Significant associations between single nucleotide polymorphisms and photosynthetic parameters and grain yield in maize

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ABSTRACT

Photosynthesis is the basis of maize (Zea mays L.) grain formation. To further understand the genetic basis of maize photosynthetic parameters and clarify the relationship between maize photosynthetic parameters and grain yield (GY), identifying quantitative trait loci (QTLs) underlying photosynthetic parameters and GY plays an important role in improving maize yield. In this study, a set of 260 maize accessions from different geographic origins were evaluated across three developmental stages in 2 yr to identify QTLs for photosynthetic parameters and grain yield using 2824 single-nucleotide polymorphisms (SNPs) via genome-wide association analysis. The analysis revealed that maize photosynthetic parameters are substantially correlated with GY at different developmental stages. Fourteen SNPs associated with photosynthetic parameters and GY were detected at the threshold of $P \leq 0.001$ in 2 yr as well as over years. Moreover, PZE-102116144 and SYN35048 associated with stomatal conductance (gs) and PZE-101152541 associated with photosynthetic rate (Pn), were identified at different developmental stages. Four loci were co-associated with two or more traits, such as PZE-110019199 with gs and GY, PZE-102116144 with GY, Pn, (at 25 and 35 d after pollination [DAP]), PZE-102116144 with gs (at 15 and 25 DAP), and PZE-109061997 with intercellular CO$_2$ concentration (C$_i$) at 25 DAP and transpiration rate (Tr) at 25 DAP, PZE-110019199 with gs at 25 DAP and GY. Based on functional annotations, two genes were considered as potential candidate genes for the identified SNPs (PZE-101152541 and PZE-109061997). The SNPs and candidate genes identified in this study might provide instrumental information for understanding the genetic mechanism of maize photosynthetic parameters and yield.

Key words: Photosynthetic parameters, SNPs, yield, Zea mays.

INTRODUCTION

Maize (Zea mays L.) is one of the world’s most important crops, serving as an essential source of food, feed, and fuel. With rapidly increasing global demands for food, bio-fuel and livestock feed, the demand for maize is increasing (Cassman and Liska, 2007; Grassini et al., 2011). Improving maize yield is the major concern of plant breeders. Photosynthesis is the primary process for crops to form grain yield. Grain yield increase in modern maize lines mainly depended on the improved chloroplast structure and more light energy caught for the photochemical reaction, thus having stronger photosynthetic capacity (Li et al., 2015). Many breeders believed that the yield increase of maize hybrids is largely attributable to the improvement of photosynthetic physiological characteristics (Chen et al., 2013). The maize yield increase largely depends on the improvement of photosynthetic characteristics in parental inbred lines (Li et al., 2013). Because photosynthesis is the basis of maize grain yield, boosting leaf photosynthesis is an important strategy to increase yield potential and biomass production (Chen et al., 2013; Li et al., 2013; Ding et al., 2014).

Many studies focused on the improvement of various crop species have revealed that photosynthetic rate (Pn) influences grain yield (GY) (Hubbart et al., 2007; Zheng et al., 2011; Peng et al., 2012). Measurements of photosynthetic gas-exchange parameters, such as Pn, stomatal conductance (gs), transpiration rate (Tr), and intercellular CO$_2$ concentration (C$_i$), are useful to assess photosynthetic capacity and gain insight into the behavior of photosynthetic machinery (Feng et al., 2009; Khan et al., 2010; Yin et al., 2010; Song et al., 2012; Yu et al., 2015; Zhong et al., 2015; Hao et al., 2017). Significant differences in photosynthetic gas-exchange parameters have been uncovered among maize varieties. A significantly positive correlation between Pn and the yields per plant has been observed in adzuki bean cultivars from the jointing to the maturing stages (Song et al., 2012), which suggests that Pn is a potential selection marker to assess their cultivar performances. Photosynthetic parameters, such gs and Tr, have an effect on crop GY under stressed and non-stressed conditions (Khazaei et al., 2010).

Genetic variations in maize photosynthesis parameters have been reported, but little is known about the genetic basis behind the traits. The development of genomics has provided alternative tools to improve selection efficiency in crop breeding programs. Molecular markers could be used to improve the efficiency and precision of crop breeding via marker-assisted selection...
to identify QTLs associated with photosynthetic parameters in inbred lines with extensive genetic variation was evaluated. In the present study, a set of 260 elite maize QTLs is stable in different environments and genetic backgrounds. Marker-assisted selection (MAS) within breeding programs, especially if the identified QTLs provide suitable information for marker-assisted selection, would improve control of both type I and type II error rates. The unified mixed model approach (MLM) is a powerful strategy, and it can account for multiple levels of relatedness simultaneously and improve control of both type I and type II error rates. The unified mixed model approach (MLM) is a powerful strategy, and it can account for multiple levels of relatedness simultaneously and improve control of both type I and type II error rates. The unified mixed model approach (MLM) is a powerful strategy, and it can account for multiple levels of relatedness simultaneously and improve control of both type I and type II error rates.

With the increased availability of genomic polymorphism data, genome-wide association studies (GWASs) based on linkage disequilibrium have become a powerful approach for dissecting quantitative traits in crops (Stich and Melchinger, 2010; Yan et al., 2011; Segura et al., 2012). A GWAS is used to exploit all recombination events that occur during the evolutionary history of a natural population (Zhu et al., 2008). Marker-trait association enables researchers to exploit natural diversity and locate valuable genes in the genome (Rafalski, 2010). The main constraints on the use of the GWAS approach are genetic relatedness and population structure, which can cause spurious marker-trait associations (Chan et al., 2011; Hao et al., 2012b). Several statistical methods have been used to account for the population structure and relatedness. The unified mixed model approach (MLM) is a powerful strategy, and it can account for multiple levels of relatedness simultaneously and improve control of both type I and type II error rates.

QTL mapping for maize photosynthetic parameters has thus far been mainly based on linkage analysis. The QTLs that have currently been detected are population specific, with few QTLs consistent across various populations. A better understanding of the genetic basis of photosynthetic parameters in different maize germplasm resources would provide suitable information for marker-assisted selection (MAS) within breeding programs, especially if the identified QTLs are stable in different environments and genetic backgrounds. In the present study, a set of 260 elite maize inbred lines with extensive genetic variation was evaluated to identify QTLs associated with photosynthetic parameters and Gy in multiple environments through a GWAS.

**MATERIALS AND METHODS**

**Plant materials and plant growth conditions**

An association mapping panel comprising 260 elite maize inbred lines (150 common maize and 110 waxy maize) was used for this study (Table 1). The trials were performed in 2014 (designated as environment E1) and 2015 (designated as environment E2) at the Experimental Farm of the Jiangsu Yanjiang Institute of Agricultural Sciences (31°58’48” N, 120°53’24” E), Nantong, China. To enable comparisons at similar growth stages, the 260 maize inbred lines were sown at different times in three groups according to their projected silking times (Table 1). All lines were arranged in a randomized complete block design with two replicates. Each line in a plot was planted in two 6-m long rows separated by 60 cm, with seeds in each row spaced 30 cm apart for a total of 20 plants per row. In order to control the border effect, the association mapping panel was surrounded by protective belt. To avoid potential nutrient and drought stresses, optimal nutrition and water were supplied throughout the whole life cycle.

All of the above experiments were conducted under natural irradiance. Daily relative humidity and daily mean temperatures throughout the growing season were based on data from the local meteorological station in Nantong, China. Mean daily relative humidities throughout the 2014 and 2015 growing seasons were 71.8% and 69.3%, respectively, with corresponding average daily temperatures of 24.7 and 23.5 °C.

**Phenotypic data collection**

Gas exchange parameters (Pn, gs, Tr, and Ci) were determined on sunny days from 09:00 to 11:30 h and 14:00 to 16:00 h with a portable photosynthesis system (LI-6400, LI-COR, Lincoln, Nebraska, USA). The air temperature of the leaf chamber was maintained at 30 °C and the photon flux density was set to 1200 μmol m−2 s−1. The CO2 concentration of the air in the chamber was controlled by the LI-COR CO2 injection system, while light used for the measurements was supplied by the LI-COR LED light source. Ear leaves of four uniform plants in the middle of each plot were measured for photosynthetic parameters at 15, 25 and 35 d after pollination (DAP). Because of the large number of tested materials, two portable photosynthesis system units were used in this study.

Grain yield was estimated from 10 consistently growing plants in the middle of each plot. Ears of corresponding plants were manually harvested at maturity, dried to constant weight and threshed.

**DNA extraction and single nucleotide polymorphism (SNP) genotyping**

Genomic DNA from maize seedling leaves was extracted using the CTAB method (Hao et al., 2012a). All 260 maize inbred lines were genotyped for 3072 SNPs via the GoldenGate assay platform (Illumina, San Diego, California, USA) at the National Maize Improvement Center of China, China Agricultural University (Beijing, China) as described by Hao et al. (2015). These 3072 SNP markers have been applied to identify QTLs for resistance to *Aspergillus flavus* in a recombinant inbred line population in maize (Yin et al., 2014). Based on the results of a genetic
Table 1. Elite maize inbred lines (150 common maize and 110 waxy maize) used for this study.

| Nr | Inbred line | Origin/Pedigree | Group |
|----|-------------|-----------------|-------|
| 1  | 4377        | (7S×YJ7)×YJ7     | I     |
| 2  | 437         | 7S×YJ7          | I     |
| 3  | T458        | Derived from Ye478 | I     |
| 4  | T750        | 8112×YJ7        | I     |
| 5  | 4S          | Derived from Huangzaosi     | I     |
| 6  | T877        | (YJ7×E28)×YJ7   | I     |
| 7  | S951        | (8711×8709)×90SEY | I     |
| 8  | T72         | (Y9×Huangzaosi)×8901 | I     |
| 9  | ND1145      | Recycled line from hybrid 78599 | I     |
| 10 | Q8319       | Recycled line from hybrid 78599 | I     |
| 11 | T249        | 5UX63C1         | I     |
| 12 | CA375       | Derived from 13QPMCO | I     |
| 13 | Dan598      | Derived from Mixed Population of Dan340 and P78599 | I     |
| 14 | HDL40       | Derived from synthetic variety | I     |
| 15 | Shen137     | Derived from 6KJ11 | I     |
| 16 | 4AYC        | 4S×YC           | I     |
| 17 | Su195       | 2163×Luyuan92   | I     |
| 18 | Wu314       | (302×HBL)×Huangzaosi | I     |
| 19 | U8112       | Derived from U8  | I     |
| 20 | F2          | Derived from Ye478 | I     |
| 21 | X19M        | Derived from mutant of Ye478 | I     |
| 22 | Zheng58     | Derived from Ye478 | I     |
| 23 | H991        | Ye478×S5003     | II    |
| 24 | N18         | Maize inbred line from 78599 | II    |
| 25 | N19         | Maize inbred line of N18 from USA | II    |
| 26 | N3          | Maize inbred line of N3 from USA | II    |
| 27 | N21         | Maize inbred line of N21 from USA | II    |
| 28 | Dan340      | Baihuazhu-9wv1d maize | II    |
| 29 | K22         | K11×Ye478       | II    |
| 30 | T803        | 8112×S5003      | II    |
| 31 | C8605       | 792×S5003       | II    |
| 32 | Ye478       | 8112×S5003      | II    |
| 33 | SMLYC       | SML×YC          | II    |
| 34 | 7922        | Recycled line from hybrid 3382 | II    |
| 35 | DH02        | Derived from Mixed Population of L178 and L199 | II    |
| 36 | Mo17        | 872×C72         | II    |
| 37 | ZG30        | OH4×Xei167      | II    |
| 38 | DHG5232     | 623×S5003       | II    |
| 39 | 8723        | Unknown         | II    |
| 40 | Xun92-2     | C7×2×C7H       | II    |
| 41 | 9801        | 502×H21         | II    |
| 42 | Y85         | 515×P78599      | II    |
| 43 | YJ7         | Recycled line from hybrid 78599 | II    |
| 44 | Tong3       | Derived from Z1330 Synthetic | II    |
| 45 | Tong31      | Derived from Z1330 Synthetic | II    |
| 46 | YHC         | (HX162×330)c×fluxpeno | II    |
| 47 | P178        | Recycled line from hybrid 78599 | II    |
| 48 | Zhong128    | 211×X2×Zhongzi7490 | II    |
| 49 | P138        | Recycled line from hybrid 78599 | II    |
| 50 | Q1261       | Q16×61          | II    |
| 51 | K12         | Huangzaosi×Weicunhuang | II    |
| 52 | ZGF         | Derived from Synthetic Population | II    |
| 53 | ZGF         | Z2×5F           | II    |
| 54 | Huangzaosi  | Tangspintgou    | II    |
| 55 | Luyuan92    | Yuanql1×133×1137 | II    |
| 56 | Ye107       | Derived from XL80 | II    |
| 57 | DH13M       | 792×C72         | II    |
| 58 | JHM         | Derived from Jinhai muban | II    |
| 59 | JHF         | Derived from Jinhai fuban | II    |
| 60 | 225M        | Z2×5F           | II    |
| 61 | J-2         | Recycled line from hybrid 78599 | II    |
| 62 | ‘66         | 8112×S5003      | II    |
| 63 | 586G        | 8112×S5003      | II    |
| 64 | L162        | 8112×75         | II    |
| 65 | Zheng37     | (138×Zheng22)×Ye52×506 | II    |
| 66 | DD10        | Derived from Liaoning | II    |
| 67 | JHS3        | Huangzaosi×Z330 | II    |
| 68 | T1003       | 84×X4S          | II    |
| 69 | T1002       | Recycled line from Xianyu | II    |
| 70 | T1004       | 4S×Zheng38      | II    |
| 71 | T1005       | Q3×X7           | II    |
| 72 | T1006       | Q3×X7×S5003     | II    |
| 73 | T1007       | Shen137×4S      | II    |
diversity analysis, 2824 SNPs with minor allele frequencies \( \geq 5\% \) and missing data \( \leq 20\% \) in the present population were used for subsequent analysis.

**Population structure and kinship analysis**

Population structure based on the 2824 SNPs was inferred using the software program STRUCTURE 2.3 (Hubisz et al., 2009). Five independent Markov chain Monte Carlo runs of 100 000 iterations (after discarding the burn-in) were performed for each hypothetical number of subpopulations \( k \) ranging from 1 to 10. To estimate the most likely number of subpopulations present, the number of subgroups \( (\Delta k) \) was maximized according to Hao et al. (2015). STRUCTURE HARVESTER (http://taylor0.biology.ucla.edu/structureHarvester/) (Earl and von Holdt, 2012) was used to visualize the STRUCTURE output and implement the Evanno method. A membership value \( (Q \text{ value}) > 0.5 \) was used as a criterion to assign each maize inbred line to a specific subgroup.
into a subpopulation. A population structure matrix (Q) was generated for further analysis.

A relative kinship matrix (K) comparing all pairs of the 260 maize accessions was calculated using the software program SPAGeDi (Hao et al., 2015), with the negative value of kinship set as zero.

Statistical and association analyses
Statistical analyses were performed using SAS 9.1 (SAS Institute, Cary, North Carolina, USA). ANOVA was performed using SAS PROC GLM. Mean values of phenotypic traits were calculated using SAS PROC MEANS. Regression coefficients and Pearson phenotypic correlations among traits were calculated using SAS PROC REG and PROC CORR, respectively. To minimize the effects of environmental factors in subsequent analyses, the best linear unbiased predictions (BLUPs) for the tested traits over years in each line were estimated using PROC MIXED (Hao et al., 2015).

To identify SNPs associated with the studied traits, an association analysis was performed using a Q+K mixed linear model (MLM) in TASSEL 4.0. Based on a threshold of \(-\log P \geq 3.00 (P \leq 0.001)\), SNPs significantly associated with phenotypic traits were identified.

To identify the best alleles of significantly associated SNP markers, average phenotypic values of the corresponding alleles were measured based on the BLUPs of the phenotypic values of each trait in the two years. The trait value for each tested maize line was the mean BLUP value calculated from observations of each line in the two years.

RESULTS

Phenotypic variations and heritability of photosynthetic parameters and GY
BLUPs, relevant statistical parameters, and broad-sense heritabilities for all five studied traits are shown in Table 2. Extensive phenotypic variations were observed among different environments (Table 2). ANOVA revealed that variances of genotypes, environments, and interactions between genotypes and environments (G×E) were highly significant at the \(P \leq 0.01\) level for all five traits (Table 2). Values of photosynthetic parameters at 25 DAP were significantly higher than at 15 DAP and 35 DAP during 2 yr. The heritability of photosynthetic parameters and GY varied across different growth stages. Except for \(C_i\), the heritabilities of photosynthetic parameters at 25 DAP were higher than those at 15 DAP and 35 DAP.

Phenotypic correlation analysis
Correlation coefficients based on the BLUP model analysis are summarized in Table 3. In all cases, correlation coefficients were significant or highly significant in different stages. GY was highly positively correlated with \(P_N\), \(C_i\), \(g_s\), and \(T_r\) at 15, 25 and 35 DAP, which suggests that photosynthetic capacity influences GY during the grain-filling process. \(P_N\), \(g_s\), and GY were highly positively correlated during the three developmental stages, with correlation coefficients ranging from 0.535 (between GY and \(g_s\) at 15 DAP) to 0.817 (between \(P_N\) and \(g_s\) at 25 DAP).

In most cases, correlation coefficients at 25 DAP were higher than those at 15 and 35 DAP.

Population structure analysis and GWAS
A Bayesian model-based method as implemented in STRUCTURE was used to infer population structure and assign individuals to subpopulations. As \(k\) increased from 1 to 10, the \(\ln (P(D))\) value corresponding to each hypothetical \(k\) increased, with no peaks evident (data not shown). As shown in Figure 1, the likelihood of \(\Delta k\) was much higher at \(k = 2\) than at \(k = 3-10\) (Figure 1), which suggests that the population could be clustered into two major subpopulations. The corresponding Q-matrix (at \(k = 2\)) was generated for the subsequent GWAS.

SNP markers associated with the five studied traits were identified using a MLM (Q+K) in TASSEL 4.0. A total of 34 marker-trait associations involving 23 SNPs were associated with maize photosynthetic parameters and yield in different years and over years at a threshold level of \(P \leq 0.001\) \((-\log P \geq 3)\) (Figure 2). Among the significant marker-trait associations, 23 marker-trait associations representing 14 SNPs remained significant in 2 yr and over years at the threshold of \(P \leq 0.001\), these SNPs were distributed among 8 of chromosomes, excluding chromosome 3 and 8 (Table 4). Of these 14 SNPs, 4 were associated with \(P_N\), 4 with \(C_i\), 5 with \(g_s\), 3 with \(T_r\) and 3 with GY. PZE-1011152541 associated with \(P_N\) was identified at 25 and 35 DAP. PZE-102116144 associated with \(g_s\) was identified at 15 and 25 DAP, and SYN35048 associated with \(g_s\) was identified at 15, 25 and 35 DAP.

Four loci were co-associated with two or three traits. PZE-1011152541 on chromosome 1 was co-associated with \(P_N\) (25 and 35 DAP) and GY. PZE-102116144 on chromosome 2 was co-associated with \(P_N\) (25 DAP), \(g_s\) (15 and 25 DAP) and GY. PZE-109061997 on chromosome 9 was co-associated with \(T_r\) (25 and 35 DAP) and \(C_i\) (25 DAP), and PZE-110019199 on chromosome 10 was co-associated with \(g_s\) (25 DAP) and GY.

Identification of candidate genes
Among the 34 significant associations \((-\log P \geq 3)\) we identified, a total of 14 SNPs were associated with 9 genes (Table 3). According to the maize gene annotation database at MaizeGDB (http://www.maizegdb.org), the putative genes, where the associated SNPs located, indicated that genes of GRMZM2G317770 on chromosome 1 and GRMZM2G002227 on chromosome 9 were the most likely candidate genes for PZE-1011152541 (associated with \(P_N\) at 25 and 35 DAP and GY) and PZE-109016787 (associated with \(g_s\) at 15), which are candidate genes encoding protein kinase (Table 4). In Arabidopsis thaliana, the protein kinase in chloroplasts is known to regulate photosynthesis (Schliebert et al., 2008).
DISCUSSION

Understanding the genetic mechanism of maize photosynthetic parameters and GY may provide a new approach for maize improvement. Identification of QTLs for photosynthetic parameters is very important for elucidation of their genetic basis and facilitation of MAS in maize genetic improvement programs. In the present study, the photosynthetic parameters were diverse in the studied population, and the heritability values of $P_N$ at 15, 25 and 35 DAP were relatively low (Table 2), consistent with results reported for soybean (Yin et al., 2010). This result indicates that photosynthetic parameters are susceptible to environmental factors, thereby hindering the improvement of these traits using conventional breeding programs. Therefore, further studies should be conducted to dissect the precise cause of controlling photosynthetic parameters for maize in our study.

Population stratification in mapping panels can cause spurious marker-trait associations (Hao et al., 2015). To account for population structure in association analysis, MLM-based (Q+K) association mapping has been found

Table 2. Descriptive statistics, results of ANOVA, and broad-sense heritabilities of grain yield and photosynthetic parameters at different growth stages among 260 maize inbred lines across 2 years.

| Traits | DAP | Year | Mean | SD  | Min | Max | G | E | GxE | h2(%) |
|--------|-----|------|------|-----|-----|-----|---|---|-----|------|
| $P_N$ | 15  | 2014 | 25.85| 5.47| 19.30| 34.69| **| **| **  | 56.45|
|       | 2015|      | 22.03| 3.65| 19.63| 33.74|   |   |     |      |
|       | BLUPs|     | 24.07| 3.96| 19.13| 34.68|   |   |     |      |
|       | 25  | 2014 | 26.64| 6.52| 15.00| 43.62| **| **| **  | 63.21|
|       | 2015|      | 30.02| 5.95| 18.39| 43.20|   |   |     |      |
|       | BLUPs|     | 28.38| 2.25| 17.67| 41.64|   |   |     |      |
|       | 35  | 2014 | 24.16| 5.45| 10.05| 43.04| **| **| **  | 59.43|
|       | 2015|      | 25.72| 6.78| 10.95| 42.54|   |   |     |      |
|       | BLUPs|     | 25.12| 2.56| 11.95| 39.72|   |   |     |      |
| $C_i$ | 15  | 2014 | 213  | 12  | 156  | 278  | **| **| **  | 51.76|
|       | 2015|      | 218  | 18  | 162  | 289  |   |   |     |      |
|       | BLUPs|     | 216  | 19  | 159  | 283  |   |   |     |      |
|       | 25  | 2014 | 238  | 17  | 164  | 287  | **| **| **  | 53.81|
|       | 2015|      | 231  | 20  | 171  | 291  |   |   |     |      |
|       | BLUPs|     | 234  | 19  | 169  | 289  |   |   |     |      |
|       | 35  | 2014 | 218  | 21  | 157  | 271  | **| **| **  | 54.82|
|       | 2015|      | 216  | 16  | 159  | 278  |   |   |     |      |
|       | BLUPs|     | 217  | 15  | 158  | 275  |   |   |     |      |
| $g_s$ | 15  | 2014 | 0.354| 0.143| 0.13 | 0.81 | **| **| **  | 47.21|
|       | 2015|      | 0.387| 0.102| 0.14 | 0.79 |   |   |     |      |
|       | BLUPs|     | 0.371| 0.118| 0.14 | 0.80 |   |   |     |      |
|       | 25  | 2014 | 0.386| 0.109| 0.15 | 0.88 | **| **| **  | 49.23|
|       | 2015|      | 0.392| 0.121| 0.16 | 0.86 |   |   |     |      |
|       | BLUPs|     | 0.389| 0.110| 0.15 | 0.87 |   |   |     |      |
|       | 35  | 2014 | 0.376| 0.152| 0.14 | 0.76 | **| **| **  | 46.42|
|       | 2015|      | 0.367| 0.173| 0.15 | 0.72 |   |   |     |      |
|       | BLUPs|     | 0.369| 0.147| 0.14 | 0.74 |   |   |     |      |
| $T_r$ | 15  | 2014 | 5.364| 1.237| 1.98 | 10.76| **| **| **  | 51.24|
|       | 2015|      | 5.765| 1.329| 1.87 | 10.87|   |   |     |      |
|       | BLUPs|     | 5.543| 1.179| 1.91 | 10.82|   |   |     |      |
|       | 25  | 2014 | 6.321| 1.987| 2.34 | 11.42| **| **| **  | 52.12|
|       | 2015|      | 6.453| 1.869| 2.01 | 11.52|   |   |     |      |
|       | BLUPs|     | 6.389| 1.141| 2.21 | 11.49|   |   |     |      |
|       | 35  | 2014 | 5.879| 2.012| 2.12 | 10.56| **| **| **  | 50.26|
|       | 2015|      | 6.213| 1.897| 2.17 | 10.87|   |   |     |      |
|       | BLUPs|     | 6.102| 1.762| 2.15 | 10.76|   |   |     |      |
| GY    | 2014|      | 320.73| 57.25| 158.75| 490.90| **| **| **  | 64.89|
|       | 2015|      | 301.71| 72.10| 147.30| 502.40|   |   |     |      |
|       | BLUPs|     | 347.72| 91.77| 151.35| 491.23|   |   |     |      |

$P_N$: Photosynthetic rate; $C_i$: intercellular CO$_2$ concentration; $g_s$: stomatal conductance; $T_r$: transpiration rate; GY: grain yield; DAP: days after pollination; G: genotype; E: environment; GxE: Genotype × Environment; $h^2$: broad-sense heritability; BLUPs: best linear unbiased predictions.

**Significant at the 1% probability level.

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to be well suited in our study, which greatly reduced false positives (Hao et al., 2015). In this study, 260 maize inbred lines were classified into subpopulations that were generally consistent with their known pedigrees. The MLM model applied in our study has been successfully used in other studies to account for population structure (Zhao et al., 2011). Consequently, an understanding of the genetic source of carbohydrates during grain filling (Liu et al., 2013). In maize, leaf photosynthesis is a major source of carbohydrates during grain filling (Liu et al., 2011). Consequently, an understanding of the genetic basis of photosynthetic parameters during the grain-filling process is needed. In the present study, 23 SNPs in the

![Figure 1. Number of subgroups (Δk) values calculated in a population structure analysis of 260 maize inbred lines using STRUCTURE.](image)

Table 3. Correlation coefficients among photosynthetic parameters and grain yield at 15 (T1), 25 (T2) and 35 d (T3) after pollination (DAP).

|       | T1 | GY | T2 | GY | T3 | GY |
|-------|----|----|----|----|----|----|
| Pn    | 0.212* | 0.699** | 0.463** | 0.589** | 0.125* | 0.705** |
| Cc    | 0.392** | 0.545** | 0.512** | 0.401** | 0.499** | 0.445** |
| gs    | 0.574** | 0.535** | 0.324** | 0.512** | 0.548** | 0.341** |
| Tr    | 0.324** | 0.341** | 0.646** | 0.339** | 0.567** | 0.452** |

Pn: Photosynthetic rate; Cc: intercellular CO₂ concentration; gs: stomatal conductance; Tr: transpiration rate; GY: grain yield; DAP: days after pollination.

* **Significant at the 0.05 and 0.01 probability levels, respectively.

These results reveal that most genes (or QTLs) controlling maize photosynthetic parameters are activated at different developmental stages, with only a handful of genes (or QTLs) stably possessing the most promising association at different developmental stages. The stable genes (or QTLs) expressed at different developmental stages can be used for marker-trait assisted selection. In the present study, the three SNPs identified at different development stages may be the most promising marker.

Previous studies of crop plants have revealed that QTLs for closely correlated traits generally map to the same or a nearby genomic region (Yang et al., 2007; Yin et al., 2012). Similar results were observed in this study. Four SNPs were significantly associated with two or more traits, a finding also supported by the significant correlation among the studied traits (Table 4). For example, PZE-101152541 and PZE-102116144 were co-associated with Pn and gs, at 25 DAP, and there were significant correlations between Pn and gs, at 25 DAP. Some putative genes for controlling these traits might be located in or near these co-associated regions, a situation that could facilitate the pyramiding of elite alleles for different traits in maize MAS schemes (Hao et al., 2012a). PZE-101152541 and PZE-102116144 were co-associated with GY and Pn in 2 yr, indicating the possible existence of a single gene with pleiotropic effects that is tightly linked with multiple genes.

Among the 14 associated SNPs, nine were located inside genes, the others were intergenic. According to the maize gene annotation database at MaizeGDB (http://www.maizegdb.org), the putative genes of GRMZM2G317770 on chromosome 1 and GRMZM2G002227 on chromosome 9 were the most potential candidate genes. The candidate genes of GRMZM2G317770 and GRMZM2G002227 encode protein kinases, which were related to the photosynthetic light reaction in the previous studies in Arabidopsis thaliana (Schliebner et al., 2008). However, other associated SNPs were not involving some putative candidate genes; the causal genes might exist within the genomic regions in LD where associated-markers located (Hao et al., 2012b). In the further studies, choice of a larger population size with more diverse genetic background, and use of more markers (especially markers from key genes of photosynthetic metabolic networks), will directly improve the scanning power and the accuracy of detection, and capture the key loci and/or candidate genes underlying the photosynthesis and yield in maize (Yan et al., 2011; Hao et al., 2012b).
CONCLUSIONS

Our results demonstrate that maize photosynthetic parameters are significantly or highly significantly correlated with grain yield (GY) at different developmental stages. Fourteen single-nucleotide polymorphisms (SNPs) associated with photosynthetic parameters and GY were detected in two years as well as over years. PZE-102116144 and SYN35048 associated with stomatal conductance ($g_s$), and PZE-101152541 associated with photosynthetic rate ($P_N$), were identified at different developmental stages. PZE-101152541 was significantly associated with GY and $P_N$ (at 25 and 35 DAP), PZE-102116144 with GY, $g_s$ (at 15 and 25 DAP), and $P_N$ (at 25 DAP), PZE-109061997 with intercellular CO$_2$ concentration (at 25 DAP) and transpiration rate (at 25 DAP), PZE-110019199 with $g_s$ (at 25 DAP) and GY. Based on functional annotations, two genes (GRMZM2G317770 and GRMZM2G002227) were considered as potential candidate genes for the identified
SNPs (PZE-101152541 and PZE-109016787). The SNPs and candidate genes identified in this study might provide instrumental information for understanding the genetic mechanism of maize photosynthetic parameters and yield.

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