

Genetic Dissection of the Photosynthetic Parameters of Maize (*Zea mays* L.) in Drought-Stressed and Well-Watered Environments

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Abstract—Photosynthesis is a critical function that allows adaptation to drought stress in maize (*Zea mays* L.). Therefore, elucidation of the genetic control of photosynthetic performance under drought stress and the associated molecular markers is of great importance for marker-assisted selection (MAS). Here, we detected 54 quantitative trait loci (QTLs) affecting the net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO₂ concentration (C_i), transpiration rate (Tr), ribulose 1,5-biphosphate carboxylase activity (RuBP), and water use efficiency (WUE) of the ear leaf across two F4 populations in drought-stressed and well-watered environments by single-environment mapping with composite interval mapping (CIM), and 43 QTLs identified under drought stress, indicating that the tolerance to photoinhibition is a key factor affecting drought stress tolerance in maize. We further dissected 54 QTLs via joint analysis of all environments with mixed-linear-model-based CIM (MCIM), including 24 involved in QTL-by-environment interactions (QEIs), 87.5% QEIs identified under drought stress, 14 pairs showing epistatic interactions with dominance-by-additive/dominance effects under contrasting environments. We further identified eight constitutive QTLs (cQTLs) across two populations by CIM/MCIM, which could be used for genetic improvement of maize via QTL pyramiding. The co-localization of five cQTLs in bin 1.07_1.10/6.05/7.02_7.04/8.03/10.03 under contrasting environments in both populations strongly supported pleiotropy. Additionally, 17 candidate genes located at the above-mentioned cQTLs were involved in photomorphogenesis, photosynthesis, and stress response. These results provide insights into the genetic mechanisms responsible for photosynthesis under different water availability conditions, and reveal alleles that could potentially be used for MAS-based development of drought tolerant maize cultivars.

Keywords: *Zea mays*, maize, QTLs, epistatic interaction, photosynthetic performance, drought

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INTRODUCTION

Water stress is one of the most important environmental factors limiting maize (*Zea mays* L.) productivity in tropical and subtropical regions. The problem of food insufficiency is worsened by the global climate change and the ever-growing population. Therefore, the improving resistance to water scarcity and breeding drought tolerant varieties are crucial for maize survival, growth, and biomass production in water scarce environments [1]. The photosynthetic performance of maize, as an important C₄ crop, is one of the main targets in breeding programs for improving grain yield and drought resistance. In recent studies, the net

photosynthetic rate (P_n), chlorophyll relative content, chlorophyll *a* content, chlorophyll *b* content, total chlorophyll content, ribulose 1,5-biphosphate carboxylase activity (RuBP), stomatal conductance (G_s), and transpiration rate (Tr) of maize were significantly reduced, whereas chlorophyll *a/b* ratio, intercellular CO₂ concentration (C_i), and water use efficiency (WUE) were significantly increased under drought stress, and compared with drought sensitive accessions, drought resistant accessions maintained higher photosynthetic capacity under drought stress [2, 3]. Moderate and severe drought stress also damaged photosystem II (PSII), and reduced electron transport in diverse maize varieties [4]. Under drought stress, even absorption of antenna chlorophyll per PSII reaction center significantly increased, along with quantum yield for electron transport, and efficiency of an electron beyond primary quinone acceptor of PSII significantly decreased in maize [5]. This suggests that once the photosynthetic performance of maize is inhibited under drought stress, it is likely to increase

Abbreviations: C_i —intercellular CO₂ concentration; CIM—composite interval mapping; cQTLs—constitutive quantitative trait loci; GEI—genotype × environment interaction; G_s —stomatal conductance; MAS—marker-assisted selection; MCIM—mixed-linear-model-based composite interval mapping; P_n —net photosynthetic rate; QEIs—quantitative trait loci-by-environment interactions; QTLs—quantitative trait loci; RuBP—ribulose 1,5-biphosphate carboxylase activity; Tr —transpiration rate; WUE—water use efficiency.

the absorption of light energy beyond the rate of photosynthetic carbon fixation, resulting in photo-oxidative damage and consequently lower grain yield.

Using identified molecular markers and quantitative trait loci (QTLs) for photosynthetic traits under different watering treatments in marker-assisted selection (MAS) is a promising way to maximize the productivity of maize under drought stress. Genetic analysis of photosynthetic traits in wheat (*Triticum aestivum* L.) [6], rice (*Oryza sativa* L.) [7], and cotton (*Gossypium* spp.) [8] under drought and high temperature stress conditions have mostly been conducted using the QTL mapping approach. In maize, however, only several experiments have been conducted for mapping QTLs affecting photosynthesis-related traits. For example Wang and Zhang [9] mapped 32 QTLs for chlorophyll *a* content, chlorophyll *b* content, total chlorophyll content, and other chlorophyll content in one F2 population derived from the A150-3-2 × Mo17 cross. Trachsel et al. [10] identified seven QTLs affecting the quantum efficiency of PSII and chlorophyll relative content in the recombinant inbred line (RIL) population derived from CML444 × SC-Malawi cross. Yu et al. [11] detected 32 QTLs associated with chlorophyll *a* content, chlorophyll *b* content, total chlorophyll content, P_n , G_s , C_i , and Tr in two F2 populations derived from Y114 × Y115 and Y105 × Y106 crosses. In addition, only two QTL mapping studies focused on photosynthetic performance have been reported in maize under drought stress conditions. These include the study of Pelleschi et al. [12], which identified 19 major QTLs controlling net CO₂-uptake, ADPglucose pyrophosphorylase activity, g_s , and Tr in a RIL population derived from the F-2 × MBS847 cross under drought-stressed and well-watered regimes, and the study of Prado et al. [13], which assessed 16 robust QTLs affecting g_s with a diversity panel of maize hybrids under water deficit conditions. Despite these studies, the molecular mechanism underlying the response of photosynthetic traits to drought stress remains unclear. Therefore, an in-depth investigation is needed to understand the genetic basis of the photosynthetic performance of maize under contrasting water availability conditions. Moreover, a better understanding of the genotype × environment interaction (GEI) will provide a foundation for the genetic improvement and genotype optimization under different environments [14].

The overall objectives of this study were to (1) identify QTLs affecting P_n , G_s , C_i , Tr RuBP, and WUE in two maize F4 populations subjected to contrasting watering regimes; (2) estimate the effects of these QTLs by single environment mapping with composite interval mapping (CIM); (3) further dissect joint QTLs, QTL-by-QTL interactions (epistasis), as well as QTL-by-environment interactions (QEI) among all watering environments via mixed-linear-model-based CIM (MCIM). By combining these experi-

ments, we aim to identify recombination hot spots, areas of QTL co-localization, and the underlying genes, which may further contribute to the mapping of QTLs, thus revealing the genetic control of photosynthetic performance under diverse watering conditions, and to develop high-yielding drought tolerance maize cultivars via MAS.

MATERIALS AND METHODS

Plant materials. Two *Zea mays* L. F4 populations (POP-CT and POP-LT) comprising 218 and 202 individuals and derived from Chang7-2 × TS141 (CH × TS) and Langhuang × TS141 (LH × TS) crosses, respectively, were used to evaluate photosynthetic parameters and detect QTLs in this study. The corresponding F2 plants were self-pollinated to generate F3 population at Pingliang maize breeding station of Gansu Agricultural University, China (106.93° N, 35.43° E; 1,204 m altitude; loessial soil) in 2013, and each F3 population was again selfed to obtain the corresponding F4 population at Zhangye breeding base of Yuyuan Co., LTD., China (38.83° N, 106.93° E; 1785 m, altitude; sandloam) in 2015 and Jingtai farm of Tiaoshan Nongken corporation, China (37.18° N, 104.03° E; 1640 m, altitude; sandloam) in 2015, respectively [15]. For the parents, Chang7-2 and Langhuang originated from Tangsipingtou germplasm, TS141 was representative of Reid yellow dent germplasm, and these three parents displayed significant differences in plant type, stomatal character, photosynthetic performance, drought tolerance, and grain yield [3, 15–17].

Field experiments and traits evaluation. Phenotypic data of the ear leaf among the two F4 families and three parental lines were evaluated in a completely randomized block design, with two replications and 10 plants at a density of 55 580 plant/ha in each plot at the Huangyang, Gansu Academy of Agricultural Sciences Proving Ground, China (http://hyc.gsagr.ac.cn/channels/channel_503_1.html) (37.67° N, 102.85° E; 1,740 m altitude; sandy loam) in 2019. The mean temperature, total sunshine duration, total rainfall, total evaporation capacity, mean relative humidity, and mean wind velocity at the experimental site were 17.9°, 1427.6 h, 118.7 mm, 1039.7 mm, 52.3%, and 2.2 m s⁻¹, respectively, during the growing seasons (April to September) in 2019 (Supplementary Fig. S1). Then, the experimental field was divided into WW and DS treatments. The DS treatment was equivalent to rainfed conditions, with rainfall of 118.7 mm during the growing seasons. The WW treatment involved irrigation with 4500 m³/ha water supply at each of the three stages (V18, R1, and R3). In addition, because of the abundant annual evaporation capacity (2164.7 mm), low annual relative humidity (45.8%), scarce annual rainfall (172 mm), and low annual mean temperature (9.3°C) at the experimental site (Supplementary Fig. S1), a plastic film (0.02 mm thick, 140 cm wide)

was laid out over the field to cover the soil surface before sowing.

Until anthesis, six photosynthetic traits of the ear leaf, including P_n ($\mu\text{mol CO}_2/(\text{m}^2 \text{ s})$), G_s ($\text{mol H}_2\text{O}/(\text{m}^2 \text{ s})$), C_i ($\mu\text{mol CO}_2/\text{mol}$), Tr ($\text{mol H}_2\text{O}/(\text{m}^2 \text{ s})$), RuBP ($\text{mol}/(\text{m}^2 \text{ s})$), and WUE ($\mu\text{mol CO}_2/\text{mol H}_2\text{O}$), were measured in the two populations and three parents using a portable photosynthesis system, namely, LI-6400 XT (LI-COR Inc., United States). Among these traits, P_n , G_s , C_i , and Tr were measured in a chamber at $1500 \mu\text{mol}/(\text{m}^2 \text{ s})$, photosynthetically active radiation, 30°C leaf temperature, and $380 \pm 5 \mu\text{mol CO}_2/\text{mol}$ [7]. All measurements were recorded using ear leaf between 9:00 and 11:00 a.m. on sunny days to avoid the of photoinhibition, and were repeated at least five times using different plants. RuBP was estimated in response to apparent mesophyll conductance, as follows [3]:

$$\text{RuBP} = P_n/C_i. \quad (1)$$

WUE was calculated as follows [12]:

$$\text{WUE} = P_n/Tr. \quad (2)$$

Then the corresponding plants were harvested and subsequently air-dried to measure the ear weight (EW, g), grain weight per ear (GW, g), and 100-kernel weight (KW, g). Relative to the WW treatment, the average rate of change (RC) of each trait in the DS treatment was estimated as follows [17]:

$$\text{RC} = (1 - T_D/T_W) \times 100, \quad (3)$$

where, T_D and T_W represent the average value of each trait in the DS and WW treatments, respectively.

Statistical analysis of phenotypic data. All phenotypic data were statistically analyzed using IBM-SPSS Statistics v. 19.0 (SPSS Inc., United States) (<https://www.ibm.com/products/spss-statistics>). The significance of total and residual variances of each trait in the two F4 populations was estimated by the general linear model for univariate data and one-way analysis of variance (ANOVA), respectively. Values of broad-sense heritability (h^2) and GEI heritability (h_{ge}^2) for the combined environments (WW and DS) were estimated as follows [17]:

$$h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{ge}^2/n + \sigma_e^2/nr), \quad (4)$$

$$h_{ge}^2 = (\sigma_g^2/n) / (\sigma_g^2 + \sigma_{ge}^2/n + \sigma_e^2/nr), \quad (5)$$

where, σ_g^2 is the genotypic variance; σ_e^2 is the environmental variance; σ_{ge}^2 represents the error variance, σ_{ge}^2 is the GEI variance; n is the number of environments ($n = 2$); and r is the number of replications ($r = 2$). Possible associations among traits were tested using several methods, based on either phenotypic Pearson correlation or principal component analysis via IBM-SPSS Statistics v. 19.0 [12].

Genetic map construction and QTL identification. A total of 205 and 199 genome-wide polymorphic SSR markers obtained from the Maize Genetics and Genomics Database (MaizeGDB, <http://www.maizegdb.org/>) were applied for developing the genetic maps of two F2 populations using JionMap v. 4.0 (<https://www.kyazma.nl/index.php/JoinMap/>). The total map length was 1648.8 and 1542.5 cM, with an average interval of 8.0 and 7.8 cM, respectively [16]. In single watering environment, QTL mapping for each photosynthetic-related trait of two F4 populations was performed using CIM implemented with Windows QTL Cartographer software v. 2.5 (<http://statgen.ncsu.edu/qtlcart/winqtlcart.htm>). Model 6 of the Zmapqtl module was used for analyzing QTLs by CIM. The window size was 10 cM, and cofactors were selected through forward and backward regressions, with in and out thresholds at $P < 0.05$. A genome-wide critical threshold value was estimated for experiment-wise type I error rate of 0.05 with 1000 random permutations.

Additionally, among all watering environments, the MCIM via QTL Network v. 2.0 (<http://ibi.zju.edu.cn/software/qtlnetwork>) was used to dissect joint QTLs, epistatic QTLs, and QEI for each photosynthetic trait of both F4 populations. The testing window, walk speed, and filtration window of the genome scan were set at 10, 2, and 10 cM, respectively. A total of 1000 permutations were performed to determine the threshold logarithm (base 10) of odds ratio (LOD) for the traits for declaring a significant QTL at $P < 0.05$. The name of the QTL was assigned according to the modified nomenclature of Zhao et al. [16]. Furthermore, the letter “J” was added to the QTL name (inserted between the trait abbreviation and chromosome number), whereas one QTL was only detected in joint analysis with MCIM but not in a single environment through the CIM program. Additionally, QTLs affecting photosynthetic traits were identified within the same marker interval or within overlapping confidence intervals, and the corresponding loci were assumed to be common QTLs with pleiotropic effects.

cQTL detection and candidate gene dissection. The cQTLs refer to QTLs detected stably and repeatedly across different mapping populations in two or more watering environments via CIM or MCIM [14]. Refer to the meta-QTL analysis [15], the corresponding cQTLs intervals were projected on the physical reference map of B73 RefGen_v3 (https://maizegdb.org/gbrowse/maize_v3), and the corresponding candidate genes were validated in B73 RefGen_v3 reference genome [17]. The functions of these genes were analyzed via the AgBase v. 2.00 (<http://agbase.arizona.edu/>) online software and public databases, namely, MaizeGDB (<http://www.maizegdb.org/>), National Center for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov/pubmed>), and China National Knowledge Infrastructure (CNKI, <http://www.cnki.net>).

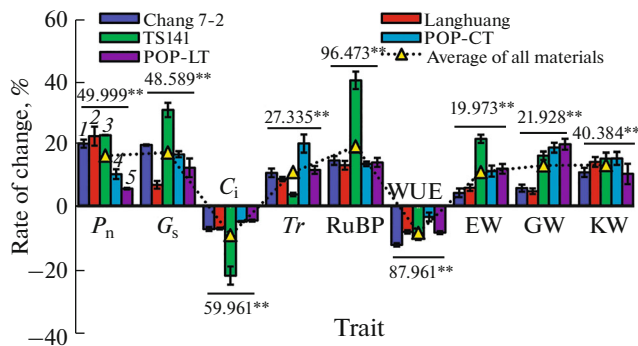


Fig. 1. Rate of change (RC) of photosynthetic performance parameters (net photosynthetic rate [P_n], stomatal conductance [G_s], intercellular CO_2 concentration [C_i], transpiration rate [Tr], ribulose 1,5-biphosphate carboxylase activity [RuBP], and water use efficiency [WUE]) and yield component traits (ear weight [EW], grain weight per ear [GW], and 100-kernel weight [KW]) in three parental lines: Chang7-2 (1), Langhuang (2), and TS141 (3) and two F4 families—POP-CT (4) and POP-LT (5) under drought-stressed (DS) environment in Wuwei in 2019. Asterisks indicate significant differences (* $P < 0.05$, ** $P < 0.01$; ANOVA).

RESULTS

We detected significant differences in the six photosynthetic performance values across three parents and two F4 populations under both DS and WW environments ($P < 0.05$) (Supplementary Table S1). Compared with the WW environment, values of P_n , G_s , Tr , and RuBP were significantly lower, and those of C_i and WUE were significantly higher in Chang7-2, Langhuang, TS141, POP-CT, and POP-LT in the DS treatment (Fig. 1). Further analysis indicated that the drought-sensitive line TS141 showed a greater rate of change of P_n , G_s , C_i , and RuBP, but smaller rate of change in Tr compared with drought-tolerant lines Chang7-2 and Langhuang (Fig. 1). Additionally, the average rate of change of the three parents and two F4 populations in P_n , G_s , C_i , Tr , RuBP, and WUE was 16.02, 17.05, -9.34, 10.80, 18.96, and -8.62%, respectively (Fig. 1). These phenotypic analyses demonstrated that the drought-induced limitation of photosynthesis in maize was primarily because of CO_2 diffusion from sub-stomatal interval internal cavities to the carboxylation site in chloroplasts and the degree of stomatal closure, which explains why RuBP activity and g_s decreased remarkably in different maize accessions under drought stress.

Moreover, values of all photosynthetic performance parameters, except RuBP, showed continuous distribution in the two F4 populations, with absolute values of skewness and kurtosis being less than 1.0 (Supplementary Table S1, Fig. S2), indicating continuous variation and a quantitative genetic basis of photosynthetic performances in these maize accessions. Analysis of variance showed that significant variation

in the photosynthetic performance parameters among genotypes (Supplementary Table S2), which further allowed their genetic dissection under drought stress. The environmental and GEI variances were also significant ($P < 0.05$; Supplementary Table S2), implying that the populations performed quite inconsistently between both watering environments. Additionally, the estimated h^2 and h_{ge}^2 values of all photosynthetic performance parameters in the two populations were 46.429 (G_s in POP-CT) ~ 91.030% (C_i in POP-LT)/1.466 (WUE in POP-CT) ~ 19.770% (Tr in POP-CT) because of the large contribution of GEI to phenotypic variance (Supplementary Table S2).

The examination of phenotypic correlations between photosynthetic traits might be useful before interpreting the co-localization of QTLs, which would be more likely to reveal genetic relationships. Principal component analysis and Pearson correlations were performed in the two populations and three parents under both watering environments. Principal component analysis revealed two significant principal components (principal component 1 and principal component 2; Eigenvalue > 1), which together explained 65.032% (WW) and 75.602% (DS) of the variance, respectively (Supplementary Fig. S3). These principal components were linear combinations of the original photosynthetic traits, which were independent of each other, and represented different combinations of the traits based on their variable loadings under different watering environments. Principal component 1 primarily represented variances in P_n , G_s , and Tr under both environments, whereas principal component 2 mainly captured variance in RuBP or C_i (Supplementary Fig. S3). Further analysis showed that pairwise Pearson correlations for six photosynthetic parameters and three yield components produced complementary information under both watering environments, and each corresponding trait showed a significant positive or negative correlation ($P < 0.05$) with 4–8 other traits in a single watering environment (Supplementary Fig. S3), thus indicating that maize yield is the result of synergistic or inhibited effects of multiple photosynthetic parameters under WW and DS environments, and the influence degree of drought stress to drought-sensitive maize plants photosynthesis and yield were larger. Moreover, photosynthetic performance parameters (P_n , G_s , C_i , RuBP, and WUE) and yield component traits (GW and KW) of the two F4 progeny populations depicted significantly positive correlations with the female parent and male parent ($P < 0.05$), respectively, whereas Tr and CW showed significantly positive correlations with both parents ($P < 0.05$) (Supplementary Table S3), indicating that the effects of both parents on different photosynthetic and yield related traits in the F4 progeny populations were inconsistent.

To dissect the genetic control of six photosynthetic performance parameters using single environment mapping with CIM, we mapped a total of 54 QTLs

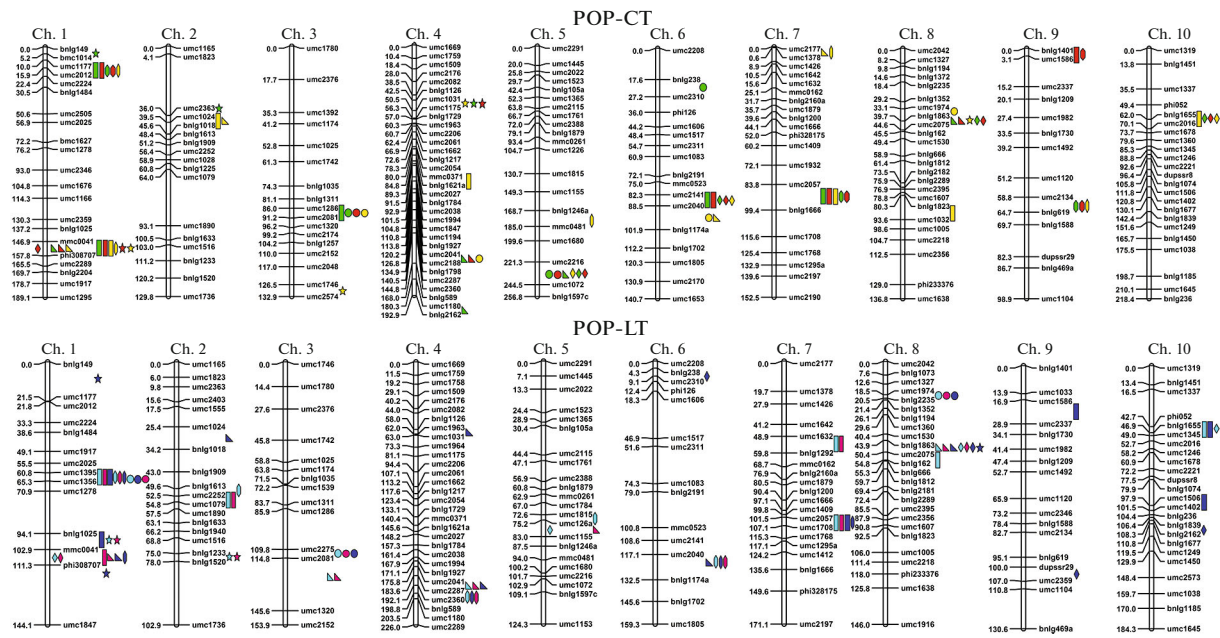


Fig. 2. Genetic map showing the QTLs affecting net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i), transpiration rate (Tr), ribulose 1,5-biphosphate carboxylase activity (RuBP), water use efficiency (WUE) in two F4 populations (POP-CT and POP-LT) by single environment mapping with compositive interval mapping (CIM) and joint analysis of all environments with mixed-linear-model-based CIM (MCIM). For POP-CT and POP-LT, green and sapphire symbols represent QTLs identified with CIM in the WW environment at Wuwei in 2019, whereas red and pink symbols represent QTLs identified in the DS environment, and yellow and blue symbols represent QTLs identified with MCIM in POP-CT and POP-LT, respectively. Rectangles, circles, triangles, rhombuses, hexagons, and pentagons represent QTLs for P_n , G_s , C_i , Tr , RuBP, and WUE, respectively.

(13 for P_n , 6 for G_s , 10 for C_i , 9 for Tr , 10 for RuBP, and 6 for WUE) across two F4 populations (POP-CT and POP-LT) in both WW and DS environments, and the phenotypic variance explained by each individual QTL ranged from 2.37% (for RuBP in POP-LT under DS) to 18.21% (for C_i in POP-CT under DS) in each environment (Fig. 2, Supplementary Table S4, Fig. S4). A total of 43 (79.63%) of the identified QTLs affecting six photosynthetic traits were detected in the DS environment (Fig. 2, Table S4, Fig. S5). QTLs affecting P_n , G_s , and RuBP displayed both additive (A) and non-additive (including partial dominance [PD], dominance [D], and over-dominance [OD]) effects; however, QTLs for C_i , Tr , and WUE showed only non-additive effects (Supplementary Table S4, Fig. S4). Moreover, approximately 33.33, 18.18, 37.50, 43.75, 35.29, and 44.44% of the alleles responsible for the increase in P_n , G_s , C_i , Tr , RuBP, and WUE, respectively, were contributed by the male parent TS141 (Supplementary Table S4).

Joint QTL analysis, with MCIM, of six photosynthetic performance parameters in all watering environments revealed 54 QTLs in POP-CT and POP-LT, which is equal to the number of QTLs detected in a single environment. Of these 54 QTLs, 25 were consistent with those of P_n , G_s , C_i , Tr , RuBP, and WUE identified through single environment mapping with

CIM. These identified QTLs explained 2.07% (for C_i in POP-CT) to 13.21% (for P_n in POP-LT) of the phenotypic variance contributed by $h^2(A)$ (Supplementary Table S5, Fig. 2). Moreover, 24 QTLs (6 for P_n , 3 for G_s , 6 for C_i , 3 for Tr , 4 for RuBP, and 2 for WUE) were involved in QEIs in two F4 populations, and accounted for 1.34% (for C_i in POP-LT) to 6.86% (for P_n in POP-LT) of the phenotypic variance explained by $h^2(AE)$ (Supplementary Table S5). Thus, the QEIs of these QTLs may exert stronger effects on photosynthetic performance in maize under contrasting environments. In addition, a total of seven stable bin intervals may be mediated by environmental factors in the two F4 populations; bin 1.08_1.10 (mmc0041-phi308707) exhibited a QEI for P_n , C_i , and RuBP; bin 3.07_3.08 (umc1286/umc2275-umc2081) showed a QEI for G_s ; bin 6.05 (umc2040-bnlg1174a) showed a QEI associated with C_i and RuBP; bin 7.00 (umc2177_umc1378) showed a QEI controlling C_i and RuBP; bin 7.02_7.04 (umc2057-bnlg1666/umv1708) showed a QEI for P_n ; bin 8.03 (bnlg1863-umc2075) showed QEI affecting WUE; bin 10.03 (bnlg1655-umc2016) displayed a QEI for P_n and Tr (Supplementary Table S5).

Further eight cQTLs were simultaneously identified in two F4 populations by single environment mapping with CIM and joint analysis through MCIM.

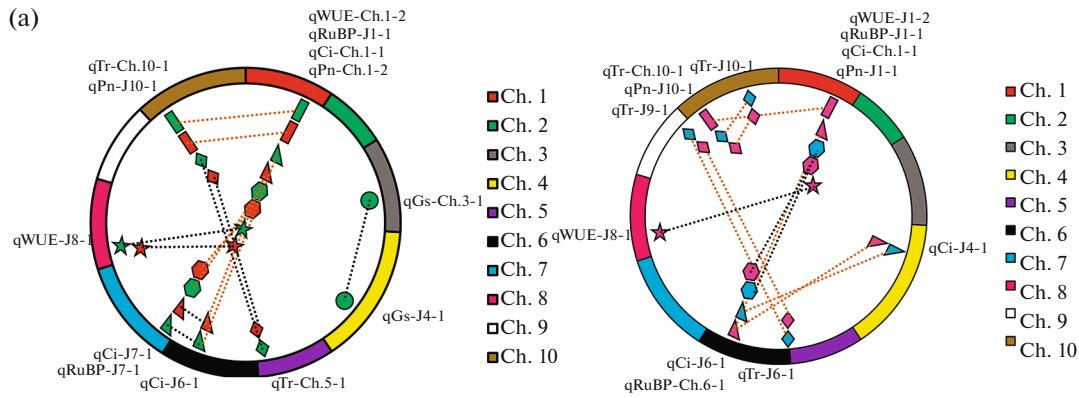


Fig. 3. Epistatic interactions among QTLs identified in maize populations by joint analysis with MCIM. (a) POP-CT; (b) POP-LT. Black/brown dotted lines represent dominance-by-additive/dominance (DA/DD) epistatic interaction effects, green and sapphire symbols represent QTLs identified in the WW environment, whereas red and pink symbols represent QTLs identified in the DS environment. Rectangles, circles, triangles, rhombuses, hexagons, and pentagams represent QTLs for P_n , G_s , C_i , Tr , RuBP, and WUE, respectively.

These cQTLs were located on chromosome 1, 6, 7, 8, and 10, and each cQTL accounted for 3.21–15.78% of the average observed phenotypic variance (Supplementary Table S6). All cQTLs, except the cQTL1 for WUE, were detected in the DS environment, indicating that these cQTLs may have several stable alleles involved in photosynthesis under stress conditions (Supplementary Table S6). Moreover, five of these eight cQTLs, including cQTL2 (for P_n , C_i , Tr , and WUE), cQTL5 (for P_n , Tr , C_i , and RuBP), cQTL6 (for P_n and RuBP), cQTL7 (for C_i , Tr , and WUE), and cQTL8 (for P_n , Tr , and RuBP) (Supplementary Table S6), showed pleiotropic effects on two or four photosynthetic traits, suggesting that these cQTL intervals control two or more tightly linked photosynthesis-related traits. In addition, these eight cQTLs were projected on the physical map of B73 RefGen_v3 (http://www.maizegdb/gbrowse/mazie_V3), resulting in the identification of 17 candidate genes involved in leaf morphology and development, photosynthesis, and stress response (Supplementary Table S6).

Among all QTLs identified, six and eight pairs of epistatic interactions for six photosynthetic traits exhibited dominance-by-additive (DA) and dominance-by-dominance (DD) effects in different watering environments in the two F4 populations (Fig. 3). Each epistatic interaction explained 2.11% (for Tr in WW) to 4.96% (for G_s in WW) of the observed phenotypic variance contributed by $h^2(\text{DA})$, and 2.09% (for C_i in DS) to 5.87% (for C_i in DS) of the observed phenotypic variance contributed by $h^2(\text{DD})$ (Supplementary Table S7), indicating that the main effects of significant QTLs may be stronger on these six photosynthetic performance parameters. Additionally, two pairs of epistatic interactions were consistently detected in the two F4 populations under different watering environments, i.e., epistatic interactions between bin 1.07_1.10 (bnlg1025-mm0041-phi308707) and bin

10.03 (bnlg1655-umc2016/umc1345) for P_n , and between bin 1.08_1.10 (mmc0041-phi308707) and bin 6.05 (umc2040-bnlg1174a) for C_i (Fig. 3, Supplementary Table S7). The two epistatic interactions may thus be critical for MAS.

DISCUSSION

Approximately 95% of the organic matter accumulated by crops is derived from photosynthesis, and the efficiency of photosynthesis directly determines the grain yield. Drought stress significantly affects crop photosynthesis, and the response of leaf photosynthesis to drought is mediated by two different physiological processes [18]. Firstly, reduction in stomatal closure and apparent mesophyll conductance is recognized as the main driver of the photosynthetic response to drought stress; to reduce transpiration under drought stress, the stomata close, which reduces the diffusion of CO_2 from the atmosphere into the substomatal cavities, thus slowing photosynthesis [18]. Additionally, apparent mesophyll conductance rapidly declines to further limit CO_2 diffusion from the substomatal cavities into the chloroplast stroma during water stress [3, 18]. Secondly, photosynthesis could be limited by biochemical processes, resulting in the inhibition of photosynthetic enzyme activity, P_n and RuBP activity decrease [4, 5, 18]. Fortunately, these findings are consistent with the results of the current study; drought-stressed Chang7-2, Langhuang, TS141, POP-CT, and POP-LT exhibited a reduction in P_n , G_s , Tr , and RuBP but a significant increase in C_i and WUE compared with corresponding plants in the WW environment. Furthermore, RuBP (average rate of change: 18.96%) and g_s (average rate of change: 17.05%) were more sensitive to drought stress than P_n (average rate of change: 16.02%), C_i (average rate of change: -9.34%), Tr (average rate of change: 10.80%), and WUE (average

rate of change: -8.62%). Therefore, it could be concluded that the drought-induced inhibition of photosynthesis in maize is primarily caused by the reduction in CO_2 diffusion from sub-stomatal interval internal cavities to the carboxylation site in chloroplasts and because of the degree of stomatal closure; this is consistent with the result of Liu et al. [2]. Therefore, genetic improvement of photosynthetic performance in maize can be applied to MAS for simultaneously improving drought tolerance and crop yield.

Although previous research considerably improved our understanding of leaf photosynthetic performance [3, 18] as well as its applications in maize MAS [2, 12], few studies considered the genetic basis of maize photosynthetic traits under water deficit stress at the molecular level [11–13]. Based on the above considerations, in this study we detected 54 QTLs for six photosynthetic traits across two F4 populations via single watering environment mapping with CIM. The QTLs identified for P_n , G_s , and RuBP showed both additive and non-additive effects in DS and WW environments, but non-additive effects were largely responsible for the genetic basis of these three traits, accounting for 95.24, 81.82, and 94.12% of the phenotypic variance, respectively. However, all identified QTLs for C_i , Tr and WUE displayed non-additive effects in both DS and WW environments. These results are consistent with previous studies on photosynthetic traits in maize [9, 19]. Furthermore, Pearson correlation analysis among F4 progenies and their parents showed that P_n , G_s , C_i , RuBP, and WUE of the two F4 populations depicted significantly positive correlation with the female parent, while Tr showed significantly positive correlation with both parents. This suggests that breeders should pay more attention to the evaluation of F1 cross combinations to effectively utilize their prominent non-additive effects and specific combining ability for the abovementioned six photosynthetic traits. More attention should also be given to the selection of parents with elite photosynthetic traits to improve these traits under drought and non-stressed environments. Additionally, 43 of the 54 identified QTLs were identified in the DS environment, suggesting that these QTLs controlling P_n , G_s , C_i , Tr , RuBP, and WUE could change under contrasting environments, and QTLs identified under drought stress may directly lead to differences in P_n , G_s , C_i , Tr , RuBP, and WUE.

GEI is critical for determining the adaptation ability and fitness of genotypes in adverse environments, resulting in phenotypic variation [14]. Therefore, QEI data is of great value for breeders and geneticists [16]. Furthermore, photosynthetic traits showed wide variation in the two F4 populations; six photosynthetic traits showed significant σ_g^2 , σ_e^2 , and σ_{ge}^2 ($P < 0.05$), with h_{ge}^2 ranging from 1.466–19.770%. Further analysis of QEIs in two F4 populations with MCIM in all watering environments also suggested that 24 QEIs

(44.44%) of the identified joint QTLs controlling P_n , G_s , C_i , Tr , RuBP, and WUE were mapped, and each QEI explained 1.3–6.86% of the phenotypic variance by $h^2(\text{AE})$ in the present study. This implies that photosynthetic traits are similar to other traits, such as yield and leaf and inflorescence architecture, in maize [15–17], of which showed extensive GEIs. Thus, GEIs may be a major challenge for the MAS of photosynthetic traits in maize.

Epistatic interactions between genetic loci are also thought to contribute to the variation in photosynthetic performance [19]. In maize, Li et al. [19] reported that chlorophyll relative content is controlled by two major genes with AD-epistatic effects and polygene with AD effects by mixed major gene plus polygene genetic model. Liu et al. [20] also suggested that chlorophyll relative content is consistent with the AD-epistatic model using Griffing's diallel cross II. In accordance with previous studies [19, 20], we identified 14 pairs of epistatic interactions, with DA and DD effects, controlling P_n , G_s , C_i , Tr , RuBP, and WUE in different watering environments in the two F4 populations. These epistatic interactions accounted for 2.09–5.87% of the observed phenotypic variance contributed by $h^2(\text{DA/DD})$, which was lower than the A effects of all photosynthetic traits. We conclude that low contributions to phenotypic variance by DA/DD effects were caused by a large number of DA/DD-QTLs with minor genetic effects, which would significantly influence the efficiency of MAS of photosynthetic traits. Notably, two stable DD-epistatic interactions were also validated in our study: one controlling P_n between bin 1.07_1.08_1.10 (bnlg1025-mmc0041-phi308707) and bin 10.03 (bnlg1655-umc2016/umc1345), which was repeatedly detected in POP-CT in both DS and WW environments and in POP-LT in the DS environment, and another affecting C_i between bin 1.08_1.10 (mmc0041-phi308707) and bin 6.05 (umc2040-bnlg1174a) in the two F4 populations under both all four environments. These results are consistent with previous studies [14, 21], which indicated that an AD/additive-additive epistatic interaction for KW, plant height, and ear length is located on Chromosome 1 and Chromosome 6/10 under multiple drought and non-drought environments. Hence, these chromosomal regions may be considered as epistatic regulators of plant development, photosynthesis, and ear formation in maize under multiple watering regimes.

The identification of cQTLs controlling photosynthetic traits in a broad genetic background under drought stress could facilitate fine mapping and MAS in maize. In this study, we identified a total of eight cQTLs affecting six photosynthetic traits using CIM/MCIM across POP-CT and POP-LT under multiple watering environments.

Five of these cQTLs were simultaneously associated with multiple traits in two F4 populations in both watering environments: cQTL2 (bin 1.07_1.10;

bnlg1025/mmc0041-phi308707/umc1847) with P_n , C_i , Tr , and WUE; cQTL5 (bin 6.05; umc2141-umc2040-bnlg1174a) with P_n , Tr , C_i , and RuBP; cQTL6 (bin 7.02_7.04; umc2057-bnlg1666-umc1708) with P_n and RuBP; cQTL7 (bin 8.03; bnlg1863-umc2075) with C_i , Tr , and WUE; cQTL8 (bin 10.03; bnlg1655-umc2016/umc1345) with P_n , Tr , and RuBP. These data indicate that these cQTLs have pleiotropic effects, which was consistent with the Pearson correlation analysis among six photosynthetic traits in both watering environments (Fig. S3). Consistent with our study, Pelleschi et al. [12] detected multiple QTLs in bin 1.08_1.10 (umc83a-umc39c), bin 6.05 (near gsy298e_pmg), bin 7.03_7.04 (gsy113_cs-gsy107_pr), and bin 10.03 (gsy321_aba-gsy329_pp) associated with photosynthetic traits (sucrose-P synthase, hexoses, sucrose, ADPglucose pyrophosphorylase, net CO₂-uptake, and Tr), leaf morphological traits (leaf number, leaf width, leaf length, and relative water content) among 120 F-2 × MBS847 RILs under drought and non-drought conditions. Li et al. [22] also mapped multiple QTLs involved in chlorophyll relative content in bin 1.08 (umc1013-umc2047), bin 7.02 (umc1585-bnlg1305), bin 8.03 (bnlg1863-bnlg2046/umc2075-phi100175), and bin 10.03 (bnlg1655) in 172 Xu172 × Zong3 single segment substitution lines under both high and low nitrogen treatments. Wang and Zhang [9] identified two pleiotropic QTLs, which were simultaneously responsible for chlorophyll *a* content, chlorophyll *b* content, and total chlorophyll content in bin 1.08 (mmc0041-bnlg1556) and bin 8.03 (umc1457-umc2199), using 189 A150-3-2 × Mo17 F2 plants under a single environment. Peng et al. [23] found a QTL affecting grain yield per plant near phi308707 (bin 1.10), a stable QTL controlling grain yield per plant and kernel number per plant located in bnlg1094-bnlg1579 (bin 7.02_7.03), and a QTL affecting 100-kernel weight in bin 8.03 (bnlg1352-umc1778) among 230 Qi319 × Huangzaosi and 235 Ye478 × Huangzaosi F_{2,3} families in six environments. These results indicate that pleiotropic cQTLs in bins 1.07_1.10, 6.05, 7.02_7.04, 8.03, and 10.03 play critical roles in leaf development, photosynthesis, and yield formation in maize under contrasting water availability conditions, and these bins may contain important genes. A total of 11 candidate genes were also confirmed at the abovementioned five pleiotropic cQTLs, as expected. These include *GRMZM2G018627* (*LHCB9*), which encodes a light-harvesting chlorophyll-binding (LHCB) protein involved in the maintenance of PSI and specific protein–chlorophyll complexes, especially under certain stress conditions [24]; *GRMZM2G162672* (*chlorophyll synthase G1*; *chlg1*), which encodes a chlorophyll biosynthesis protein, as shown by GO analysis via the AgBase v. 2.00 (<http://agbase.arizona.edu/>) online software; *GRMZM2G039113* (*tangled 1*; *tan1*), which is required for the spatial control of cytoskeletal arrays associated

with cell division during maize leaf development, and for cell division orientation during leaf development without altering leaf shape as well as for maize photosynthesis capacity; *GRMZM2G013657* (*dwarf & irregular leaf 1*; *dwil1*), which regulates leaf base, tip, and sheath development in maize (https://maizegdb.org/gene_center/gene); *GRMZM5G809292* (*PYG7*), which encodes a tetratricopeptide repeat domain-containing protein, is a component of the chloroplast and thylakoid membrane in mesophyll component involved in the assembly of PSI, as shown by GO analysis; *GRMZM2G042592*, encoding thioredoxin-like 6, which interacts with the CHL1 subunit of Mg²⁺ chelatase to regulate the chelation of Mg²⁺ chelatase and the chlorophyll precursor protoporphyrin IX [25]; *GRMZM2G042592*, whose mutant shows abnormal chloroplast, lack of pigments, and reduced PSII [26]; *GRMZM2G163437* (*ADP glucose pyrophosphorylase small subunit leaf 1*; *agps11*), which is expressed in maize grain and source leaf during grain filling; *GRMZM2G033885* (*psb29*), encoding photosystem II subunit 29, a part of a novel mechanism of photoprotection, which is reversibly phosphorylated in maize upon exposure to high light intensity under cold stress, and its phosphorylation is dependent on the redox state of the plastoquinone pool; and *GRMZM2G045431* (*bHLH150*) and *GRMZM2G058451* (*bHLH164*), which encode bHLH transcription factors that play important roles in various developmental processes in maize, such as, root differentiation, photomorphogenesis and light signal transduction, and stress response.

We also noted that cQTL3 in bin 3.07_3.08 (umc1286/umc2275-umc2081) affected G_s in two F4 populations in both DS and WW environments. Li et al. [22] reported a QEI for chlorophyll relative content in bin 3.08 (umc1844-bnlg1182) under high nitrogen condition, and Guo et al. [27] reported a meta-QTL (mQTL; bin 3.08) for leaf width in 28 original populations under multiple environments via mQTL analysis. Further analysis validated two candidate genes, *GRMZM2G159937* (*bHIH57*) and *GRMZM2G117851* (*bZIP1*), in bin 3.07_3.08 in our study. *GRMZM2G159937* may have all the functions of *bHLH* transcription factor family. Previously, Wallappa et al. [28] reported a basic leucine zipper (*bZIP*) transcription factor, *ZBF2*, which regulates blue light-mediated photomorphogenic growth in *Arabidopsis thaliana*. This suggests that *GRMZM2G117851*, also as a *bZIP* transcription factor, plays an important role in maize photomorphogenesis.

Additionally, we also identified two new cQTLs (cQTL1 and cQTL4) in this study. Among these, cQTL1, which controls WUE, was identified by CIM/MCIM in bin 1.00_1.01 (bnlg149-bmc1014/umc1177) in both populations. At cQTL1, *GRMZM2G042250* (*rolled leaf 2*; *rld2*.) was predicted, and its orthologous gene *ATHB23* (which encodes a phytochrome B-interacting protein), plays an important role in phytochrome

B-mediated red light signaling in *Arabidopsis* [29]. The other cQTL, cQTL4, is responsible for C_i and was identified in bin 4.08_4.09 (umc2041-umc2188/umc2287) with CIM in both populations in DS and WW environments. At cQTL4, three genes were identified including *GRMZM2G446426* (*MADS52*), *GRMZM2G038479* (*bHLH8*), and *GRMZM2G074122* (*pep3*). *GRMZM2G446426* encodes the *MADS52* transcription factor, which affects chlorophyll content and osmotic stress response, similar to *ZmMADS4* [30]. *GRMZM2G074122* encodes phosphoenolpyruvate carboxylase isoform 1 (PEPC1), which is involved in carbon fixation and the tricarboxylic acid cycle and influences PEPC activity as shown by GO analysis. These two cQTLs may explain the genetic basis of the photosynthetic performance of maize under both DS and WW conditions.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving animals or human participants as objects of research. The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Authors X.Q. Zhao and Y. Zhong contributed equally to this work.

SUPPLEMENTARY MATERIAL

Supplementary materials are available for this article at <https://doi.org/10.1134/S1021443721060236> and are accessible for authorized users.

REFERENCES

1. Zhao, X.Q., Ren, B., Peng, Y.L., Xu, M.X., Fang, P., Zhuang, Z.L., Zhang, J.W., Zeng, W.J., Gao, Q.H., Ding, Y.F., and Chen, F.Q., Epistatic and QTL \times environment interaction effects for ear related traits in two maize (*Zea mays*) populations under eight watering environments, *Acta Agron. Sin.*, 2019, vol. 45, p. 856.
2. Liu, J., Guo, Y.Y., Bai, Y.W., Li, H.J., Xue, J.Q., and Zhang, R.H., Response of photosynthesis in maize to drought and re-watering, *Russ. J. Plant Physiol.*, 2019, vol. 66, p. 424.
3. Zhao, X.Q., Lu, Y.T., Bai, M.X., Xu, M.X., Peng, Y.L., Ding, Y.F., Zhuang, Z.L., Chen, F.Q., and Zhang, D.Z., Response analysis of maize genotypes with different plant architecture to drought stress, *Acta Pratacult. Sin.*, 2020, vol. 29, p. 149.
4. Zhang, R.H., Zhang, X.H., Camberato, J.J., and Xue, J.Q., Photosynthetic performance of maize hybrids to drought stress, *Russ. J. Plant Physiol.*, 2015, vol. 62, p. 788.
5. Liu, J., Guo, Y.Y., Bai, Y.W., Camberato, J.J., Xue, J.Q., and Zhang, R.H., Effects of drought stress on the photosynthesis in maize, *Russ. J. Plant Physiol.*, 2018, vol. 65, p. 849.
6. Pshenichnikova, T.A., Doroshkov, A.V., Osipova, S.V., Permyakov, A.V., Permyakova, M.D., Efimov, V.M., and Afonnikov, D.A., Quantitative characteristics of pubescence in wheat (*Triticum aestivum* L.) are associated with photosynthetic parameters under conditions of normal and limited water supply, *Planta*, 2019, vol. 249, p. 839.
7. Vivitha, P., Raveendran, M., Vijayalakshmi, C., and Vijayalakshmi, D., Genetic dissection of high temperature stress tolerance using photosynthesis parameters in QTL introgressed lines of rice cv. improved white Ponni, *Ind. J. Plant Physiol.*, 2018, vol. 23, p. 741.
8. Levi, A., Lianne, O., Paterson, A.H., and Saranga, Y., Photosynthesis of cotton near-isogenic lines introgressed with QTLs for productivity and drought related traits, *Plant Sci.*, 2009, vol. 177, p. 88.
9. Wang, A.Y. and Zhang, C.Q., QTL mapping for chlorophyll content in maize, *Hereditas*, 2008, vol. 30, p. 1083.
10. Trachsel, S., Messmer, R., Stamp, P., Ruta, N., and Hund, A., QTLs for early vigor of tropical maize, *Mol. Breed.*, 2010, vol. 25, p. 91.
11. Yu, T.T., Liu, C.X., Mei, X.P., Wang, J.G., Wang, G.Q., and Cai, Y.L., Correlation and QTL analyses for photosynthetic traits in maize, *J. Southwest Univ.*, 2015, vol. 37, p. 1.
12. Pelleschi, S., Leonardi, A., Rocher, J.-P., Cornic, G., de Vienne, D., Thevenot, C., and Prioul, J.-L., Analysis of the relationships between growth, photosynthesis and carbohydrate metabolism using quantitative trait loci (QTLs) in young maize plants subjected to water deprivation, *Mol. Breed.*, 2006, vol. 17, p. 21.
13. Prado, S.A., Cabrera-Bosquet, L., Grau, A., Coupel-Ledru, A., Millet, E.J., Welcker, C., and Tardieu, F., Phenomics allows identification of genomic regions affecting maize stomatal conductance with conditional effects of water deficit and evaporative demand, *Plant Cell Environ.*, 2018, vol. 41, p. 314.
14. Zhao, X.Q., Zhang, J.W., Fang, P., and Peng, Y.L., Comparative QTL analysis for yield components and morphological traits in maize (*Zea mays* L.) under water-stressed and well-watered conditions, *Breed. Sci.*, 2019, vol. 69, p. 621.
15. Zhao, X.Q., Fang, P., Zhang, J.W., and Peng, Y.L., QTL mapping for six ear leaf architecture traits under

- water-stressed and well-watered conditions in maize (*Zea mays* L.), *Plant Breed.*, 2018, vol. 137, p. 60.
16. Zhao, X.Q., Peng, Y.L., Zhang, J.W., Fang, P., and Wu, B.Y., Mapping QTLs and meta-QTLs for two inflorescence architecture traits in multiple maize populations under different watering environments, *Mol. Breed.*, 2017, vol. 37, p. 91.
 17. Zhao, X.Q., Peng, Y.L., Zhang, J.W., Fang, P., and Wu, B.Y., Identification of QTLs and meta-QTLs for seven agronomic traits in multiple maize populations under well-watered and water-stressed conditions, *Crop Sci.*, 2018, vol. 58, p. 507.
 18. Zhou, L., Wang, S.Q., Chi, Y.G., Li, Q.K., Huang, K., and Yu, Q.Z., Response of photosynthetic parameters to drought in subtropical forest ecosystem of China, *Sci. Rep.*, 2015, vol. 5, p. 18254.
 19. Li, H.T., Xu, H.Y., Li, J.F., Zhu, Q., Chi, M., and Wang, J., Analysis of gene effect on chlorophyll content in maize, *Crops*, 2019, vol. 5, p. 46.
 20. Liu, P.F., Jiang, F., Chen, Q.C., Zeng, M.H., Zhang, Y., Zhang, Z.L., and Wang, X.M., Genetic analysis on chlorophyll content of leaf in fresh-eatable sweet corn, *J. Anhui Agric. Univ.*, 2013, vol. 40, p. 134.
 21. Sa, K.J., Park, J.Y., Woo, S.Y., Ramekar, R.V., Jang, C.S., and Lee, J.K., Mapping of QTL traits in corn using a RIL population derived from a cross of dent corn \times waxy corn, *Genes Genome*, 2015, vol. 37, p. 1.
 22. Li, Y.D., Wang, Y., Tang, H., Xu, H., Tan, J.F., and Han, Y.L., QTL mapping for ear leaf stay-green in maize under high and low N conditions, *J. Plant Nutr. Fertil.*, 2019, vol. 25, p. 115.
 23. Peng, B., Wang, Y., Li, Y.X., Liu, C., Zhang, Y., Liu, Z.Z., Tan, W.W., Wang, D., Sun, B.C., Shi, Y.S., Song, Y.C., Wang, T.Y., and Li, Y., Correlation analysis and conditional QTL analysis of grain yield and yield components in maize, *Acta Agron. Sin.*, 2010, vol. 36, p. 1624.
 24. Zhao, L., Cheng, D.M., Huang, X.H., Chen, M., Osoto, L.D., Xing, J.L., Gao, L.Y., Li, L.Y., Wang, Y.L., Bassi, R., Peng, L.W., Wang, Y.C., Rochaix, J.D., and Huang, F., A light harvesting complex-like protein in maintenance of photosynthetic components in *Chlamydomonas*, *Plant Physiol.*, 2017, vol. 174, p. 2419.
 25. Luo, T., Fan, T., Liu, Y., Rothbart, M., Yu, J., Zhou, S., Grimm, B., and Luo, M., Thioredoxin redox regulates ATPase activity of magnesium chelatase CHLI subunit and modulates redox-mediated signaling in tetrapyrrole biosynthesis and homeostasis of reactive oxygen species in pea plants, *Plant Physiol.*, 2012, vol. 159, p. 118.
 26. Wang, F., Duan, S.M., Li, T., Wang, R.N., and Tao, Y.S., Fine mapping and candidate gene analysis of leaf color mutant in maize, *J. Plant Genet. Resour.*, 2018, vol. 19, p. 1205.
 27. Guo, S.L., Zhang, J., Qi, J.S., Yue, R.Q., Han, X.H., Yan, S.F., Lu, C.X., Fu, X.L., Chen, N.N., Ku, L.X., and Tie, S.G., Analysis of meta-quantitative trait loci and their candidate genes related to leaf shape in maize, *Chin. Bull. Bot.*, 2018, vol. 53, p. 487.
 28. Wallappa, C., Yadav, V., Negi, P., and Chattopadhyay, S., A basic leucine zipper transcription factor, G-box-binding factor 1, regulates blue light mediated photomorphogenic growth in *Arabidopsis*, *Biol. Chem.*, 2006, vol. 281, p. 22190.
 29. Choi, H., Jeong, S., Kim, D.S., Na, H.J., Ryu, J.S., Lee, S.S., Nam, H.G., Lim, P.O., and Woo, H.R., The homeodomain-leucine zipper *ATHB23*, a phytochrome B-interacting protein, is important for phytochrome B-mediated red light signaling, *Physiol. Plant*, 2014, vol. 150, p. 308.
 30. Schreiber, D.N., Bantin, J., and Dresselhaus, T., The *MADS* box transcription factor *ZmMADS2* is required for anther and pollen maturation in maize and accumulates in apoptotic bodies during anther dehiscence, *Plant Physiol.*, 2004, vol. 134, p. 1069.