Received: 07 Jul 2020 | Revised: 10 Jul 2020 | Accepted: 10 Jul 2020

Electronic Journal of Plant Breeding

Research Article

QTL analysis for yield-related traits under water stress at flowering in tropical maize (*Zea mays.L*)

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Abstract
In the present study 300 doubled haploid lines from six populations were phenotyped with four yield related traits under water stress at flowering. Quantitative trait loci analysis was done for 263 lines which were genotyped successfully based on individual location and combined locations. For the four yield related traits *viz*., Anthesis silking interval, ear weight, ears per plant and chlorphyll content a total of 48 significant QTLs were detected based on the separate individual location analysis, of which 26 were detected for Hyderabad location data and 22 detected for Aurangabad location data. In the combined analysis there were 18 QTLs detected in which four QTLs found in population group 1(for ASI), four QTLs in population group 2 (one each for ASI and ears per plant and two for ear weight)and 10 QTLS detected in Pooled population analysis (two for ASI, one for chlorophyll content, five for ears per plant and two for ear weight). However, in combined location QTL analysis one common overlapping QTL was identified on chromosome 8 with same interval for ears per plant and ear weight. Meanwhile eight and three major QTLs were identified in individual location and combined location analysis. These genomic regions could be candidate targets for further fine mapping and marker-assisted breeding in maize.

Keywords
Doubled haploid, Quantitative trait loci, Anthesis silking interval, chromosome

INTRODUCTION

Maize (Zea mays L.) is one of the most important cereal crops of the world and contributes to food security in most of the developing countries. In India, maize is emerging as the third most important crop after rice and wheat. Its importance lies in the fact that it is not only used for human food and animal feed, but also widely used in corn starch industry, corn oil production *etc*. Maize is affected by drought at different growth stages in different regions. Drought stress at seedling and flowering stages of maize has been estimated to cause annual yield losses of about 13% in the tropics (Edmeades *et al*., 1993). When drought stress occurs before or during flowering in maize, a delay in silk emergence is observed, resulting in an increase in anthesis-silking interval (ASI) (Hall *et al*., 1982).

Development of improved maize varieties which are tolerant to drought is one important approach to enhance the yield reduction under drought, because genetic improvement can probably reduce 20-25% of the yield gaps between drought-affected and optimal conditions (Edmeades, 2013). In the past, much breeding research was conducted to improve performance under drought conditions, with some achievements (Campos *et al*., 2004). Conventional selection by CIMMYT specifically for drought tolerance by focusing on yield and associated secondary traits has resulted in a gain of around 100 kg/ha/yr, in tropical maize populations (Edmeades, 2013). However, the breeding progress of drought tolerance improvement has been slow as the decreasing heritability

of phenotypes under drought stress (Messmer *et al*., 2009). Marker-assisted selection (MAS) is now having a significant impact, and proper execution could double gains from conventional drought tolerance selection (Edmeades, 2013). Thus, more understanding of the genetic basis of yield-related traits under different water regimes is necessary for molecular breeding for drought tolerance (Mir *et al.,* 2012).

Molecular markers and doubled haploids (DH) have emerged as two most powerful technologies that are revolutionizing the way homozygous lines are developed in maize breeding programs (Mayor and Bernardo, 2009; Babu *et al*., 2013). Molecular marker-assisted breeding (referred commonly as molecular breeding), which seeks to accelerate the pace of phenotype-based breeding in resource-efficient manner, is gaining significance as more and more marker-trait associations are discovered, validated and becoming available for integration into product-oriented breeding pipelines. DH technology significantly reduces the time required to obtain genetically homozygous and pure lines compared to conventional inbreeding. Besides maximum genetic variance and increased precision in estimating the genotypic value of DH lines, this approach permits early selection of prospective hybrids, simplifies the logistics of inbred seed increase and maintenance and allows quick fixation of favorable alleles at quantitative trait loci (QTL) (Mayor and Bernardo, 2009; Lubberstedt and Ursula, 2012). Secondary traits that are easy and inexpensive to measure have been adopted in the breeding programs (Ribaut *et al*.2009). However, QTL information pertaining to such key secondary traits that are associated with drought tolerance in maize is scarce (Messmer *et al*. 2011). Though QTL mapping experiments successfully identified a number of small effect genomic regions, it did not translate into tangible germplasm products especially for complex traits such as abiotic stress tolerance or polygenic biotic stress resistance (Bernardo, 2008). Therefore, the main aim of this study was to identify QTLs using SNP markers and detect and map QTLs controlling yield-related traits under water stress at flowering Conditions using doubled haploid progenies derived from different populations in single and across locations and evaluate their effects. The results obtained in this research could contribute to the development of effective approaches for fine mapping and breeding maize for the future.

MATERIALS AND METHODS

During *Rainy* 2016 the three lines ZL113812, ZL135133 and ZL135154 were crossed to known tester CML 479 (Neutral for drought) and ZL 113908, ZL 135137 and ZL 135158 were crossed to known tester CML 451 (Neutral for drought) to develop breeding crosses within heterotic group. The developed six single crosses ZL113812 X CML479, ZL135133 X CML479, ZL135154 X CML479, ZL113908 X CML451, ZL135137 X CML451 and ZL135158 X CML451 were subjected to production of double haploid lines at Pioneer Hi-Bred private limited facility Bangalore and developed 50 DHL's from each population. During *Rainy* 2018 all the DHL's (300) were crossed to opposite heterotic group testers (CML 451 and CML 479) to get 300 hybrids. Three hundred hybrids were planted under normal well-watered (WW) and water stress at flowering conditions (WSF) and screened for moisture stress tolerance by following Augmented complete block design during Nov-March 2018.

The experimental material was grown in two separate sets in two different locations *viz*, Hyderabad, Telangana (lat. 17^0.46 N, long. 78^0.46 E) and Aurangabad, Maharashtra (lat. 19^0.72 N, long. 75^0.20 E) which are under the control of Pioneer Hi-Bred Pvt Ltd. One set was sown under moisture stress and another set under normal conditions by following 60 × 30 cm spacing and 100:50:25 kg ha^{-1} N:P:K. Irrigation was given to both the sets up to forty days after sowing with a regular interval of seven to ten days. Moisture stress was induced by withholding the irrigation between 55-75 DAS (*i.e*. during anthesis). To avoid barren cobs and ensure optimum plant stand, a protective irrigation was given at 75 days after sowing whereas, normal field received irrigation at an interval of seven to ten days, till physiological maturity.

Data were recorded from each plot at Hyderabad and Aurangabad in Nov-March 2018. Days to 50 % anthesis (DTA) and days to 50 % silking (DTS) were recorded as the number of days from planting to when 50 % of plants in a plot shed pollen, and had emerged silks, respectively. Anthesis silking interval (ASI) was computed as the difference between DTS and DTA. The relative chlorophyll content of the third leaf from the top was measured at 70 days after sowing (DAS) on three randomly selected competitive plants using chlorophyll meter (SPAD-502, Konica Minolta make). Stay-green could be evaluated at the leaf level using portable chlorophyll metres, such as the Minolta SPAD (Cai *et al*. 2012a, The SPAD values (CHLSPAD) were recorded as the average value of chlorophyll content at lower, upper and middle portion of the leaf from each entry in both the treatments. The ear weight (EARWT) was measured in kg/plot and ears per plant (EARPLT) was counted in each plot and averaged to total plant count. For all the traits Best Linear Unbiassed Estimates (BLUEs) were estimated for all progenies of the population which are further being used for QTL analysis.

A total of 3352 SNPs covering maize whole genome was used for genotyping of 300 doubled haploid lines from the six crosses and their parents. Genomic DNA was extracted from each DH line and SNP genotyping was performed at Corteva Agri sciences Jhonston, USA. In this study, the Infinium XT method was used to genotype all 300 lines and their parents. The 3352 markers were selected from several published and unpublished sources. Out of 300 maize DH lines, 263 were successfully genotyped after removal of unsuccessful allele calls.

QTL signal detection was done using Bayes-B model by the software BT-SAT which selects significant markers by

forward least-squares, using F-tests to add detected signals to the final model (or χ2-tests if a MME term is random). This approach has been chosen to avoid fitting detected signals that are in high LD or are even colinear, which would otherwise render the equation system unstable. By default, a high significance level of α = 0.8 is used to keep most of the original signal in the final model, effects of detected signals are re-estimated simultaneously and -log10(p) is recalculated. For better understanding and existence of less number of progenies per population we have grouped the bi-parental populations in two groups based on the presence of common tester *viz*., population group 1 consist of CML451 as a common parent among 3 populations similarly population group 2 consist CML479 as a common parent among 3 populations. All the traits under WSF have been subjected to analysis but the good threshold -log10 (p) value of >3 was observed for four traits *viz*., ASI, chlorophyll content, ear weight and ears per plant. Finally, QTLs were identified for the BLUEs of each trait for individual locations and also across locations under WSF condition.

RESULTS AND DISCUSIONS

The combined analysis shows that the variability for the different drought tolerant parameters under study and highly significant differences between genotypes (P≤ 0.01) were recorded for all the four traits in individual location analysis. The effect due to genotypes x environment interaction showed highly significant differences for all the four traits under water stress at flowering condition. The traits *viz.,* anthesis silking interval, chlorophyll content, ears per plant and ear weight are determined as yield-related traits and essential for maize breeding targets (Edmeades *et al*., 2000 and Campos *et al*., 2004). The grain yield was positively correlated with ears per plot, chlorophyll content and ear weight and negatively correlated with anthesis silking interval (Kumar *et al*., 2006, Pavan *et al*., 2011, Almeida et al., 2013, Adebayao and Menkir., 2014, Dar *et al*., 2015, Jakhar *et al*., 2017a and Gazal *et al*., 2018) indicating the importance of these secondary traits in selection for yield. Similar results have been found in the current investigation where in EARPLNT and EARWT reveals highly significant positive correlation (r = 0.75) where as CHLSPAD and EARWT shows a significant positive correlation (r = 0.57). EARPLNT and ASI showed a strong negative correlation (r= -0.7). The significant negative correlation (r = -0.59) found between ASIGDU and EARWT (fig.1). In general Ear weight is a direct measure of yield which is significantly correlated with grain yield in maize. Therefore, the genetic dissection of these traits are considered to be important for drought tolerance in maize.

A total of 2158 out of 3353 SNP markers which reveals expected 1:2:1 ratio as tested by chi-square test were used to construct a linkage map from 143 DH progenies from population group 1 (Table 1) and 2202 out of 3353 markers were used for constructing a linkage map from 119 DH progenies from population group 2 (Table 2).

Table 1. Summary of complete marker data per chromosome and polymorphic markers for the population group-1

	Population group-1 CML451			
	complete marker data per chr.		polymorphic SNP markers	
Chr.	**SNPs**	**Length(cM)**	**SNPs**	**Length(cM)**
Ch1	522	322	302	317.88
Ch2	379	257	246	253.57
Ch3	396	251	269	246.19
Ch4	384	248	248	246.17
Ch5	347	228	229	219.26
Ch6	262	184	150	180.79
Ch7	269	214	163	206.19
Ch8	304	213	224	208.95
Ch9	260	185	167	178.88
Ch10	229	164	160	162.26
Total	**3352**	**2266**	**2158**	**2220.14**

QTLs were considered to be significant when the -log10 (p) values exceed the threshold 3.0 and data on lesser than threshold has not been tabulated and not considered for analysis. Major and minor QTLs were classified with percentage of phenotypic variance (R^2) more than 10.0 as major QTL and QTL with R^2 less than 10.0 as minor QTL. For the four yield related traits, a total of 48 significant QTLs were detected based on the separate individual location analysis, of which 26 QTLs were detected for Hyderabad location data and 22 QTLs detected for Aurangabad location data. There were 18 significant QTLs detected when we run combined QTL analysis (Table 4). Out of 66 QTLs (Individual locations + combined locations), 8 QTLs were considered as major QTLs for four yield related traits as these R^2 values are more than 10 and all these QTLs were also common in Hyderabad location analysis. Whereas one QTL identified as major QTL for ASI, EARPLNT and EARWT in the population

group 2 under combined analysis. To validate QTLs, the first scheme is to confirm them in other mapping populations, the second scheme is to confirm QTLs using the same population evaluated in multiple locations and in multiple years. Hence in this study, location wise and across location QTL analysis is done to identify the most prominent QTLs in different locations.

Population Group-1 (PGCML451)

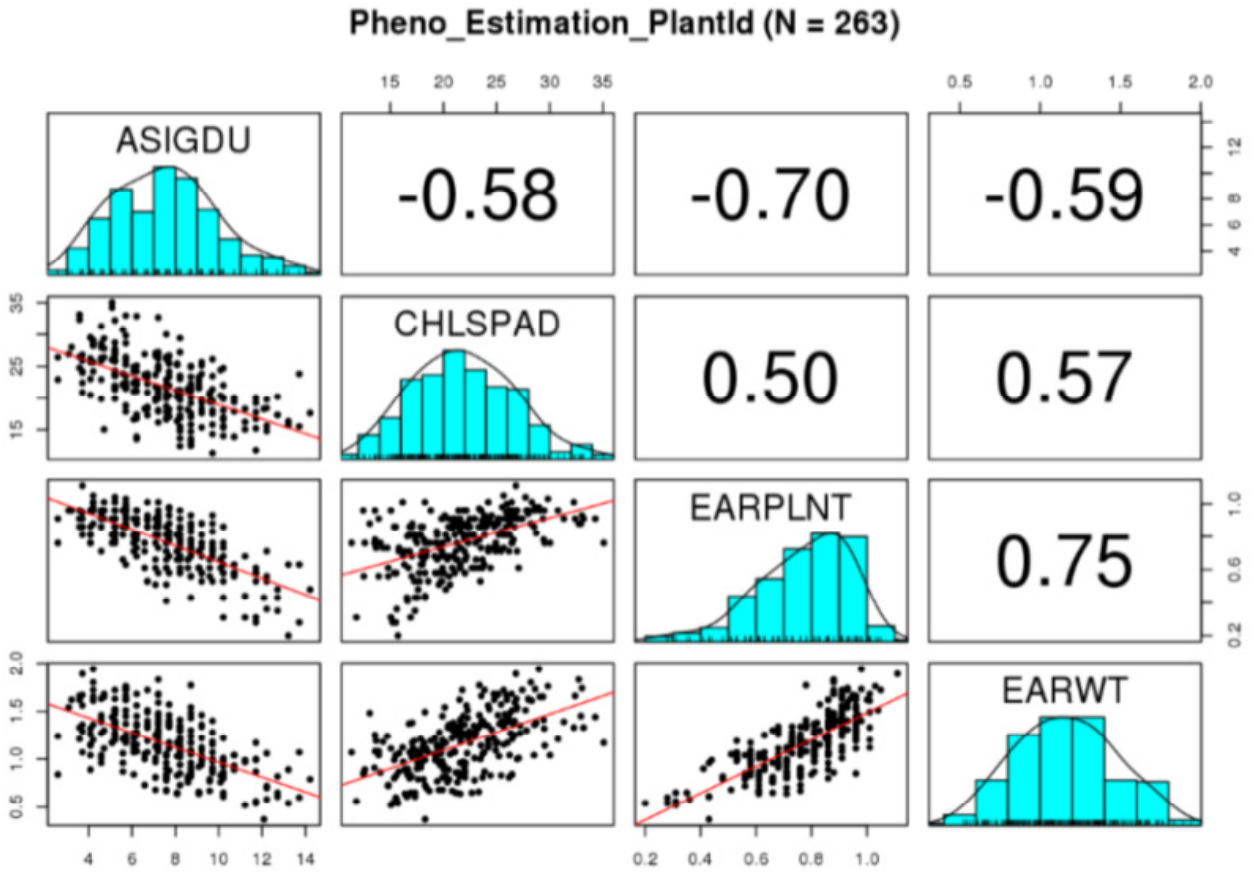

Fig. 1 The correlations between different traits across population under joint stress environment. The numbers in the upper right panel refer to the correlation coefficients between the four traits. Correlations between traits were significant at 1%.

Table 2. Summary of complete marker data per chromosome. and polymorphic markers for the population group -2

	Population group-2 CML479			
	complete marker data per chr.		polymorphic SNP markers	
Chr.	SNPs	Length(cM)	SNPs	Length(cM)
Ch1	522	322	353	317.88
Ch2	379	257	240	253.57
Ch3	396	251	273	245.58
Ch4	384	248	255	246.17
Ch5	347	228	227	219.26
Ch6	262	184	171	180.79
Ch7	269	214	157	206.19
Ch8	304	213	203	208.95
Ch9	260	185	171	178.88
Ch10	229	164	152	162.26
Total	**3352**	**2266**	**2202**	**2219.53**

The single location analyses revealed QTLs for ASI on chr.4 and 5 when Hyderabad data was analyzed with -log10 (p) score of 6.7 (R^2=12%) and 3.4 (R^2=7%) respectively (Table 3). However, by using Aurangabad data, a non significant QTLs were found on the chr. 4 and 5. while one more significant QTL was identified on chr.3 with phenotypic variance of 7 percent. When combined QTL analysis across location was done there were four QTLs on chr.2,3,4 and 5 with -log10 (p) scores of 3.8, 3.0, 3.7 and 3.8 respectively, explaining phenotypic variance of 8%,5%,8% and 8 %respectively (Table 4). For the trait Ear weight (EARWT) there were two significant QTLs detected on chr. 4 for Hyderabad data with -log10 (p) score of 4.7 and 5.3 explaining phenotypic variance of 9% and 11% respectively. When Aurangabad data investigated there were 2 QTLs detected on chr.3 and 8 with -log10 (p) value of 3.7 and 3 explaining 8% and 6% phenotypic variance respectively. There was no QTLs detected for the traits EARPLNT and CHLSPAD when Hyderabad data was investigated. However, by analyzing Aurangabad data a significant QTLs found for EARPLNT on chr. 4 and 8 with phenotypic variance of 6% and 8% respectively. For the trait CHLSPAD there were two significant QTLs detected on chr. 2 and 10 explaining phenotypic variance of 6 % and 8% respectively. There were no QTLs for the population group 1 found in pooled analysis across locations for the traits EARWT, EARPLNT and CHLSPAD.

Significant QTLs for ASI were detected on chr. 2,3 and 4 when Hyderabad data was analyzed. one QTL found on chr. 2 with -log10 (p) score of 3.0 explaining phenotypic variance of 4%. Two QTLs were detected on chr. 3 with -log10 (p) values of 4.5 and 3.2 explaining 10% and 6% phenotypic variances respectively. There was one QTL detected on chr. 4 with -log10 (P) value of 5.5 explaining 10% phenotypic variance (Table 3). Investigating Aurangabad data only one significant QTL was found for the ASI on chr. 7 with -log10 (p) value of 3. When combined QTL analysis across location was done there was one QTL on chr.7 for the trait ASI with -log10 (p) scores of 5 which is explaining 13% phenotypic variance (Table 4). There were 2 QTLs detected for EARPLNT on chr. 5 and 8 with 10% phenotypic variance on each. However, there was no QTL detected for the EARPLNT when Aurangabad location data was analyzed. While investigating combined location analysis one significant QTL was identified on chr. 8 with -log10 (p) score of 4.4 which is explaining 10% phenotypic variance. For the trait chlorophyll content (CHLSPAD)3 QTLs detected by using Hyderabad data of which one on chr. 3 with phenotypic variance of 8% and two QTLs found on chr. 7 with 12% (-log10 (p)=5.1) and 7% (-log10 (p)=3.1) phenotypic variance. There was no single QTL found for Aurangabad data similarly combined location data also posed no QTLs for EARPLNT. When analysis was done for the trait EARWT there were one, two and two QTLs detected for Hyderabad, Aurangabad and Combined location analysis respectively. The QTL on chr. 8 exhibited -log10 (p) score of 4 with phenotypic variance of 12% for Hyderabad data. Two QTLs found on chr. 3 and 8 with -log10 (p) values of 3.7 and 3 respectively when Aurangabad data was considered. Combined analysis across location for EARWT reveals two QTLs on chr.3 and 8 with -log10 (p) scores of 3.3 (R^2=8%) and 5.1 (R^2=12%) respectively. Hence in the current study the pooled population analysis is considered to be the best for the interpretation of results.

QTLs mapped in one population might not be detected in another population. Thus, it is critical important for QTLs to be confirmed to rule out statistical anomalies while used in marker assisted breeding (Gelli *et al*.,2017). The results exhibited in this study says QTLs detected in population group 1 are not similar in population group 2 which is because of the lack of commonality between the QTLs identified in different populations could be attributed to combination of various factors *viz*., the type and size of the mapping population used, segregation of different sets of QTLs in different crosses, detection of QTLs in a segregating population and epistatic interaction between QTLs in different mapping populations (Beavis and Keim, 1996 and Bohn *et al*., 1997).

Individual location (Hyderabad) analysis revealed that there were two QTLs for the ASI on chr. 2 and 7 with -log10 (p)scores of 3.1 and 4.1 respectively. There was only one QTL detected on chr. 8 with -log10 (p) value of 3 explained phenotypic variance is 3% when Aurangabad data was investigated. Across location analysis revealed that there were two QTLs on chr. 7 and 8 with phenotypic variance of 3 percent each for the trait ASI. Combined analysis across location revealed that there were QTLs found on chr. 2,3,4,5,7 and 8 for the trait ASI (Table 4). Similar results were also evidenced by previous investigations (Agrama and Moussa., 1996, Xin-Hai *et al*., 2003, Guo *et al*., 2008 and Almeida *et al*., 2013). When analysis was done for the EARPLNT using Hyderabad data, there were three significant QTLs identified on chr. 5,8 and 10 with -log10(p) scores of 3.1, 5.8 and 3.4 respectively. Similarly, five QTLs detected on chr. 2,3,6 and 8 of which two QTLs detected on chr. 2 with -log10 (p) score of 3 and 3.3 while on chr. 3,6 and 8 one QTL each detected with 8%, 4% and 5% phenotypic variance when Aurangabad data was examined for the trait EARPLNT. In the pooled analysis across locations, there were five significant QTLs identified for the EARPLNT on 2,4,5,7 and 8 chr. with -log10 (p) values of 4.7, 4.3, 4.6, 3.5 and 3.4 respectively these results are similar with the study conducted by Milena *et al*., 2006. For the trait EARWT, a total of seven QTLs were detected of which two QTLs found on chr. 1 with -log10 (p) values of 3.2 and 3.8. One QTL each was found on chr. 3,4,5,7 and 8 with -log10 (p) scores of 3.8, 3.7, 3.5, 4.2 and 4.5 respectively when Hyderabad data was examined. Similarly when Aurangabad data was analysed, there were two QTLs found for EARWT on chr. 3 and 7 with -log10 (p) scores of 4.1 and 3.5 respectively. However, The ear weight is directly contributing to the grain yield and many QTLs has been identified in different studies previously (Milena *et al*., 2006) are close proximity with the current study with

Table.3 QTLs detected for four drought related traits for population group 1, population group 2 at Hyderabad and Aurangabd locations.

Population groups	Trait	Flanking Markers	Chr.	Pos	(-LOG10(p))	N	Add. Effect	R2 (%)
			Hyderabad					
Population Group-1 (PGCML451)	ASI	MSNP01TDG-001	4	53.71	6.7	142	-1.14	12
		MSNP0225D-001	5	115.82	3.4	143	-0.72	7
	EARWT	MSNP01YXP-001	4	107.25	4.7	143	0.1	9
		MSNP026U4-001	4	168.95	5.3	143	-0.11	11
Population group-2 (PGCML479)	ASI	MSNP01JUR-001	2	215.74	3.0	116	0.8	4
		MSNP01HUU-001	3	36.55	4.5	115	0.94	10
		MSNP01KJY-001	3	121.1	3.2	119	-0.75	6
		MSNP01KPR-001	4	37.66	5.5	117	-0.93	10
	EARPLNT	MZA1325-11	5	141.66	4.0	118	0.07	10
		MSNP01XBD-001	8	136.05	4.2	117	0.11	10
	CHLSPAD	MSNP01KJY-001	3	121.1	3.8	119	1.75	8
		MSNP01WDM-001	7	50.69	5.1	117	-1.85	12
		MSNP027PB-001	7	195.1	3.1	103	1.48	7
	EARWT	MSNP01X69-001	8	134	4.0	119	0.14	12
Pooled analysis (PG1+PG2)	ASI	MSNP01K3Y-001	2	171.91	3.1	260	0.45	3
		MSNP01V73-001	7	64.38	4.1	261	-0.5	3
	EARPLNT	MZA1325-11	5	141.66	3.1	261	-0.04	5
		MSNP01XBD-001	8	136.05	5.8	261	0.13	8
		MSNP02DPY-001	10	57.33	3.4	260	-0.04	4
	EARWT	MSNP015X7-001	1	46.52	3.2	259	-0.08	3
		MSNP018YT-001	1	130.45	3.8	262	-0.07	4
		MSNP01K8T-001	3	224.23	3.8	263	0.07	4
		MSNP01TBT-001	4	67.38	3.7	259	-0.08	5
		MSNP02220-001	5	134.61	3.5	254	0.07	4
		MSNP01V73-001	7	64.38	4.2	261	0.09	7
		MSNP01X69-001	8	134	4.5	263	0.08	6
			Auranagbad					
Population Group-1 (PG1CML451)	ASI	MSNP01HX5-001	3	38.61	3.2	142	-0.84	7
	EARPLNT	MSNP02BNK-001	4	219.3	3.2	142	0.05	6
		MSNP02UYK-001	8	18.12	4.0	142	-0.08	8
	CHLSPAD	MSNP01BA5-001	2	28.93	3.2	142	2.54	6
		MSNP0334-01	10	85.03	4.1	143	-1.84	8
	EARWT	MSNP01JY9-001	3	65.93	3.7	140	0.09	8
		MSNP02744-001	8	32.83	3.0	140	-0.08	6
Population group-2 (PG2CML479)	ASI	MSNP027PB-001	7	195.1	3.0	118	-0.08	6
	EARWT	MSNP0154X-001	1	3.14	3.6	101	-0.14	8
		MSNP02CNW-001	4	204.48	3.4	116	-0.09	6
		MSNP01X69-001	8	134	4.6	119	0.13	9
Pooled analysis (PG1+PG2)	ASI	MSNP022K3-001	8	132.25	3	262	-0.59	3
	CHLSPAD	MSNP01AG7-001	1	285.99	3.7	258	1.89	5
		MSNP01JRY-001	2	156.64	3.0	258	-1.42	4
		MSNP0334-01	10	85.03	3.0	257	-1.29	4
	EARPLNT	MSNP0322K-001	2	7.66	3.0	269	0.04	3
		MSNP01JC3-001	2	148.52	3.3	261	-0.05	5
		MSNP01JAM-001	3	62.87	4.1	259	-0.06	8
		MSNP023GE-001	6	135.65	3.2	260	0.05	4
		MSNP01X69-001	8	134	3.9	263	0.05	5
	EARWT	MSNP01JY9-001	3	65.93	4.1	258	-0.09	6
		MSNP023PP-001	7	77.79	3.5	262	0.07	4

Table 4. QTLs detected for four drought related traits for pooled population analysis under combined location.

Population groups	Trait	Flanking Markers	Chr.	Pos	(-LOG10(p))	N	Add. Effect	R2 (%)
Combined location (Hyderabad and Auranagbad)								
Population Group-1 (PG1CML451)	ASI	MSNP01J1J-001	2	154.73	3.8	144	0.82	8
		MSNP01HX5-001	3	38.6	3	142	-0.58	5
		MSNP01TDG-001	4	53.7	3.7	142	-0.89	8
		MSNP0225D-001	5	115.8	3.8	143	-0.73	8
Population group-2 (PG2CML479)	ASI	MSNP027PB-001	7	195.1	5	103	-0.97	13
	EARPLNT	MSNP01XBD-001	8	136.1	4.4	117	0.1	10
	EARWT	MSNP01NHD-001	3	105.1	3.3	119	0.11	8
		MSNP01X69-001	8	134	5.1	119	0.14	12
Pooled analysis (PG1+PG2)	ASI	MSNP01WTB-001	7	70.96	3	263	-0.49	3
		MSNP02ERJ-001	8	89.03	3.1	263	-0.58	3
	CHLSPAD	MSNP016C6-001	1	9	3.4	261	-1.01	5
	EARPLNT	MSNP01K3Y-001	2	171.91	4.7	260	-0.04	5
		MSNP02629-001	4	178.85	4.3	261	-0.03	4
		MZA1325-11	5	141.66	4.6	261	-0.04	5
		MSNP01V73-001	7	64.38	3.5	261	0.04	5
		MSNP01X69-001	8	134	3.4	263	0.03	4
	EARWT	MSNP023PP-001	7	77.79	3.8	262	0.07	5
		MSNP01X69-001	8	134	3.4	263	0.07	5

QTLs detected on chr. 1,3,4,5,7 and 8. But combined location analysis reveals significant QTLs found on chr. 7 and 8. Combined analysis across locations for the trait CHLSPAD reveals that there were 3 QTLs on chr. 1,2 and 10 explained 5%,4% and 4% respectively when Aurangabad location is analyzed. Chlorophyll content is the trait correlate directly with the accumulation of photosynthates there by yield will elevate, while studying this trait QTLs were found on chr. 1,2,3,7 and 10 when individual location being analysed, but one significant QTL on chr. 1 observed in combined location analysis. These findings confirmed similar results previously by (Cai *et al*., 2012 and Wang and Zang., 2008). Interestingly, some common chromosome regions were found for Hyderabad data that contained overlapping QTL (Pleiotropy) for ASI and CHLSPAD on chr. 3 in population group 2. Similarly, one more common QTL identified on chr. 7 for ASI and EARWT in the pooled population analysis. But these common QTLs are not observed in Aurangabad location and one more common QTL detected for EARPLNT and EARWT in across location analysis with pooled population combined location analysis. However, because the epistatic analysis would be more powerful when larger populations are used (Carlborg and Haley, 2004), considerable size of mapping populations in combination with high-density mapping markers are desired to clarify the epistasis of QTLs for quantitative traits such as yield-related traits.

Grain yield-related traits have an extremely complicated genetic mechanism in maize due to their complex genetic networks and strong genotype by environment interactions. In the present study, the combined location analysis identified 18 yield-related QTLs under water-stressed environment, one QTL on Chr. 8 is supposed to be a pleiotropic QTL conferring ears per plant and ear weight. And one QTL on Chr. 3 is also probably one pleiotropic QTL conferring Anthesis silking interval and chlorophyll content in population group 2 while another one QTL on chr. 7 conferring as a common QTL for Anthesis siliking interval and Ear weight in the pooled population analysis. These genomic regions could be candidate targets for further fine mapping and marker-assisted breeding for drought tolerance in maize.

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