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Full Length Article

Correlation Analysis of Yield and Photosynthetic Traits with Simple Repeat Sequence (SSR) Markers in Maize

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Abstract

In order to obtain high yield and photosynthetic utilization rate maize hybrids, and develop the functional markers which associated with 10 yield and photosynthetic traits (leaf number of plant, chlorophyll content of three-ear-leaves, leaf area of ear leaf, leaf area of three-ear-leaves, leaf area index, plant height, ear height, ear length, row number of ear and 100 seed weight), 64 pairs of SSR markers were used to genotype the population of 257 maize inbred lines, analyzed the linkage disequilibrium loci and population structure, and determine the contribution rate of markers to phenotypic traits. The results showed that: (1) SSR markers displayed a certain extent of linkage disequilibrium (LD) in the public map. (2) Genetic structure analysis of SSR data demonstrated that the gene population can be divided into 5 subgroups. (3) 18 marker loci were identified to associate with 10 traits, in which 4 loci concentrated in chromosome 9. Seven marker loci were found to associated with 5 photosynthesis traits (leaf number of plant, chlorophyll content of three-ear-leaves, leaf area of ear leaf, leaf area of three-ear-leaves, leaf area index), which were bnlg439, bnlg2291, umc1524, bnlg1154, umc1545, umc1231 and bnlg1450. Seven loci were identified to associated with 5 yield traits (plant height, ear height, ear length, row number of ear and 100 seed weight), which were bnlg1175, umc1946, umc1496, bnlg249, umc2084, bnlg1191 and umc2122. There were other 4 markers loci (umc1065, phi116, bnlg162, phi065) were found associated with both yield traits and photosynthesis traits. The contribution rates of any locus to phenotypic date range from 0.0566 to 0.2145. Our results indicated that SSR marker could be useful for genotyping the population of maize inbred lines. Correlation analysis was useful to identify the markers which associated with phenotypic traits, which can be used for molecular maker assisted selection to improve the breeding efficiency. © 2017 Friends Science Publishers

Keywords: Maize; SSR; Correlation analysis; Yield; Photosynthetic traits

Introduction

Crop yield and photosynthetic traits basically belong to quantitative traits. Understanding and mastering crop genotype is the premise for the scientific research and breeding of new hybrids. Currently, the main method to study plant quantitative traits is drawing genetic linkage map using different molecular markers and proper segregation populations (Houmanat *et al.*, 2016; Wang *et al.*, 2017). However, there are some shortages about genetic linkage mapping, including difficult in constructing appropriate segregating population in short time, and only could involve two allele loci. In addition, the gene recombination times were relatively less during obtaining separation populations, which may limit the accuracy of mapping (Qi *et al.*, 2014).

To overcome these shortages of genetic linkage mapping, a correlation analysis method for genetic linkage and recombination was developed, a correlation analysis of LD (Linkage Disequilibrium) mapping for traits and specific alleles based on disequilibrium of genetic linkage (Gupta *et al.*, 2005; Yang *et al.*, 2007; Li *et al.*, 2016). Compared to linkage mapping, the advantage of correlation analysis included that shorter term required to obtain population, requirement of fewer certain molecular markers, wider detection range, capability of scanning multiple specific alleles simultaneously, wider range for material selection (natural population was also acceptable), and higher detection accuracy(Miao *et al.*, 2010).

Thornsberry *et al.* (2001) analyzed the relation between *Dwarf8* and maize flowering period using correlation analysis which introduced the method to maize. Aranzana *et al.* (2005) studied the flowering period-related gene *FRI* and anti-disease genes (*Rpm, Rps5, Rps2)* of

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arabidopsis (*Arabidopsis thaliana* L.) using whole genome correlation analysis. In recent years, many achievements of correlation analysis of important traits of soybean (*Glycine max* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), chrysanthemum (Chrysanthemum× morifolium) and cassava (Manihot esculenta crantz) have been acquired using whole genome correlation analysis method (Jun *et al.,* 2008; Wen *et al.,* 2008; Dou *et al.,* 2009; Wang *et al.,* 2010; He *et al.,* 2010; Li *et al.,* 2012; Amjid *et al.,* 2015). The candidate genes related to maize ear height and row number of ear have been explored (Li *et al.,* 2013; Zhang *et al.,* 2014). With the completion of whole genome sequencing of many crops, the method of correlation analysis has been increasingly widely applied in quantitative traits positioning and molecular breeding.

Photosynthetic traits also belong to quantitative trait controlled by multiple genes, which has complex genetic basis and vulnerable to environment factors. In recent years, genetic linkage mapping has been usually used for QTL mapping of photosynthetic traits (chlorophyll content, leaf area, photosynthetic rate) in many crop (soybean, wheat, rice, and maize) (Li *et al.,* 2010; Mao, 2010; Kong and Hu, 2010; Cai *et al.,* 2011; Wang, 2012; Yu *et al.,* 2015; An *et al.,* 2016). However, very few researches have reported the QTL mapping of photosynthetic traits by correlation analysis. With advantages of high polymorphism, co-dominance, easy to detect, SSR marker has been widely applied in molecular marker assisted breeding.

In this study, 64 pairs of SSR markers and 257 maize inbred lines with abundant genetic basis were used to analyze the correlation of SSR markers, yield and photosynthetic traits. The population structure of maize inbred line was analyzed by whole genome correlation analysis based on linkage disequilibrium and analysis of genetic structure. The markers, which associated with yield and photosynthetic traits were determined. The gene areas responsible for plant height, ear height, 100-seed weight, chlorophyll content, leaf area, leaf area index and other important yield and photosynthetic traits were mapped, which contributed to explore and apply excellent alleles, promote the genetic modification and innovation of maize germplasm, and speed up the breeding of new maize hybrids.

Materials and Methods

Materials

In this research 257 excellent maize inbred lines were selected, which were mainly originated from northeast region of China (88), Hebei province (11), Henan province (15), Shangdong province (9), Shan'xi province (7), Sichuan province (8), Yunnan and Guizhou province (6), overseas (10), Beijing (14), maize department of Crop Research and Breeding Center of Land Reclamation of Heilongjiang Province (75) and other known sources (14).

Methods

Detection of yield and photosynthetic traits: The experiments were conducted at test site of Heilongjiang agricultural reclamation and scientific research breeding center from 2013 to 2015. The maize inbred lines were randomly planted, with 2 lines in each block, 5 m long of each line, repeating for 3 times. Consecutive 5 plants in the middle were selected for test material, and conducted field investigation and indoor analysis of the traits such as plant height, ear height, ear length, row number of ear, 100 seed weight, and growing period; during silking period. We also selected 3 plants of each row for photosynthetic traits measurement, where in the chlorophyll content was measured using portable SPAD-502, single leaf area=leaf length × leaf width×0.75, whole plant leaf area=sum of single leaf areas, leaf area index=total leaf areas in planting block/planting block area.

SSR Analysis

Young fresh leaves of 3–5 plants were selected at three-leaves stage to extract DNA using CTAB, and all extracted DNA were detected. DNA mother liquor was diluted to 10 ng/μL (working solution concentration). 100 pairs of SSR primers covering the whole maize genome were selected for screening. All gene sequence information was accessed from http://www.maizegdb. org. By preliminarily screening, 64 SSR markers were determined to use in this study (Table 1).

PCR reaction system: Every 10 μL of PCR reaction system contained 1.3 μL 10×Buffer, 1.0 μL (2.5 mM) dNTP, 0.3 μL (25 μM) Primer, 0.3 μL (5 U/μL) Taq enzyme, 1 μL (10 ng/μL) DNA, and 6.1 μL ddH_2O. PCR reaction procedures include pre-degeneration at 95℃ for 5min, degeneration at 95℃ for 30s, annealing(different marker sites) at 55–57℃ for 30s, extending at 72℃ for 30s, repeating 35 times, extending at 72℃ for 5 min, stop reaction at 10℃, storage at 4℃. PCR products were dected using 6% denatured polyacrylamide gel electrophoresis. With regarding to the observation and statistics of expand spectrum bands, the spectrum bands were marked from up to bottom with number 1, 2, 3, 4……N according to the molecular weights of expanded products from large to small.

Data Analysis

The statistic function of Excel was used to analyze the max value, min value, average value, kurtosis, and skewness of photosynthetic and yield traits.

Coefficient of variable (CV%) = standard deviation/average value×100.

Software STRUCTURE2.2 was use to divide test groups (Falush *et al*., 2003), determine the number of subgroups (k value) (Evanno *et al*., 2005), characterize SSR sites of each group by a set of allelic variation frequency, and calculate Q values of individuals in each subgroup (the variation of individual specific allele stems from the frequency of certain subgroup). Software TASSEL was used to detect LD distribution status and levels of SSR sites, calculate matrix graph of LD matching detection and observe the LD distribution between collineation SSR sites and non-collineation SSR sites (Bradbury *et al*., 2007). The D′ values of all possible site combinations were calculated according to related equations. Chromatic aberration was introduced to make D′values in matching matrix graph more intuitively reflected. GLM procedure in TASSEL software was used to identify the marker sites and corresponding contribution rates of all yield and photosynthetic traits. The test observed the significant correlation standard at $P<0.01$.

Results

Variation and Distribution of Phenotypic Traits

The variation and distribution of completed phenotypic traits of test materials were shown in Table 2. From this figure we can see that the skewness and kurtosis meet standard level, the coefficients of variables could be sequenced from large to smallas chlorophyll content of three-ear-leaves＞ear height＞leaf area index＞100 seed weight＞ leaf area of three-ear-leaves＞ear height＞ear length＞row number of ear＞leaf number of plant ＞plant height. The results showed that the test maize inbred lines show relatively large genetic difference, with abundant genetic basis excellent allelic variation may involve in the group of test materials (Table 2).

LD Analysis of SSR Loci

Normally, linkage unbalance coefficient D’ was adopted to measure LD degree, which varies from 0 (no LD) - to 1 (totally LD). The results of Fig. 1 showed the linkage disequilibrium (LD) of SSR markers in linkage group of test maize inbred lines, where D’ value showed above diagonal line. The support probability of site pairs LD showed under diagonal line, and the square color was consistent with the right side color code. Markers of higher LD levels were distributed on linkage group of "Ch6, Ch7 and Ch9", which including the combination of these several sites. The numbers of SSR sites used for scanning of these groups were 8, 9 and 9, respectively. The combination number of all SSR marker sites used for scanning was 2016. No matter co linearity combination (the same chromosome group) or non co-linearity combination (different chromosome group), there always existed LD in certain degree, shown as the non-white square above diagonal line in Fig. 1.

The number of sites related to D’value and its proportion of total number of sites were shown in Table 3. Of all 2016 combinations of site pairs, the number of sites over $P<0.01$ (the non-white square under diagonal line in Fig. 1) was 393, accounting for 19.49%.

Group Structure Analysis of Materials

On mathematical model, the group structure of test materials was analyzed using STRUCTURE software, and the number of subgroups was determined. When K=5, the LnP(D) had the maximum value, therefore there were 5 subgroups of test maize inbred lines (See Fig. 2), and the test group has clear group structure. Using such software could achieve a quick and effective division of maize inbred line groups, and increased the usage efficiency of heterosis. To avoid the existence of group structure which can affect correlation analysis accuracy via affecting site LD, in this research, the Q values of all species (the probability of classifying individuals in all species into subgroups), as concomitant variables, were taken into the regression analyses of SSR marker and phenotypic traits variation.

The geographic ecological types of test materials, number of individuals in subgroup, and its proportion are given (Table 4). By conducting biological analysis of number of individuals in subgroups as well as the geographic sources (i.e. detection of independence), which can find the division of subgroups was independent from geographic ecological types of test materials($X^2=5.58$, less $X^2_{0.01,8}=20.09$), therefore it was suggested to select more representative test materials.

SSR Markers Associated with Phenotypic Traits

Of all SSR marker sites involved in detection, it was identified that 18 sites were correlated with 10 traits, wherein the accumulation number of yield traits correlated sites is 15, while the accumulation number of photosynthetic traits correlated sites was 18. The phi065 located at the 9th chromosome has the lowest contribution to plant height, which was 0.0566; while the bnlg1175 located at the 2nd chromosome has the highest contribution to ear height, which is 0.2145. In addition, the photosynthetic and yield traits correlated sites, phenotype, and contribution rate were respectively umc1065 (number of plant leaves, 0.1233; plant height,0.1219), (phi116 (number of plant leaves, 0.09; leaf area of ear leaf, 0.0789; leaf area of ear three leaves, 0.0736; leaf area index, 0.1126; plant height, 0.1504; ear height, 0.1118; ear length, 0.068; 100 seed weight, 0.0764) and most photosynthetic and yield traits correlation (excluding three leaf chlorophyll and row number of ear) bnlg162 (leaf area of ear three leaves, 0.076; plant height, 0.0916); phi065 (number of

Table 1: SSR markers used for the whole genome scanning

Linkage Group	Position (cM)	Locus	Linkage Group	Position (cM)	Locus	Linkage Group	Position (cM)	Locus
Ch1	170.1	bnlg1007	Ch5	560.0	bnlg2305	Ch8	416.0	bnlg240
	259.1	bnlg439		641.4	umc1225		483.4	bnlg1823
	596.0	umc1335	Ch6	23.2	bnlg161		571.5	phi080
	700.5	bnlg1025		98.4	bnlg249		609.1	phi233376
	842.3	umc2047		220.1	mmc0523	Ch9	0.0	umc1957
	913.4	bnlg1671		253.0	bnlg1154		65.0	umc2084
Ch2	154.8	bnlg125		320.7	bnlg1702		142.6	bnlg244
	216.5	umc1185		450.7	phi299852		226.0	phi065
	295.1	bnlg1175		502.9	umc2165		308.0	umc1492
	368.1	umc1065		548.7	umc1127		342.0	umc1231
	394.6	umc1946	Ch7	55.0	umc1545		461.6	bnlg1191
Ch3	2.0	umc2105		118.5	umc2160		603.5	umc1137
	511.5	bnlg197		155.8	umc1016		805.1	umc1277
	757.0	bnlg1754		190.4	bnlg1792	Ch10	30.9	phi041
	835.9	umc1136		392.1	bnlg1805		86.8	bnlg1451
Ch4	116.4	umc1294		444.7	umc1029		120.1	umc1432
	237.8	bnlg490		472.9	phi328175		217.8	bnlg1712
	392.2	bnlg2291		530.0	phi260485		392.5	umc2122
	670.2	bnlg589		611.5	phi116		483.7	bnlg1450
	737.8	umc1058	Ch8	269.9	umc1741		505.5	bnlg1185
Ch5	49.8	umc1496		337.2	umc1309			
	493.5	umc1524		367.0	bnlg162			

Table 2: Variation and distribution of ten phenotypic traits in maize

Traits	Minimum	Maximum	Mean	CV%	Skewness	Kurtosis
leaf number of plant	7.0	15.0	11.3	14.1	-0.1	-0.3
chlorophyll content of three-ear-leaves	13.9	73.3	40.7	29.8	0.1	-0.4
leaf area of ear leaf	359.4	940.5	635.3	17.4	0.0	-0.1
leaf area of three-ear-leaves	726.3	2646.4	1551.4	20.8	0.3	0.5
leaf area index	1.6	4.2	2.8	22.2	0.2	-0.9
plant height	110.0	258.0	183.1	14.1	0.0	-0.1
ear height	22.0	150.0	61.8	26.9	0.8	2.8
ear length	8.4	20.5	13.4	17.0	0.2	0.0
row number of ear	10.0	22.0	13.9	15.0	0.6	1.5
100-seed weight	11.8	38.8	22.9	22.2	0.3	0.0

Table 3: LD of SSR markers

Rate of D'>0.5	Number of LD locus pairs	Freq. dis. of D'(p<0.01)			Mean of D′
		0-0.2	0.21-0.4	0.41-0.6	
3(0.15%)	393(19.49%)	44(2.18%)	343(17.01%)	6(0.30%)	0.2587

Table 4: Geographic ecotype and population structure analysis of maize inbred

Geographic ecotype	Sub-population					
	Q1	Q2	Q3	Q4	Q5	Total
Northern spring maize region	78	67	57	17	9	228
Huanghuaihai summer maize region	5	3	4	2	1	15
Southwest maize region	3	2	5	2	2	14
Number of sub-population	86	72	66	21	12	257
Rate of sub-population,%	33.5	28.0	25.7	8.1	4.7	

plant leaves, 0.1126; leaf area of ear leaf, 0.0792; leaf area of ear three leaves, 0.0799; leaf area index, 0.1253; plant height, 0.0566).

Eighteen phenotypic traits correlated SSR sites were unevenly distributed on 10 chromosomes (No. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10), wherein the 9th chromosome had the most markers, which was 4. There can be see one site correlating with multiple traits (phi116 and phi065) and also multiple sites correlating with multiple traits. The maize phenotypic traits were correlated with internal genetic factors.

Analysis of Yield Traits Correlated Loci

There were 6 plant height correlated sites, including

Table 5: Loci data and their explained phenotypic variation of SSR markers associated with photosynthesis and agronomic traits

Markers	Chr.	Photosynthesis trait					Agronomic trait				
		Leaf number of plant	Chlorophyll content of three-ear-leaves	Leaf area of ear leaf	Leaf area of three-ear-leaves	Leaf Area index	Plant height	Ear height	Ear length	Row number of ear	100-seed weight
bnlg439	1.03				0.1235						
bnlg1175	2.04							0.2145			
umc1065	2.05	0.1233					0.1219				
umc1946	2.07						0.0738				
bnlg2291	4.06		0.0749								
umc1496	5.00							0.0782			
umc1524	5.06				0.081	0.1041					
bnlg249	6.01										0.1124
bnlg1154	6.05			0.0916							
umc1545	7.00					0.0711					
phi116	7.06	0.09		0.0789	0.0736	0.1126	0.1504	0.1118	0.068		0.0764
bnlg162	8.05				0.076		0.0916				
umc2084	9.01						0.0761	0.0648			
phi065	9.03	0.1126		0.0792	0.0799	0.1253	0.0566				
umc1231	9.05					0.0775					
bnlg1191	9.06									0.0728	
umc2122	10.06							0.0678			
bnlg1450	10.07			0.091		0.0925					

umc1065, umc1946, phi116, bnlg162, umc2084, phi065, with contribution rates of 0.1219, 0.0738, 0.1504, 0.0916, 0.0761, 0.0566, respectively. There were 5 maize ear position correlated sites, including bnlg1175, umc1496, phi116, umc2084, umc2122, with contribution rates of 0.2145, 0.0782, 0.1118, 0.0648, 0.0678, respectively. The maize ear length correlated site was phi116, with contribution rate of 0.068. The number of maize ear row correlated site was bnlg1191, with contribution rate of 0.0728. The 100 grain weight correlated sites were bnlg249, phi116, with contribution rates of 0.1124, 0.0764, respectively. The SSR marker of phi116 was significantly correlated with 4 yield traits, therefore it can excavate yield traits correlated major gene in the marked segment of the 7th chromosome. Of the 6 plant height correlated markers, umc1065 and umc1946 belong to the 2nd chromosome and the genetic distance between them was relatively short; umc2084 and phi065 locate at the 9th chromosome, between which the genetic distance was short. There may also exist gene controlling plant height in such marker segment, which remains to be further determined. The 5 maize ear position correlated sites and 2 hundred-grain weight correlated sites respectively come from different chromosomes, with very different contribution rates, wherein the probability for recombination and mutation was relatively larger; The sites correlated with ear length and row number of ear were in different chromosomes, with relatively lower contribution rates.

Analysis of Photosynthetic Traits Correlated Loci

Of photosynthetic traits, only maize ear three leaf chlorophyll was correlated with 1 marker (bnlg2291), with low contribution rate (0.0749), other 4 traits (number of whole plant leaves, maize ear position leaf area, maize ear three leaf area, leaf area index) were correlated with multiple markers simultaneously. The phi116 and phi65, locating on different chromosomes, were significantly correlated with the 4 photosynthetic traits, therefore there should exist function gene that controls the key process of photosynthesis in such marker segment. Of all 6 leaf area index correlated markers, umc1545 and phi116 belong to the 7th chromosome, while phi065 and umc1231 belong to the 9th chromosome, between two of each the genetic distance is short, there may exist function gene regulating leaf area indexes (i.e., controlling total leaf area). The three markers that were correlated with number of whole plant leaves are umc1065(0.1233), phi116(0.09), phi065(0.1126), which locate at the 2^{nd,} 7th and 9th chromosome, respectively. The four markers correlated with maize ear position leaf area were umc1154(0.0916), phi116(0.0789), bnlg065(0.0792), phi1450(0.091), and located at the 6th, 7th, 9th and 10th chromosome, respectively; The four markers correlated with maize ear three leaf area were umc1524(0.081), phi116(0.0736), bnlg162(0.076), phi065(0.0799), which locate at the 5th, 7th, 8th and 9th chromosome, respectively; The markers that were respectively correlated with number of whole plant leaves, maize ear position leaf area, maize ear three leaf area locate at different chromosomes, with different genetic distances, and there may exist gene recombination and mutation.

Discussion

According to Flint-Garcia *et al*. (2003) and Aranzana *et al.* (2005), we know that conducting whole genome correlation analysis for different species requires marker sites of human

(70 000), Arabidopsis (6 000), land race of maize (750 000), and excellent maize inbred line (50 000). Strictly speaking, conducting whole genome correlation analysis requires thousands of markers and individuals without genetic relationship as many as possible. In this study, although the number of SSR marker sites is less, the selected test materials have an extensive germplasm basis and larger difference. Conducting whole genome correlation analysis for the natural group consisting of the selected materials is conductive to further understanding the LD degree and distribution of maize whole genome. In principle, markers in high dense are needed for whole genome correlation analysis, and the group consisting of proper test materials is the key to the success of correlation analysis.

Thornsberry *et al.* (2001) detected maize whole genome using 47 SSR markers, holding that it is a feasible way to analyze maize group structure using SSR markers. Camus-Kulandaivelu *et al.* (2006) conducted correlation analysis between 650 maize inbred lines and locally grown D8 seats, which also shows that the group structure can affect the correlation analysis results. Selection of independent individuals and proper mating design are two key points for correlation analysis (Myles *et al.*, 2009). Yu *et al.* (2006) developed relevant statistical model which was used to control group structure, so that the probabilities of I error and II errors were reduced. LD occurs accompanying gene recombination and mutation, while decreases with the increases of decay time and genetic distance between genetic sites. In some special groups, LD exists in a very long distance. These groups are ideal groups which can be potentially used for high efficient correlation analysis and gene positioning.

To eliminate group structure's negative effect on correlation analysis accuracy and LD degree of marker sites, the probabilities of classifying test maize inbred lines into subgroups (Q values which have not been listed) were taken as concomitant variables into the regression analysis of SSR marker and traits variation. Using mathematics model to analyze group structure can eliminate the human factors in division of subgroups, which can avoid spurious correlation caused by subgroups mixing in certain degree. In addition, there is no correlation between the subgroups divided based on math model and the geographical ecology category of test maize inbred line, which is probably due to the test materials mostly come from northern spring maize area, those from HuangHuaiHai summer maize region and southwest maize region are less, while those from southern hills maize area and northwestern irrigated maize area are nearly none.

In this study, many markers that were strongly correlated with yield and photosynthetic traits concentrate in the same or adjacent loci. For example, regarding the plant height correlated markers, there are 2 from the 2nd chromosome, 2 from the 9th choromosome, and 2 from the adjacent seat of 7th and 8th chromosome; regarding leaf area index correlated markers, the 7th and 9th chromosome respectively provides 2; regarding maize cob leaf area correlated markers, the 7th and 8th chromosome respectively provides 1.

According to Yu *et al.* (2009), the QLT of maize yield and related traits were unevenly distributed on all linkage groups, with the most on the 1st linkage group, and the least on the 10th linkage group. QTL for ear length, axial diameter, row grains, grain weight, and grain yield are mainly distributed on the 1st chromosome, QTL for kernel rows is mainly distributed on the 9th chromosome, QTL for ear diameter and grain length are approximately evenly distributed on chromosomes. The results of this research are roughly consistent with of above studies that the 18 positioned sites are distributed on 10 chromosomes (No. 1, 2, 4, 5, 6, 7, 8, 9, 10), with the number of sites as 1, 3, 1, 2, 2, 2, 1, 4, 2, respectively. It can be seen that the 9th chromosome has the most sites, which is mainly due to that the 9th chromosome has relatively more SSR markers, and sites for correlation positioning are unevenly distributed.

Liu *et al.* (2010) took 218 F8 recombinant inbred lines (RIL) as mapping population, and conducted QTL mapping analysis for maize leaf area (three ear leaves). There were totally 7 leaf area correlated QTL sites being positioned, including 4 in 2006 and 3 in 2007. In the marker interval of umc1542-umc1518 on the 2nd chromosome, a main-effect QTL site was observed, which can be detected in both years, accounting for 12.5% and 17.3% of phenotypic variation. An *et al.* (2006) used 259 single-plant F2 as mapping population and positioned 36 leaf area QTL using CIM (composite interval mapping), which are mainly distributed on the 1st, 2nd, 3th, and 5th chromosome. In this study, the sites correlated with maize three area leaves located on the 1st, 5th, 7th, 8th and 9th chromosome, which play a complementary role with QTL positioning for excavating excellent alleles together.

Cai *et al.* (2011) performed QTL positioning for chlorophyll content of maize under different environments and detected a total of 17 QTL correlated with chlorophyll content, which were distributed on the 2nd, 3th, 4th, 6th, 7th, 8th, 10th chromosome; Wang (2012) detected 21 QTL controlling chlorophyll content, which were distributed on the 1st, 4th, 6th and 10th chromosome. Yu *et al.* (2015) analyzed Y group of maize, finding that the total chlorophyll mass fraction was distributed on the 4th and 10th chromosome in five leaf stage and milk ripe stage, respectively. In this study, the loci that were strongly correlated with three ear leaves chlorophyll locates on the 4th chromosome, is contribution rate of 0.0749, which is consistent with other QTL mapping results. By screening according to significant level of $P<0.01$ can eliminate more correlation markers, in order to improve test accuracy.

Through comprehensive comparison, it can be concluded that the excellent alleles controlling plant height locate at umc1065-umc1946 section of chromosome 2 and umc2084-phi065 section of chromosome 9; the excellent alleles controlling leaf area index (i.e. total leaf area) locate at umc1545-phi116 section of chromosome7 and phi065-

umc1231 section of chromosome9; phi116 and phi065 are significantly correlated with most photosynthetic traits and some of yield traits, which can be used to excavate excellent candidate alleles especially photosynthetic traits correlated alleles through combining with QTL mapping. Based on these obtained excellent alleles, specific PCR primers were designed for gene clone, so as to realize molecular mark assisted breeding.

Conclusion

The results of this study showed that using Structure software and based on mathematical model, which can quickly and effectively divide groups, and thus to improve the utilization efficiency of heterosis; By selecting natural groups consisting of materials with extensive genetic basis and through correlation analysis, which can quickly obtain phenotypic traits correlated SSR markers, so as to further excavate excellent alleles, reduce positioning cost, and promote commercialization; Combining with QTL positioning, molecular breeding was applied to provide basis for cloning certain gene(s) controlling excellent traits, so as to accelerate the pace for new variety breeding.

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