger and Schreiber 1986). Fv/Fm is determined in dark-adapted samples to measure the intrinsic maximum efficiency of the photosynthetic apparatus. The other MF parameters are determined in light-adapted samples, to measure electron transport and energy distribution within the photosynthetic apparatus under a light-adapted steady state (Roháček and Barták 1999; Baker and Rosenqvist 2004).

Another measurement method of chlorophyll *a* fluorescence using a continuous excitation fluorometer (PEA, Handy PEA, and Pocket PEA) has been developed (Strasser and Govindjee 1992a, b). This method offers additional information that cannot be obtained by modulated fluorometry. The time course of the chlorophyll fluorescence yield measured by this approach is termed the fast chlorophyll *a* fluorescence (OJIP) transient (Strasser and Govindjee 1992a, b). The procedure developed for quantification of OJIP transients is called the JIP-test (Strasser et al. 1995). Based on the JIP-test, many parameters have been derived. These include light energy absorbed by the reaction center (ABS/RC), flux ratio of trapping per absorption ($TR_o/ABS$) ($\Phi_{Po}$), probability that a trapped exciton moves an electron into the electron transport chain beyond $QA^-$ ($ET_o/TR_o$) ($\Psi_{Eo}$), probability that an electron beyond $QA^-$ reduces an end acceptor at the PSI electron acceptor side ($RE_o/ET_o$) ($\delta_{Ro}$), and performance index on absorption basis ($PI_{ABS}$) (Strasser and Strasser 1995). These JIP-test parameters are determined during state transition of the photosynthetic apparatus from a dark-adapted to a light-adapted state. They reflect the electron transfer and energy distribution within the photosynthetic apparatus during primary photochemistry (Strasser et al. 2004).

For many years, the use of molecular markers has enabled the identification of quantitative trait loci (QTLs) of traits associated with yield, quality and resistance to disease and pathogens (Fu et al. 2006; Cui et al. 2008). QTLs may help in the identification of new genes or development of marker-assisted selection (MAS) for plant breeding. QTL analysis not only provides information on the number and position of loci involved in the expression of a trait, but can also test the relationships between physiological traits (Prioul et al. 1997). The coincidence of QTLs for two traits, with allelic differences corresponding to the expected relationship between the traits, is strong evidence that the two traits are causally related (Thumma et al. 2001). Many studies have investigated the relationships between physiological traits through QTL mapping (Lebreton et al. 1995; Quarrie et al. 1997; Thumma et al. 2001). QTLs for photosynthetic rate ($P_N$) have been identified in many crops, including sunflower (Herve et al. 2001), soybean (Vieira et al. 2006), and rice (Teng et al. 2004). $P_N$ is a final in vivo photosynthetic activity that depends on many traits (parameters). As chlorophyll fluorescence parameters can provide qualitative and quantitative information about photosynthetic processes in chloroplasts (Roháček and Barták 1999; Baker 2008), they might play an important role in determining $P_N$. To date, only a few studies have mapped loci associated with MF parameters (Fracheboud et al. 2002, 2004; Hund et al. 2005; Jompuk et al. 2005). QTLs for JIP-test parameters have not yet been reported.

Mapping QTLs for chlorophyll *a* fluorescence parameters and $P_N$ could provide information on their genetic relationships, identify genes that control them, and improve crop yield by increasing photosynthetic capacity. In the present study, four MF parameters (Fv/Fm, Fv′/Fm′, ΦPSII,

and qP), five JIP-test parameters (ABS/RC, $TR_o$/ABS, $ET_o$/$TR_o$, $RE_o$/$ET_o$, and $PI_{ABS}$), and $P_N$ were measured in a soybean (*Glycine max* (L.) Merr.) recombinant inbred line (RIL) population under multiple environments. The aims of the study were (1) to identify QTLs associated with the five JIP-test parameters, four MF parameters, and $P_N$, and (2) to dissect the relationships among these photosynthetic traits.

## Materials and methods

### Plant materials

The RIL population used to map QTLs was derived from a cross between soybean varieties Kefeng No.1 and Nannong1138-2, and was designated as NJRIKY. This population, which consists of 184 $F_{7:11}$ lines derived via single-seed descent at the National Center for Soybean Improvement of China, has been used for mapping QTLs of several traits (Fu et al. 2006; Cui et al. 2008). RILs and the two parent lines were grown both in pots and in the field.

### Experimental condition

All experiments were conducted under natural irradiance at Jiangpu Experimental Station, Nanjing Agricultural University, Nanjing, China. To control environmental effects on phenotypic evaluation, the RILs were divided into three groups according to their maturity observed from three previous years (data not shown). Each group was sown at a different time in either pot or field experiments, so that when collecting phenotypic data, all RILs were at a similar growth stage.

For pot experiments, plants were individually grown in plastic pots containing 3.0 L soil. The RIL population was grown in a completely randomized design with six replications (each consisting of one plant per pot) during two successive years. Sowing was carried out on 8, 15, and 22 May 2007, and 12, 19, and 26 May 2008. For field experiments, the RIL population was grown in hill plots in a completely randomized design with five replications (each consisting of a hill of three plants), with lines of a similar maturity time planted nearby. Hills were planted every 40 cm along rows spaced 60 cm apart. Sowing was carried out on 12, 19, and 26 June 2008. For the purposes of abbreviation in this study, the environments under which the 2007 and 2008 pot experiments were carried out were designated as E1 and E2, respectively; the environment under which the 2008 field experiment was carried out was designated as E3.

Nutrition and water were supplied sufficiently throughout the experiments to avoid potential nutrition and drought stresses. The average temperatures throughout the experiments of E1, E2, and E3 were 25.8, 25.7, and 26.6°C, respectively. The global radiation levels were 15.0, 16.3, and 15.1 MJ $m^{-2}$ $d^{-1}$, respectively (data adapted from the local meteorological station of Nanjing, China). The average temperatures 14 days before trait measurements (see below) were 29.2°C (E1), 28.2°C (E2), and 24.0°C (E3). The average global radiation levels 14 days before trait measurements were 18.0, 15.3, and 13.9 MJ $m^{-2}$ $day^{-1}$ for the three experiments, respectively.

### Trait measurement

All traits were measured using the upper third leaf at the R6 development stage of soybean plants. $P_N$ and MF parameters were measured in the genotypes grown in E1 and E2, whereas JIP-test parameters were determined in E1, E2, and E3. $P_N$-photon flux density (PFD) curves were determined using the two parents of the NJRIKY population.

$P_N$ was measured using a LI-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA). Two plants from two replications were determined per genotype. All measurements were done in the morning (9:00–11:30) to avoid high afternoon temperatures and air vapor pressure deficit. The PFD within the cuvette was supplemented with a LED lighting system set at 1,200 μmol $m^{-2}$ $s^{-1}$. Leaf temperature was kept at 25°C. The large number of tested materials meant that two sets of LI-6400 portable photosynthesis systems were used. Measurements were carried out on two successive sunny days with similar temperatures and global radiation levels. $P_N$ was measured on 6 and 7 August 2007 (E1), and 6 and 7 August 2008 (E2). Weather conditions for these days are listed in Supplementary Table S1.

$P_N$-PFD curves of the two parents, Kefeng No.1 and Nannong1138-2, were also measured. Steady-state values of $P_N$ were maintained for 5 min at each PFD from 2,000 down to 0 μmol $m^{-2}$ $s^{-1}$, at which point $P_N$ was recorded. Light level was modified using a LED lighting system. Measurements were carried out on three plants for both parents in air (340 μmol $CO_2$ $mol^{-1}$) at room temperature (25°C). The apparent dark respiration rate (Rd), light compensation point (LCP), apparent quantum yield (AQY), maximum photosynthetic rate (Pmax), and light saturation point (LSP) of the two parents were calculated by modeling the response of leaf $P_N$ to PFD by a non-rectangular hyperbola, as described previously (Prioul and Chartier 1977).

MF parameters were measured at 25°C using a PAM fluorometer (PAM2100, Heinz Walz, Effeltrich, Germany). Two plants from two replications per genotype were used. Leaves were cut from the plant and kept in wet gauze for dark adaptation for more than 2 h before measurement.

Initial fluorescence (Fo) in dark-adapted leaves was determined using a low-intensity light (0.1 μmol $m^{-2}$ $s^{-1}$). A 1-s flash of saturating white light (7,500 μmol $m^{-2}$ $s^{-1}$) was given to determine maximal fluorescence (Fm). When steady-state fluorescence (Fs′) was achieved, 5 min after the onset of actinic light (600 μmol $m^{-2}$ $s^{-1}$), a saturating pulse was given to determine the maximal fluorescence (Fm′). The minimal fluorescence (Fo′) was calculated according to the method described previously (Oxborough and Baker 1997; Baker and Rosenqvist 2004). The parameter Fv/Fm was calculated as (Fm − Fo)/Fm, Fv′/Fm′ as (Fm′ − Fo′)/Fm′, qP as (Fm′ − Fs′)/(Fm′ − Fo′), and ΦPSII as (Fm′ − Fs′)/Fm′. Measurements were carried out on four to five successive sunny days with similar temperatures and global radiation levels. MF parameters were measured on 6, 7, 8, 9, and 10 August 2007 (E1), and 5, 6, 7, and 8 August 2008 (E2). Weather conditions for these days are listed in Supplementary Table S1.

OJIP transient measurements were performed using a plant efficiency analyzer (Handy PEA, Hansatech Instruments, UK) on dark-adapted leaves at 25°C. Four plants from four replications per genotype were used for measurement. Leaves were cut from plants and kept in wet gauze for dark adaptation for 30 min before sunrise. OJIP transients were recorded for a period of 1 s at an actinic irradiance of 3,000 μmol $m^{-2}$ $s^{-1}$. The JIP-test parameters of ABS/RC, $TR_o$/ABS, $ET_o$/$TR_o$, $RE_o$/$ET_o$, and $PI_{ABS}$ were derived from the OJIP transients according to Strasser et al. (2004). Measurements were completed within one day. OJIP transients were measured on 6 August 2007 (E1), 7 August 2008 (E2), and 17 September 2008 (E3). Weather conditions for these days are listed in Supplementary Table S1.

### Genetic mapping

A previously described linkage map (Fu et al. 2006) was used for QTL analysis. The map of this RIL population, covering 2,625.9 cM of the soybean genome, converged into 24 linkage groups consisting of 221 simple sequence repeat markers and one *R* gene (resistance to soybean mosaic virus). Most of the linkage groups were consistent with those of Cregan et al. (1999). The average distance between markers was 11.8 cM.

### Statistical analysis and QTL mapping

All phenotypic data were analyzed using SAS V9.0 (for Windows). The means of each RIL were calculated by SAS PROC MEANS, and checked for normality of distribution by SAS PROC UNIVARIATE. Pearson phenotypic correlations between traits were calculated by SAS PROC CORR. Outliers were judged by residual analysis.

QTL detection was performed using a procedure similar to that described by Jompuk et al. (2005). The means of each RIL from each environment were used for QTL analysis. All QTL analyses for individual environments were performed using Cartographer version 2.5 (Wang et al. 2005). Composite interval mapping (CIM) (model 6) was used to map QTLs. The genome was scanned at 2 cM intervals and the window size was set at 30 cM. Cofactors were chosen using the forward and backward regression method at $P(\text{Fin}) = P(\text{Fout}) = 0.05$. A joint analysis of the phenotypic data for all environments was used to determine the QTL by environment (Q × E) interaction (Jiang and Zeng 1995).

For all traits examined in this study, the Log10 of the likelihood odds ratio (LOD) scores for declaring a significant QTL were 2.6–2.8 and 3.2–3.5, for the single-trait and joint QTL analysis, respectively, by permutation test analyses (1,000 permutations, significance level 5%) described previously (Churchill and Doerge 1994). However, to find more putative QTLs and obtain a better understanding of the relationships among traits, LOD scores of 2.0 and 3.0 for declaring a QTL were employed for the single-trait and joint QTL analysis, respectively. Low LOD thresholds may not be useful in plant breeding programs, but they help in understanding the relationships among traits (Thumma et al. 2001). Similar strategies have been used in other reports (Simko et al. 1997; Thumma et al. 2001). For Q × E interaction analysis, LOD thresholds of 1.3 and 0.83 were used for traits examined under three and two environments, respectively (Jompuk et al. 2005).

The maximum LOD score along the interval was taken as the position of the QTL. The region in the LOD score, within one LOD unit of maximum, was taken as the confidence interval. The additive effects of the detected QTL were estimated from CIM results as the mean effect of replacing both Nannong1138-2 alleles at the locus studied by Kefeng No.1 alleles. Thus, for a QTL to have a positive effect, the Kefeng No.1 allele must increase the trait value. The contribution of each identified QTL to the total phenotypic variance ($R^2$) was estimated by variance component analysis. QTL nomenclature was adapted as described previously (Cui et al. 2008), which starts with 'q' followed by an abbreviation of the trait name, the linkage group (LG), and the number of QTLs affecting the trait.

## Results

### Correlation of JIP-test parameters, MF parameters and $P_N$

Table 1 lists the correlations among all traits. $P_N$ was positively correlated with ΦPSII, qP, Fv′/Fm′, $PI_{ABS}$, and

**Table 1** Correlation coefficients and significance of correlations among JIP-test parameters, MF parameters, and PN in the recombinant inbred line (RIL) population under two environments, E1 (the environment for pot experiments in 2007) on the upper right, E2 (the environment for pot experiments in 2008) on the lower left

| Traits | $TR_o/ABS$ | $ET_o/TR_o$ | $RE_o/ET_o$ | ABS/RC | $PI_{ABS}$ | Fv/Fm | Fv′/Fm′ | qP | ΦPSII | $P_N$ |
|---|---|---|---|---|---|---|---|---|---|---|
| $TR_o/ABS$ | | 0.592** | −0.228** | −0.425** | 0.771** | 0.714** | 0.561** | −0.196** | 0.149* | 0.163* |
| $ET_o/TR_o$ | 0.636** | | 0.381** | −0.569** | 0.903** | 0.517** | 0.358** | −0.003 | 0.204** | 0.270** |
| $RE_o/ET_o$ | −0.071 | 0.394** | | −0.346** | 0.236** | −0.158** | −0.133 | 0.199** | 0.094 | 0.126 |
| ABS/RC | −0.509** | −0.556** | −0.175* | | −0.760** | −0.456** | −0.243** | 0.108 | −0.046 | −0.134 |
| $PI_{ABS}$ | 0.787** | 0.915** | 0.251** | −0.769** | | 0.621** | 0.457** | −0.105 | 0.170* | 0.233** |
| Fv/Fm | 0.646** | 0.342** | −0.174* | −0.323** | 0.510** | | 0.772** | −0.296** | 0.187* | 0.126 |
| Fv′/Fm′ | 0.290** | 0.232** | 0.047 | −0.200* | 0.281** | 0.816** | | −0.062 | 0.513** | 0.193** |
| qP | 0.006 | 0.014 | −0.037 | 0.012 | −0.005 | −0.126 | 0.080 | | 0.824** | 0.278** |
| ΦPSII | 0.144 | 0.121 | −0.009 | −0.088 | 0.131 | 0.286** | 0.545** | 0.878** | | 0.349** |
| $P_N$ | 0.132 | 0.205** | 0.115 | −0.168* | 0.205** | 0.115 | 0.174* | 0.409** | 0.431** | |

*$TR_o/ABS$* maximum quantum yield of primary photochemistry, *$ET_o/TR_o$* probability that a trapped exciton moves an electron into the electron transport chain beyond $QA^-$, *$RE_o/ET_o$* probability that an electron beyond $QA^-$ reduces an end acceptor at the PSI electron acceptor site, *ABS/RC* light energy absorbed by RC of photosynthetic apparatus, *$PI_{ABS}$* performance index on absorption basis, *Fv/Fm* maximum quantum yield of PSII primary photochemistry in the dark-adapted state, *Fv′/Fm′* maximum quantum yield of PSII primary photochemistry in the light-adapted state, *qP* photochemical quenching coefficient, *ΦPSII* actual quantum yield in the light-adapted state, *$P_N$* photosynthetic rate

* $P < 0.05$; ** $P < 0.01$

$ET_o/TR_o$ under both environments, and with $TR_o/ABS$ under E1, and negatively with ABS/RC under E2. This suggests that $P_N$ could be increased by increasing ΦPSII, qP, Fv′/Fm′, $PI_{ABS}$, $ET_o/TR_o$, and/or $TR_o/ABS$, and/or decreasing ABS/RC.

ΦPSII showed a significant positive correlation with Fv/Fm, Fv′/Fm′, and qP under both environments, and with $TR_o/ABS$, $ET_o/TR_o$, and $PI_{ABS}$ under E1. Fv′/Fm′ was positively correlated with Fv/Fm, $TR_o/ABS$, $ET_o/TR_o$, and negatively with ABS/RC, and thus resulted in a positive correlation between $PI_{ABS}$ and Fv′/Fm′ under both environments. qP was positively correlated with $RE_o/ET_o$, and negatively with Fv/Fm and $TR_o/ABS$ under E1. Fv/Fm was positively correlated with $TR_o/ABS$, $ET_o/TR_o$, and $PI_{ABS}$, and negatively with $RE_o/ET_o$ and ABS/RC under both environments. The five JIP-test parameters, ABS/RC, $TR_o/ABS$, $ET_o/TR_o$, $RE_o/ET_o$, and $PI_{ABS}$, were significantly correlated with each other under both environments, with the exception of $TR_o/ABS$ and $RE_o/ET_o$ under E2.

## Quantitative variation in RIL families and characterization of the parental lines

Ten traits ($TR_o/ABS$, $ET_o/TR_o$, $RE_o/ET_o$, ABS/RC, $PI_{ABS}$, Fv/Fm, Fv′/Fm′, qP, ΦPSII, and $P_N$) were evaluated for the RIL population and the parent lines. The results of the statistical analysis are shown in Supplemental Table S2. There were significant differences among genotypes and genotype × environment interactions for all traits. The skewness and kurtosis for the distribution of these traits in NJRIKY were less than 1.0 in absolute value. This indicated that all traits approximately fit normal distributions, and that the data were suitable for QTL mapping.

Compared with Nannong1138-2, Kefeng No.1 had significantly higher $TR_o/ABS$, $ET_o/TR_o$, $RE_o/ET_o$, $PI_{ABS}$, Fv/Fm, Fv′/Fm′, qP, ΦPSII, and $P_N$, and lower ABS/RC (Supplemental Table S2). In addition, though the two parent lines had similar Rd, LCP, and AQY, Kefeng No.1 also had an obviously higher Pmax and LSP (Table 2; Fig. 1). These results show that remarkable differences in JIP-test parameters, MF parameters and photosynthetic capacity existed between the two parents, which could therefore provide a good genetic background for the QTL mapping study.

## QTLs for JIP-test parameters, MF parameters, and $P_N$

QTL analysis revealed 26 QTLs for all traits examined, with 13, 9 and 4 QTLs for JIP-test parameters, MF parameters and $P_N$, respectively (Tables 3, 4; Fig. 2). Thirteen of these QTLs were stable across different environments. Each trait was controlled by one to four QTLs dispersed among the LG. QTLs with positive and negative allelic effects were identified; a positive effect implied a higher value for the trait conferred by the Kefeng No.1 allele, and vice versa.

By comparing the detected QTLs (Tables 3, 4; Fig. 2), four major genomic regions with high LOD scores and $R^2$ were identified. First, the marker interval sat_162–AW132402 on LG A2 (19.81 cM) was identified as controlling $TR_o/ABS$, $ET_o/TR_o$, ABS/RC, and $PI_{ABS}$. Nannong1138-2 alleles increased value of all traits except

**Table 2** Simulation parameters of the light response in soybean Nannong1138-2 and Kefeng No.1

| Parents | AQY | Rd (μmol $CO_2$ $m^{-2}$ $s^{-1}$) | LCP (μmol $m^{-2}$ $s^{-1}$) | LSP (μmol $m^{-2}$ $s^{-1}$) | Pmax (μmol $CO_2$ $m^{-2}$ $s^{-1}$) | *K* |
|---|---|---|---|---|---|---|
| Kefeng No.1 | 0.0628 | −2.38 | 37.9 | 420 | 24 | 0.619 |
| Nannong1138-2 | 0.0724 | −2.76 | 38.1 | 321 | 20.5 | 0.284 |

Data are the means calculated from three independent tests

*AQY* apparent quantum yield, *Rd* apparent dark respiration rate, *LCP* 1ight compensation point, *LSP* light saturation point, *Pmax* maximum photosynthetic rate, *K* a convexity or shape parameter representing the ratio of the physical to total resistance to $CO_2$ diffusion into a leaf

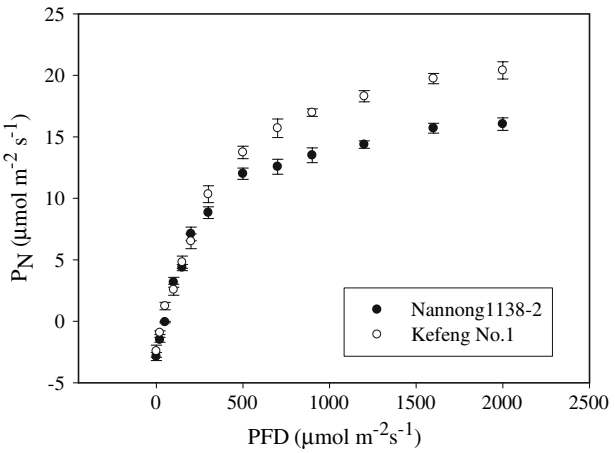


**Fig. 1** Light response curves of leaves from Nannong1138-2 and Kefeng No.1. Steady-state values of photosynthetic rate ($P_N$) were maintained for 5 min at each photon flux density (PFD) from 2,000 down to 0 μmol $m^{-2}$ $s^{-1}$, at which point $P_N$ was recorded

ABS/RC at this locus. Second, within the marker interval sat_274–sat_196 on LG O (30.61–49.91 cM), seven QTLs were detected: *qetO.1* for $ET_o/TR_o$, *qreO.1* for $RE_o/ET_o$, *qabO.1* for ABS/RC, *qpiO.1* for $PI_{ABS}$, *qqpO.1* for qP, *qΦPSO.1* for ΦPSII, and *qP*$_N$*O.1* for $P_N$. At this locus, Kefeng No.1 alleles were positive for all traits except ABS/RC. Third, within the marker interval sat_311–AI794821 on LG C1 (94.31 and 97.61 cM), three QTLs (*qqpC1.1* for qP, *qΦPSC1.1* for ΦPSII, and *qP*$_N$*C1.1* for $P_N$) coincided and had the same direction of additive effect with positive alleles from Nannong1138-2. Finally, the marker interval satt655–satt210 on LG M (100.51 cM) was identified for $TR_o$/ABS, Fv′/Fm′ and Fv/Fm. Kefeng No.1 alleles increased value of all traits at this region.

The LOD scores for the individual environments and the Q × E interaction (Tables 3, 4) indicated that the QTLs on LG O (30.61–49.91 cM) were mainly expressed in the E2 environment, whereas those on the other three genomic regions were almost stable across different environments. Besides these four major loci, additional QTLs were also identified on LG K (11.21 cM) for $TR_o$/ABS, LG K (49.51 and 51.51 cM) for $ET_o/TR_o$ and $PI_{ABS}$, LG A2 (119.01 cM) for $RE_o/ET_o$, LG A1 (90.21 cM), and LG H (4.01 cM) for Fv′/Fm′, LG D2 (8.01 and 14.01 cM) for ΦPSII and $P_N$, and LG E (52.11 cM) for $P_N$.

## Discussion

Of the 10 traits measured in this study, only $P_N$ had previously been used for QTL analysis in soybean. Using a RIL population derived from soybean varieties BARC-8 and Garimpo, Vieira et al. (2006) detected two QTLs for net assimilation rate of $CO_2$. These QTLs were located in intervals satt241–satt345 on LG O and satt215–satt183 on LG J, respectively. In this study, we identified four QTLs for $P_N$: *qP*$_N$*C1.1*, *qP*$_N$*D2.1*, *qP*$_N$*E.1*, and *qP*$_N$*O.1* (Table 4; Fig. 2). Although *qP*$_N$*O.1* was also located on LG O, it should be classified as a new QTL; the marker sat_196, which is closely linked to *qP*$_N$*O.1*, is about 60 cM away from satt345 or satt241 on the consensus linkage map (Song et al. 2004). It seems that the photosynthetic rate alleles in Kefeng No.1 and Nannong1138-2 are different from those in BARC-8 and Garimpo.

Previous studies mapping QTLs for chilling tolerance of photosynthesis-related traits in maize revealed few stable QTLs across environments (Fracheboud et al. 2002, 2004; Jompuk et al. 2005). In this study, however, 13 QTLs (up to half of the total detected QTLs) were stable across environments (Tables 3, 4). This might be due to the similarity in climate conditions between environments in our study. For example, the temperature throughout our experiments ranged from 25.7 to 26.6°C, whereas other studies reported ranges of 13–25°C (Fracheboud et al. 2002, 2004). However, even with such a small difference in climate conditions, the environments still resulted in differential expression of many QTLs, especially of those within the marker interval sat_274–sat_196 on LG O (Tables 3, 4). This clearly shows that JIP-test parameters, MF parameters, and $P_N$ were under different genetic controls in different environments. As pointed out by previous studies (Fracheboud et al. 2002, 2004; Jompuk et al. 2005), this indicates that changes in environments can induce constitutive structural alterations of the photosynthetic apparatus, and ultimately changes in photosynthetic activity.

In this study, although JIP-test parameters, MF parameters, and $P_N$ were measured at the same R6 soybean

**Table 3** Main characteristics of QTLs for JIP-test parameters of soybean under three environments

| Trait | QTL | Linkage group | Marker interval | Position (cM) | Confidence intervals | LOD score | | | | | $R^2$ | | | Add. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | E1 | E2 | E3 | Joint | Q × E | E1 | E2 | E3 | E1 | E2 | E3 |
| $TR_o$/ABS | *qtrA2.1* | A2 | sat_162-AW132402 | 19.81 | 8.2–30.1 | 2.55 | 3.67 | 2.34 | 3.96 | 0.13 | 6.29 | 8.96 | 7.16 | −0.002 | −0.003 | −0.004 |
| | *qtrK.1* | K | satt326–sat_363 | 11.21 | 6.0–21.0 | 3.86 | 1.38 | 1.60 | 4.13 | 0.68 | 10.83 | 2.50 | 1.65 | 0.003 | 0.002 | 0.001 |
| | *qtrM.1* | M | satt655–satt210 | 100.51 | 97.2–112.9 | 2.75 | 4.71 | 3.88 | 6.21 | 0.44 | 6.51 | 11.18 | 8.01 | 0.002 | 0.003 | 0.003 |
| $ET_o/TR_o$ | *qetA2.1* | A2 | sat_162–AW132402 | 19.81 | 13.4–25.1 | 5.12 | 6.37 | 3.23 | 8.97 | 0.49 | 9.83 | 10.70 | 7.28 | −0.009 | −0.010 | −0.009 |
| | *qetK.1* | K | satt273–satt260 | 49.51 | 41.3–61.4 | 0.55 | 3.81 | 3.64 | 5.28 | 1.42 | 1.62 | 8.55 | 8.20 | 0.004 | 0.009 | 0.009 |
| | *qetO.1* | O | satt331– sat_196 | 31.91 | 21.8–44.9 | 2.44 | 3.69 | 0.40 | 4.25 | 0.58 | 4.61 | 5.97 | 0.74 | 0.006 | 0.007 | 0.003 |
| $RE_o/ET_o$ | *qreA2.1* | A2 | sat_232–satt707 | 119.01 | 116.3–132.5 | 4.81 | 6.39 | 5.23 | 8.97 | 0.18 | 9.89 | 13.27 | 11.60 | 0.014 | 0.014 | 0.016 |
| | *qreO.1* | O | sat_274–satt331 | 30.61 | 20.5–43.9 | 3.18 | 1.07 | 0.47 | 3.22 | 0.97 | 12.19 | 2.03 | 0.24 | 0.014 | 0.006 | 0.002 |
| ABS/RC | *qabA2.1* | A2 | sat_162–AW132402 | 19.81 | 10.5–25.1 | 1.77 | 2.66 | 3.64 | 5.09 | 1.43 | 3.53 | 2.90 | 6.71 | 0.026 | 0.026 | 0.064 |
| | *qabO.1* | O | satt331–sat_196 | 39.91 | 19.7–49.4 | 2.70 | 5.63 | 2.78 | 6.00 | 1.77 | 5.59 | 18.43 | 5.00 | −0.032 | −0.064 | −0.054 |
| $PI_{ABS}$ | *qpiA2.1* | A2 | sat_162–AW132402 | 19.81 | 11.1–25.4 | 5.15 | 4.73 | 3.07 | 7.73 | 0.20 | 10.16 | 7.95 | 6.04 | −0.301 | −0.269 | −0.263 |
| | *qpiK.1* | K | satt273–satt260 | 51.51 | 39.1–64.7 | 1.87 | 3.31 | 2.04 | 3.47 | 0.26 | 0.78 | 10.29 | 3.95 | 0.087 | 0.302 | 0.209 |
| | *qpiO.1* | O | satt331– sat_196 | 31.91 | 21.0–48.6 | 1.85 | 3.62 | 1.25 | 3.72 | 0.66 | 2.14 | 6.78 | 1.46 | 0.140 | 0.241 | 0.127 |

*E1* the environment for pot experiments in 2007, *E2* the environment for pot experiments in 2008, *E3* the environment for field experiments in 2008, *LOD (Log10 of the likelihood odds ratio)* the probability associated with the most likely location of the detected QTL, *Marker interval* the interval within which QTLs were mapped, *Position* the position of the peak of the QTL in linkage group, *Confidence intervals* the map interval corresponding to a 1-LOD decline either side of the LOD peak, *Joint* LOD score in the joint analysis of E1, E2, and E3, $Q \times E$ LOD score value for QTL–environment interaction in the joint analysis of E1, E2, and E3, $R^2$ percentage of the phenotypic variance explained by genotype class at LOD peak, *Add.* estimated phenotypic effect of substituting both Nannong1138-2 alleles with Kefeng No.1 alleles at the QTL, $TR_o/ABS$ maximum quantum yield of primary photochemistry, $ET_o/TR_o$ probability that a trapped exciton moves an electron into the electron transport chain beyond $QA^-$, $RE_o/ET_o$ probability that an electron beyond $QA^-$ reduces an end acceptor at the PSI electron acceptor site, *ABS/RC* light energy absorbed by RC of photosynthetic apparatus, $PI_{ABS}$ performance index on absorption basis

**Table 4** Main characteristics of QTLs for modulated fluorescence (MF) parameters and photosynthetic rate ($P_N$) under two environments

| Trait | QTL | Linkage group | Marker interval | Position (cM) | Confidence intervals | LOD score | | | | $R^2$ | | Add. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | E1 | E2 | Joint | Q × E | E1 | E2 | E1 | E2 |
| Fv/Fm | *qfvM.1* | M | satt655–satt210 | 100.51 | 96.4–112.0 | 4.86 | 2.70 | 4.98 | 0.55 | 9.91 | 5.05 | 0.005 | 0.003 |
| Fv′/Fm′ | *qfv′A1.1* | A1 | satt300–satt276 | 90.21 | 76.5–92.5 | 0.54 | 2.77 | 3.76 | 1.96 | 2.10 | 10.03 | 0.004 | 0.008 |
| | *qfv′H.1* | H | satt434–sat_218 | 4.01 | 0.0–9.1 | 2.11 | 3.73 | 4.22 | 0.72 | 5.77 | 11.49 | 0.006 | 0.009 |
| | *qfv′M.1* | M | satt655–satt210 | 100.51 | 95.9–118.0 | 3.28 | 1.16 | 3.29 | 0.56 | 7.94 | 2.22 | 0.007 | 0.004 |
| qP | *qqpC1.1* | C1 | sct_191–AI794821 | 97.61 | 92.1–104.1 | 9.20 | 7.17 | 11.52 | 0.18 | 23.99 | 13.74 | −0.018 | −0.014 |
| | *qqpO.1* | O | satt331–sat_196 | 31.91 | 20.1–47.7 | 1.06 | 4.06 | 5.27 | 2.79 | 5.29 | 8.67 | 0.008 | 0.011 |
| ΦPSII | *qΦPSC1.1* | C1 | sct_191– AI794821 | 97.61 | 90.1–105.1 | 4.21 | 5.90 | 6.06 | 0.04 | 9.43 | 11.27 | −0.009 | −0.010 |
| | *qΦPSD2.1* | D2 | sat_296–sat_277 | 8.01 | 0.0–18.1 | 3.57 | 2.81 | 4.32 | 0.61 | 9.64 | 2.58 | −0.010 | −0.005 |
| | *qΦPSO.1* | O | satt331–sat_196 | 31.91 | 17.4–51.1 | 0.30 | 3.29 | 3.43 | 1.25 | 0.54 | 8.12 | 0.002 | 0.008 |
| $P_N$ | *$qP_N$C1.1* | C1 | sat_311–sct_191 | 94.31 | 88.4–104.4 | 3.74 | 3.58 | 3.95 | 0.65 | 6.98 | 6.69 | −0.897 | −0.811 |
| | *$qP_N$D2.1* | D2 | sat_296– sat_277 | 14.01 | 0.0–30.4 | 2.73 | 2.71 | 3.11 | 0.02 | 9.35 | 6.67 | −1.032 | −0.779 |
| | *$qP_N$E.1* | E | satt268–sat_331 | 52.11 | 44.0–63.6 | 4.17 | 0.14 | 4.92 | 3.33 | 12.17 | 0.35 | 1.186 | 0.193 |
| | *$qP_N$O.1* | O | satt331–sat_196 | 49.91 | 44.1–56.0 | 0.13 | 6.35 | 7.94 | 1.75 | 0.95 | 12.37 | 0.328 | 1.097 |

*E1* the environment for pot experiments in 2007, *E2* the environment for pot experiments in 2008, *LOD (Log10 of the likelihood odds ratio)* the probability associated with the most likely location of the detected QTL, *Marker interval* the interval within which QTLs were mapped, *Position* the position of the peak of the QTL in linkage group, *Confidence intervals* the map interval corresponding to a 1-LOD decline either side of the LOD peak, *Joint* LOD score in the joint analysis of E1and E2, *Q* × *E* LOD score value for QTL–environment interaction in the joint analysis of E1 and E2, $R^2$ percentage of the phenotypic variance explained by genotype class at LOD peak, *Add.* estimated phenotypic effect of substituting both Nannong1138-2 alleles with Kefeng No.1 alleles at the QTL, *Fv/Fm* maximum quantum yield of PSII primary photochemistry in the dark-adapted state, *Fv′/Fm′* maximum quantum yield of PSII primary photochemistry in the light-adapted state, *qP* photochemical quenching coefficient, *ΦPSII* actual quantum yield in the light-adapted state, $P_N$ photosynthetic rate

development stage, they were not measured at the same time, or using identical plants, or using the same number of replications. This might reduce the number of common QTLs detected for these traits, and thus the power to examine the relationships among different traits. However, the two parameters Fv/Fm and $TR_o$/ABS, determined by two different chlorophyll fluorescence techniques, should be equal for a certain sample (Strasser et al. 2004). These two parameters were significantly correlated ($r = 0.741^{**}$ and $0.646^{*}$ under E1 and E2) (Table 1), and had a common significant QTL on LG M (100.51 cM) across different environments (Tables 3, 4). This indicates that the genetic relationships among different traits could be appropriately deduced from the data collected in our experiments.

$P_N$ had three common QTLs with chlorophyll *a* fluorescence parameters: one on LG C1 with ΦPSII and qP under E1 and E2; another on LG O with $ET_o/TR_o$, ABS/RC, $PI_{ABS}$, qP, and ΦPSII under E2; and the last on LG D2 with ΦPSII under E1 and E2. All common QTLs showed the expected additive direction predicted by correlations using phenotypic data (Tables 1, 3, 4). For example, $P_N$ was positively correlated with ΦPSII, and all common QTLs between the two traits showed the same additive direction. This suggests that the parameters ΦPSII, qP, $ET_o/TR_o$, ABS/RC, and $PI_{ABS}$, play an important role in determining $P_N$ in the NJRIKY population. Compared with other parameters, ΦPSII and qP had QTLs in common with $P_N$ at more loci, and under more environments. ΦPSII coincided with $P_N$ at all three loci and under both environments, whereas qP coincided with $P_N$ at two loci and under both environments. This suggests that these two parameters were more important than other parameters in affecting $P_N$. Since numerous traits (parameters) could impact on $P_N$, the relationships between $P_N$ and chlorophyll fluorescence parameters are expected to be complex and variable. ΦPSII might not have a linear relationship with $P_N$ as a function of changes in photorespiration. However, a close association between $P_N$ and chlorophyll fluorescence parameters, especially ΦPSII and qP, was observed in this study through both QTL mapping and correlation analysis. This might indicate that genotypic differences for photorespiration might not exist in the NJRIKY population.

According to Paillotin (1976) and Genty et al. (1989), ΦPSII is calculated by the formula: ΦPSII = Fv′/Fm′ × qP. Consequently, ΦPSII showed a significantly positive correlation with Fv′/Fm′ and qP (Table 1); it also coincided with qP on LG C1 under both E1 and E2, and on LG O under E2, respectively. Both QTLs had the same additive direction (Table 4). Unexpectedly, no common QTLs were found between ΦPSII and Fv′/Fm′. This suggests that genotypic differences in qP, but not Fv′/Fm′, may explain the genotypic difference in ΦPSII within the NJRIKY population. The mathematical relationships among JIP-test parameters were also confirmed in this study. $PI_{ABS}$ was

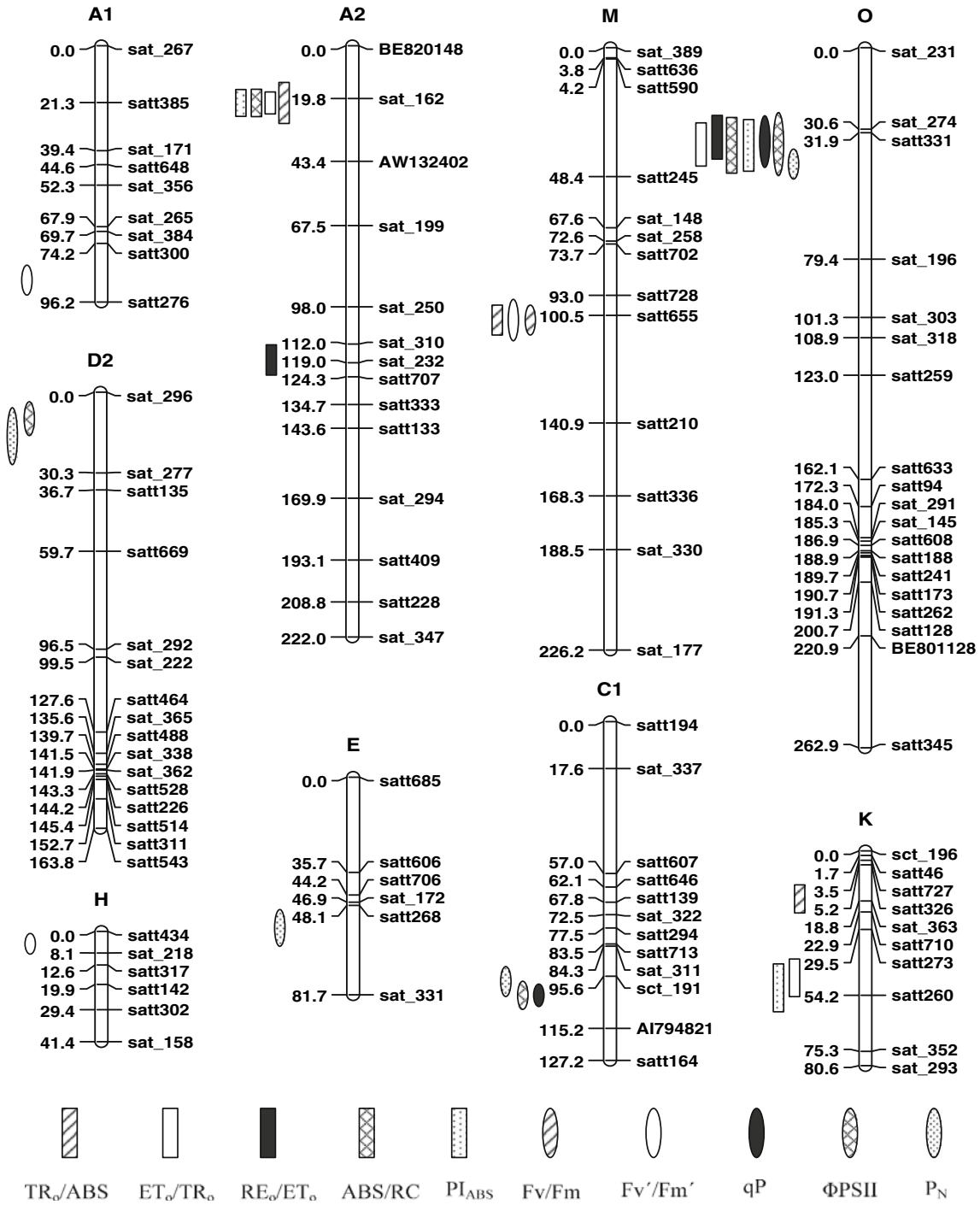


**Fig. 2** Summary of QTL locations detected in this study. *Markers* are shown to the right of the linkage groups (chromosomes) and cumulative distances in centimorgans on the left, as presented by Fu et al. (2006). QTLs, represented by *bars* or *ovals*, are shown to the left of the chromosomes, close to their corresponding markers. The *lengths of the bars* (*ovals*) are proportional to the confidence interval of corresponding QTL as shown in Tables 3, 4

combined from three independent parameters, ABS/RC, $TR_o$/ABS ($\Phi_{Po}$), and $ET_o/TR_o$ ($\Psi_{Eo}$), by the formula: $PI_{ABS} = (RC/ABS) \times [\Phi_{Po}/(1 - \Phi_{Po})] \times [\Psi_{Eo}/(1 - \Psi_{Eo})]$ (Strasser et al. 2004). $PI_{ABS}$ coincided with ABS/RC, $TR_o$/ABS, and/or $ET_o/TR_o$ at three genomic regions: LG A2 (19.81 cM), LG K (49.51 and 51.51 cM) and LG O (30.61–39.91 cM). All involved QTLs showed the expected additive direction (Tables 2, 3). Compared with the other two parameters, $ET_o/TR_o$ coincided with $PI_{ABS}$ at more loci and under more environments, suggesting that

$ET_o/TR_o$ was more important in determining $PI_{ABS}$ in the NJRIKY population.

JIP-test parameters (we refer to Fv/Fm, though determined by PAM technology, as a JIP-test parameter herein, since it theoretically equals the JIP-test parameter $TR_o$/ABS) were measured during a 1 s or less exposure of a dark-adapted leaf to light. During this time, the steady state of photosynthesis was not achieved; therefore, the JIP-test parameters might mainly reflect the intrinsic function and structure of the photosynthetic apparatus during primary photochemistry reactions (Strasser et al. 1995, 2004). In contrast, MF parameters were measured when the light-adapted steady state was reached, where both light and dark reactions of photosynthesis play a role. Therefore, MF parameters depend not only on the intrinsic features of the photosynthetic apparatus, but also on many biochemical or physiological processes associated with light beyond the primary photochemistry reactions (Maxwell and Johnson 2000; Li et al. 2007). In this study, we detected QTL regions in common between JIP-test parameters and MF parameters on LG O (30.61–49.91 cM) and LG M (100.51 cM). We also detected a region on LG A2 (19.81 cM) for JIP-test parameters only, and a region on LG C1 (94.31 and 97.61 cM) for MF parameters only (Tables 3, 4; Fig. 2). This indicates that JIP-test and MF parameters could be controlled by the same or different genes. The genomic regions associated with JIP-test parameters (including the common regions of JIP-test and MF parameters) may contain genes underlying the intrinsic features of the photosynthetic apparatus. This could affect the value of JIP-test and/or MF parameters. However, within the regions associated only with MF parameters, genes involved in biochemical or physiological processes beyond the primary photochemistry reactions may exist. This could change the value of MF parameters, but not JIP-test parameters.

The major QTL region on LG M (100.51 cM) was identified for $TR_o$/ABS (Fv/Fm) and Fv′/Fm′ (Table 4; Fig. 2). As these two parameters measure the maximum quantum yield of the photosynthetic apparatus in a dark-adapted and a light-adapted state, respectively, we assume that this region might contain a gene encoding the intrinsic maximum efficiency of the photosynthetic apparatus RC. Another major common QTL region between JIP-test and MF parameters was located on LG O (30.61–49.91 cM) (Tables 3, 4; Fig. 2). A favorable allele at this locus would result in an increase of $ET_o/TR_o$, $RE_o/ET_o$, and qP, and a decrease in ABS/RC, which could consequently increase $PI_{ABS}$, ΦPSII, and ultimately $P_N$. The parameter $ET_o/TR_o$ represents the fraction of open RCs at the J point of the OJIP transient; and $RE_o/ET_o$ the ratio of open RCs at the I and J points of the OJIP transient (Strasser et al. 1995, 2004), while the parameter qP monitors the fraction of open RCs in the light-adapted state (Havaux et al. 1991).Therefore, a gene responsible for regulating the fraction of open RCs might be present at this major QTL region.

A major region on LG C1 (94.31 and 97.61 cM) was detected for qP, ΦPSII and $P_N$ (Table 4; Fig. 2), where one would expect that a gene encoding key enzymes of photosynthesis-related biochemical processes, which could regulate these three traits simultaneously, might exist. Another major QTL region, on LG A2 (19.81 cM), was identified for $TR_o$/ABS, $ET_o/TR_o$, ABS/RC, and $PI_{ABS}$ (Table 3; Fig. 2). It has been known that the parameters $TR_o$/ABS, $ET_o/TR_o$, and ABS/RC represent three independent energy fluxes (Strasser et al. 1995, 2004). The coincidence of QTLs suggests that the underlying gene could have a pleiotropic effect. Therefore, changes in the structure of the photosynthetic apparatus resulting from this underlying gene could indeed have an effect on these three parameters, and consequently the $PI_{ABS}$.

QTL pyramiding is the process of assembling several QTLs from different loci for a specific trait to produce superior genotypes (Xu 1997). A similar way of improving chlorophyll fluorescence parameters, and therefore photosynthesis, by MAS under drought conditions has been discussed (Guo et al. 2008). In this study, 13 QTLs were shown to be stable across different environments (Tables 3, 4). These QTLs might be useful for the improvement of photosynthetic capacity in soybean, an aim to which considerable attention should be given.

**Acknowledgments** This work was supported in part by National High-Tech Research Programs (2006AA10Z1C1), National Natural Science Foundation (30771362), National Basic Research Programs (2004CB117206, 2009CB118400), and the 111 Project from the Ministry of Education of China (B08025). We thank two anonymous reviewers for their critical and highly appreciated comments on this manuscript.

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