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Original scientific article

IDENTIFICATION OF QTLS FOR YIELD AND CONTRIBUTING TRAITS IN MAIZE-TEOSINTE DERIVED BILS UNDER DISEASED-STRESSED AND CONTROL CONDITIONS

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In maize, grain yield is the most important trait having a complex inheritance pattern. Yield contributing traits are more stable and have higher heritability than yield. Therefore, the present study was conducted to identify quantitative trait loci (QTLs) associated with grain yield and its components by using simple sequence repeat (SSR) markers. A population of 169 BC_1F_5 lines was derived from the crossing between maize inbred line DI-103 and teosinte-*parviglumis* was utilized for genotyping and phenotyping. In diseased stressed condition (E_1), ear length (EL), ear diameter (ED), kernel rows per ear (KR/E), kernels per row (K/R), test weight (TW), and grain yield per plant (GY/P) had 7, 6, 7, 4, 6 and 5 QTLs whereas, in controlled condition (E_2) 5, 2, 5, 4, 5 and 3 QTLs were detected for enlisted characters, respectively. Consistent QTLs across the environments were detected for 5 of the 6 investigated traits and number of QTLs were EL (2), ED (1), KR/E (3), TW (1), and GY/P (1) whereas, for K/R none of the QTLs were common between E_1 and E_2. By mapping analysis, we have identified genomic regions

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associated with two traits in a manner that was consistent with phenotypic correlations among traits, supporting either pleiotropy or tight linkage among QTLs. Three co-localized QTLs were identified between grain yield and contributing traits. Notably *umc1720*-linked QTL at bin 4.10 was simultaneously responsible for GY and EL, ED, KR/E, K/R; *umc1215*-linked QTL at bin 6.03 was simultaneously responsible for GY and ED, KR/E, K/R, TW; *umc1279*-linked QTL was responsible for GY and ED, TW. The findings suggest that the chromosomal region containing co-localized QTLs governing multiple yields associated traits are potential targets for selection. In addition for 6 studied traits, 44 superior lines were identified, and along with both the parents i.e. maize (DI-103) and teosinte they were clustered in 11 groups. Therefore, lines clustered independently can be utilized in a hybridization programme for the accumulation of yield contributing traits for yield maximization.

Key words: Maize, Teosinte, QTL, SSR, Genotyping

INTRODUCTION

Maize is the crop of world repute with wider adaptation around the year. Due to diverse applications like maize as a food, feed, and industrial raw material its demand keeps on increasing. To meet out rapidly increasing global maize demand obtaining higher grain yield is the main objective of maize breeders. Yield and its contributing traits are complex in nature as govern by quantitative trait loci (QTLs) (AUSTIN and LEE, 1996). For yield improvement, it is important to dissect yield contributing traits at the molecular level.

Grain yield in maize is a complex, continuous trait that might be modified by a large number of genes including those controlling ear architecture parameters. Ear and kernel traits along with test weight (TW) are important yield components in maize (HUO *et al*., 2016; YANG *et al*., 2020). Ear traits include ear length (EL) and ear diameter (ED) and kernel traits are composed of kernel rows per ear (KR/E) and kernels per row (K/R). Therefore for achieving a higher yield in maize, improvement of these traits is necessary. In comparison to grain yield itself, the yield contributing traits tends to display higher heritability and better stability across environments (MESSMER *et al*., 2009; PENG *et al.*, 2011; YANG *et al*., 2020). Yield traits (EL, ED, KR/E, K/R, and TW) are reported to demonstrate a significant positive correlation with grain yield (SABADIN *et al*., 2008; LI *et al*., 2010; BARTAULA *et al*., 2019). Henceforth for dissection of genetic basis and improvement of grain yield in maize, identification of QTLs for yield contributing traits instead of the grain yield itself would be more effective (HUO *et al*., 2016).

Several researchers have reported that yield contributing traits usually exhibit stable QTLs across environments (MESSMER *et al.*, 2009; LIU *et al*., 2014; ZHANG *et al*., 2017). Till today various researchers carried out QTL analysis for various yield traits, namely those related to ear morphology (EL, ED) (MENDES-MOREIRA *et al*., 2015; CHEN *et al*., 2016; SU *et al*., 2017), KR/E (VELDBOOM and LEE, 1994; AUSTIN and LEE, 1996), K/R (CHEN *et al*., 2016; SU *et al*., 2017), TW (CHEN *et al*., 2016; PAN *et al*., 2017; SU *et al*., 2017; ZHAO *et al*., 2018) and GY/P (VEIGA *et al*., 2012; YANG *et al.*, 2016; CHEN *et al*., 2016; SU *et al*., 2017; NIKOLIC *et al*., 2018; RIBEIRO *et al*., 2018). To date, many QTLs for yield contributing traits were discovered of which 45 QTLs were associated with ED, 149 with TW, 46 with EL, and 23 with KR/E (https://archive.gramene.org/qtl/).

Although numerous mapping experiments were conducted for yield traits but all were based on contrasting maize parents. Only a few QTL mapping studies were conducted by targeting wild relatives of maize (CALDERÓN *et al.*, 2016; LIU *et al.*, 2016b; KARN *et al.*, 2017; FU *et al.*, 2019). Due to domestication followed by selection and trait-specific breeding, there is less allelic variability in present-day maize (WARBURTON *et al.*, 2008; SINGH *et al.*, 2017) therefore for yield enhancement there is an urgent need for novel genetic resources (LE CLERC *et al.*, 2005). Wild relatives particularly wild progenitor of maize i.e. teosinte (*Zea mays* spp. *parviglumis*) reported possessing wide variation for desirable traits that were lost during domestication (LIU *et al.*, 2016a; JOSHI *et al.*, 2021; ADHIKARI *et al.*, 2019, 2021; SAHOO *et al.*, 2021). Hence such variation could be exploited in maize improvement either through introgression or pre-breeding programmes.

In this study, mapping populations previously used to map QTLs for banded leaf and sheath blight (BLSB) resistance, flowering, and plant architecture-related traits were used to identify QTLs governing EL, ED, KR/E, K/R, TW, and GY/P and to identify markers that could be utilized in marker-assisted selection. Thorough knowledge of the genes affecting these yield components would lead to better yield modelling. As teosinte is distinct from maize in many aspects be morphology, yield traits (SMITH and LESTER, 1980; DOEBLEY, 2004; SINGH *et al.*, 2017; YANG *et al.*, 2019; ADHIKARI *et al.*, 2021) or at the molecular level (ADHIKARI *et al.*, 2019) therefore teosinte derived population could be targeted for mapping of several traits together. In addition, teosinte was reported to possess more diverse alleles for yield contributing traits hence teosinte introgression facilitates the identification of superior lines for yield traits also.

MATERIAL AND METHOD

Material development

The present investigation was undertaken with wild progenitor teosinte (*Z. mays* ssp. *parviglumis*) and a maize inbred line DI-103. The maize inbred line was crossed with teosinte as pollen parent to produce F_1s and one backcross with the maize inbred line as a recurrent parent to produce BC_1F_1. Subsequently, four generations of selfing were carried out in to produce BC_1F_5 mapping population. Thus, the 169 BC_1F_5 backcrossed inbred lines (BIL) (encoded as MT-1 to MT-169) constitute the population for the present investigation.

Experimental layout and recoding of traits

The experimental materials were planted in Randomized Complete Block Design (RCBD) with two replications under artificially inoculated with *Rhizoctonia solani* (E_1) and control condition (E_2) in the *Kharif* season of the year 2018-2019. Each line was planted in a single row (2 m long and 75 cm apart). The material was evaluated for six yield and contributing traits namely ear length (EL), ear diameter (ED), kernel rows per ear (KR/E), kernels per row (K/R), test weight (TW), and grain yield per plant (GY/P). These traits were recorded by averaging values of three randomly selected ears that were harvested from three randomly tagged plants of each line.

Genotyping procedure

DNA isolation was carried out from 30 days old seedling by CTAB (Cetyl trimethyl ammonium bromide) method (DOYLE and DOYLE, 1990) with some modification. The DNA quantity, as well as quality, was insured by spectrophotometer (Systronics PC Based Double Beam Spectrophotometer 2202) and electrophoresis in 0.8% agarose gel. The DNA was diluted to the working concentration of 200ng/ µl. For genotyping, 168 SSR markers widely distributed throughout the maize genome were selected from the maize database: http://maize.gdb (PORTWOOD *et al.*, 2018). For PCR reaction in total 13.8 µl reaction mixture were prepared that constitutes 3 µl (200 ng/µl) genomic DNA, 0.35 µl dNTPs mix (2.5 mM each), 1.5 µl reaction buffer with 15mM MgCl2 (10X), 1.5 µl each forward and reverse primer (40 ng/µl), 0.25 µl Taq DNA polymerase (3U/µl), and 7.2 µl deionized water. The PCR cycles were performed with the flow of initial denaturation (94°C, 5 min) followed by denaturation (94°C, 40 s), primer annealing (55°C- 68°C, 40 s varied with primer), elongation (72°C,1 minute). From denaturation to elongation cycle repeated 35 times then final elongation was performed at 72°C for 10 min. Thereafter electrophoresis was carried out in 3% agarose gel for resolving PCR products of each genotype. Further, the gel image was captured in PC based gel documentation system (Alpha Innotech Corporation, USA). The product length for each marker was determined by comparing it with the 100bp DNA ladder. Following coding system were used for scoring of marker data of each genotype for all markers.

Scoring of SSR banding pattern in BC_1F_5 population

S. No.	Code	Type of Band	Description
1	A	AA	Homozygote for parent 1
2	H	Aa	Heterozygote
3	B	aa	Homozygote for parent 2
4	E	--	Missing data

Statistical analysis and QTL mapping

R statistical software (R CORE TEAM, 2020) was utilized for analysis of variance (ANOVA) and construction of Box plots of six yield and contributing traits in two different environments. Pearson correlation coefficient between the analyzed traits was estimated using OPSTAT (SHEORAN *et al*., 1998). Ten superior lines that showed higher estimates under the control environment (E_2) for six studied traits were identified. These superior lines were further classified based on the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) method of PAleontological STatistics (PAST) software (HAMMER *et al*., 2001). Then the dendrogram was generated to portray clustering patterns of lines by using dissimilarity matrix. For each polymorphic marker, polymorphism information content (PIC) value was calculated in Microsoft Excel by the following formula: PIC= $1-\sum_{i=1,n}^{n} fi^2$ where *fi* is the frequency of the i^{th} allele (SMITH *et al*., 1997). QTL analysis was performed using the single marker analysis (SMA) method of WinQTL cartographer, 2.5 version software (WANG *et al*., 2012). It quickly scans the association between the targeted trait and a single marker at a time to identify linked marker. For each marker individuals were grouped into different genotypic classes. Then to detect linked marker mean value of the trait of interest calculated for each genotypic class by summing up the

estimates of each individual of respective genotypic class. Then the mean value of each genotypic class for a marker at a time is compared with the help of 't' test. If the difference found significant then the maker on the basis of which individuals were grouped into different genotypic classes is likely to be linked with the targeted trait.

RESULTS AND DISCUSSION

Phenotyping of morphological traits and identification of superior line

ANOVA revealed significant variance among 169 lines for all studied characters. This showed the presence of sufficient amount of variability among the experimental material (Table 1 and Table 2).

Table 1. Analysis of variance (ANOVA) for different characters in parents and their BC_1F_5 maize lines under artificially inoculated environment (E_1)

S.V.	d.f.	Mean squares					
		EL	ED	KR/E	K/R	TW	GY/P
Replication	1	0.49	0.03	0.02	2.34	4.39	5.45
Treatment	170	44.61**	2.73**	34.04**	135.42**	8483.10**	838.09**
Error	170	0.95	0.31	3.61	0.21	6.49	413.37
SEm±		0.16	0.04	0.22	0.31	1.80	0.83
CD (at 1%)		0.60	0.15	0.81	1.19	6.64	3.05
CD (at 5%)		0.46	0.11	0.61	0.91	5.03	2.31
CV (%)		8.44	8.35	12.99	10.16	6.79	12.49

** 1% level of significance EL-Ear length, ED-Ear diameter, KR/E- Kernel rows per ear, K/R- Kernels per row, TW-Test weight, GY/P-Grain yield per plant

SEm± -standard error of the mean, CD- Critical difference, CV- Coefficient of variation

Table 2. Analysis of variance (ANOVA) for different characters in parents and their BC_1F_5 maize lines under control environment (E_2)

S.V.	d.f.	Mean squares					
		EL	ED	KR/E	K/R	TW	GY/P
Replication	1	7.38	0.16	0.39	32.62	400.92	144.35
Treatment	170	10.87**	1.05**	7.51**	101.23**	1853.85**	900.99**
Error	170	0.49	0.05	2.38	1.51	24.79	434.74
SEm±		0.49	0.15	1.08	0.87	3.52	4.17
CD (at 1%)		2.11	0.58	1.95	2.75	10.34	37.18
CD (at 5%)		1.59	0.44	1.47	2.08	7.81	28.09
CV (%)		4.93	8.24	6.33	5.69	3.46	13.31

** 1% level of significance EL-Ear length, ED-Ear diameter, KR/E- Kernel rows per ear, K/R- Kernels per row, TW-Test weight, GY/P-Grain yield per plant

SEm± -standard error of the mean, CD- Critical difference, CV- Coefficient of variation

Introgression of significant variation through teosinte in maize lines also depicted by wider range for all the studied traits (Table 3). Similarly, in previous studies aiming diversification of maize through teosinte-*parviglumis* allelic introgression, significant variation for yield traits was also observed (SINGH *et al.*, 2017; KUMAR *et al.*, 2019; ADHIKARI *et al.*, 2021). WANG *et al.*, (2020) recorded huge variation in *Zea mays* subsp *nicaraguensis* and *Zea mays* subsp *parviglumis* derived RIL populations for 31 morphological traits. Likewise, AKAOGU *et al.*, (2020) recorded sufficient variation for yield traits among maize lines that were derived from another wild species of maize (*Zea diploperennis*). These findings suggest the possible role of wild species in the diversification of the maize genome. In E_2 maximum lines had EL between 10.00 to 11.00 cm, however, under E_1 most of the lines scored "0" value (Fig.1). Most of the lines possessed ED of 2.50 to 3.00 cm in E_2 but in E_1, the "0" score for ED was prominent (Fig.2). Frequencies of lines with 10.00 K/R were higher in E_2 while in E_1 most of the lines were scored "0" KR/E (Fig.3). BC_1F_5 lines possessing KR/E between 15.00 to 20.00 was maximum in frequency in E_2 whereas in E_1, "0" score frequency was prominent (Fig.4). In E_2, most of the lines possessed TW of 150.00 g to 170.00 g whereas under E_1 maximum lines showed "0" TW (Fig.5). In E_1 maximum lines clustered at "0" score whereas under E_2 for grain yield per plant maximum lines clustered at 140.00-185.00 g (Fig.6). Under a disease environment susceptible lines produced rudimentary or no ear at al resulted in reduced EL, ED, KR/E, and K/R. Whereas due to full mycelia growth of *Rhizoctonia solani* (causal organism of BLSB) under high disease pressure maximum lines showed reduced seed set which resulted in low TW and GY/P under E_1.

Table 3. Range and heritability of different morphological traits in BC_1F_5 mapping population under artificially inoculated and control environment along with parents

Characters	DI-103		Teosinte		Inoculated environment (E_1)		Control environment (E_2)	
	(E_1)	(E_2)	(E_1)	(E_2)	Range	h^2(b)	Range	h^2(b)
L (cm)	11.33	14.25	4.06	3.98	0-13.83*	95.82	3.16-19.16	91.64
ED (cm)	2.81	3.33	0.68	0.70	0-3.66*	79.90	0.81-7.16	91.13
KR/E	10.00	12.66	2.00	2.00	0-14.00*	80.84	2.66-16.00	60.22
K/R	14.00	14.00	3.16	3.16	0-30.50*	99.69	3.50-44.83	96.72
TW(g)	189.50	191.75	63.65	63.25	0-211.40*	99.85	98.19-254.85	97.76
GY/P(g)	48.50	64.66	136.83	133.81	0-76.50*	33.94	6.60-105.00	38.73

*Values ranges from "zero" due to rudimentary or no ear formation under higher disease incidences that is ranges from 8-9 in disease rating scale; EL-Ear length, ED-Ear diameter, KR/E- Kernel rows per ear, K/R- Kernels per row, TW- Test weight, GY/P-Grain yield per plant, E_1 = Artificially inoculated environment, E_2=Control environment, , h2(b)= broad-sense heritability

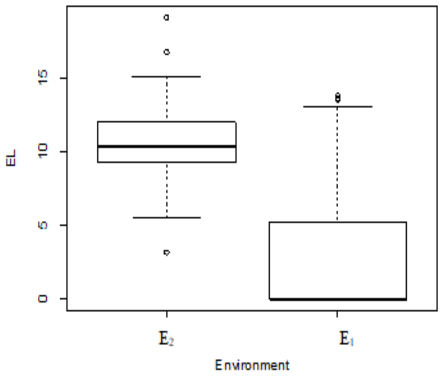

Fig: 1 Box plots of ear length among BC_1F_5 maize lines under control and disease environment

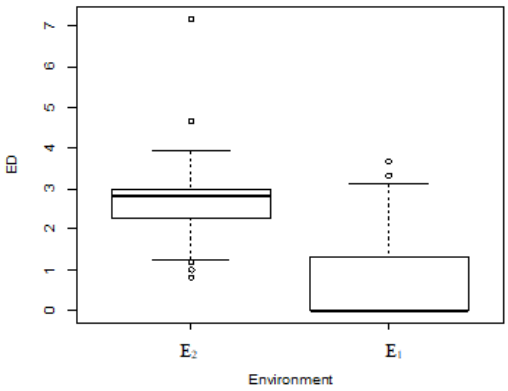

Fig: 2 Box plots of ear diameter among BC_1F_5 maize lines under control and disease environment

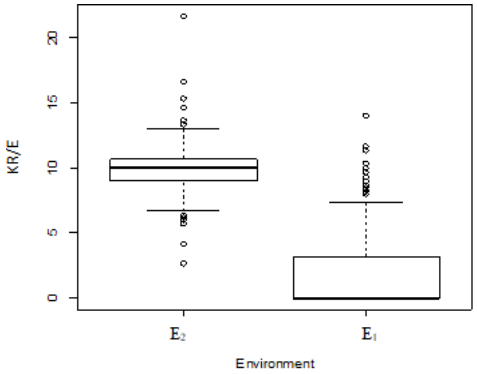

Fig: 3 Box plots of kernel rows per ear among BC_1F_5 maize lines under control and disease environment

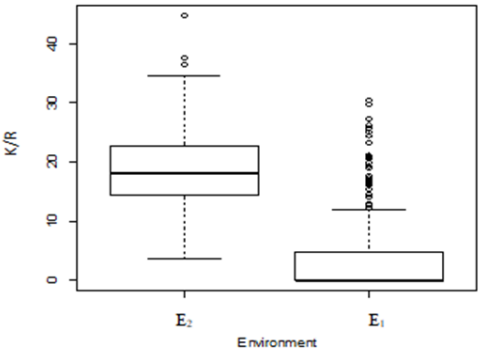

Fig: 4 Box plots of kernels per row among BC_1F_5 maize lines under control and disease environment

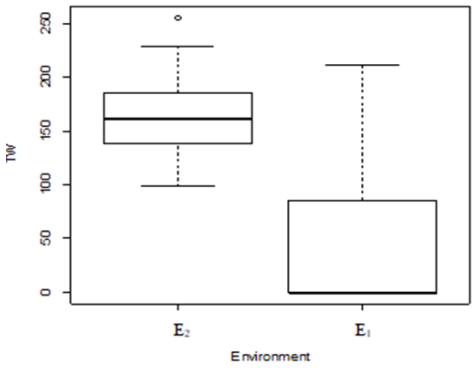

Fig: 5 Box plots of test weight among BC_1F_5 maize lines under control and disease environment

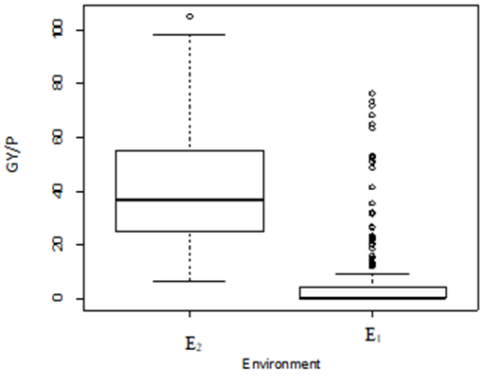

Fig: 6 Box plots of grain yield per plant among BC_1F_5 maize lines under control and disease environment

Based on data under controlled conditions for six yield and contributing traits top 10 superior lines were selected (Table 4). Of the total of 44 selected lines, three lines namely MT-37, MT-39, and MT-49 were found superior for four different traits. Seven lines namely MT-20, MT-24, MT-130, MT-136, MT-142, MT-155, and MT-156, found superior for two traits each. However, the remaining 34 lines consisted of one superior trait only. These lines were further clustered by the unweighted pair group method with arithmetic averages (UPGMA) to demonstrate molecular diversity among them and that will facilitate parental selection for desirable trait accumulation. Based on molecular profiling with 76 SSR markers, at 0.47 Jaccard similarity coefficients, these 46 lines including both the parents were clustered into eleven groups (Table 5, Fig.7).

Table 4. Trait-wise list of top ten superior teosinte introgressed maize lines

S. No.	Characters	Teosinte introgressed maize lines
1	EL	MT-24, MT-37, MT-41, MT-49, MT-52, MT-112, MT-130, MT-142, MT-145, MT-155
2	ED	MT-10, MT-37, MT-39, MT-49, MT-63, MT-87, MT-130, MT-136, MT-143, MT-156
3	KR/E	MT-20, MT-37, MT-39, MT-49, MT-53, MT-61, MT-64, MT-133, MT-138, MT-154
4	K/R	MT-20, MT-24, MT-37, MT-38, MT-39, MT-49, MT-76, MT-79, MT-142, MT-155
5	TW	MT-16, MT-39, MT-59, MT-73, MT-89, MT-90, MT-98, MT-136, MT-149, MT-164
6	GY/P	MT-25, MT-29, MT-36, MT-80, MT-96, MT-105, MT-122, MT-147, MT-156, MT-162

EL-Ear length, ED-Ear diameter, KR/E- Kernel rows per ear, K/R- Kernels per row, TW- Test weight, GY/P-Grain yield per plant

Table. 5 Clustering patterns of 46 lines including the parent's viz., Maize (DI-103), Teosinte and identified 44 superior teosinte derived maize lines

Cluster	No. of genotypes	Genotypes
1	1	Teosinte
2	2	MT-80, MT-182
3	8	MT-24, MT-36, MT-49, MT-59, MT-112, MT-147, MT-149, Maize (DI 103)
4	2	MT-90, MT-138
5	7	MT-87, MT-89, MT-105, MT-130, MT-133, MT-136, MT-145
6	2	MT-98, MT-142
7	1	MT-73
8	2	MT-20, MT-29
9	11	MT-25, MT-37, MT-38, MT-39, MT-41, MT-52, MT-53, MT-64, MT-79, MT-162 , MT-164
10	8	MT-16, MT-61, MT-63, MT-76, MT-143, MT-154, MT-155, MT-156,
11	2	MT-10, MT-96

As appose to previous studies which were based on clustering of maize germplasm (PATTO *et al.,* 2004; ADU *et al.,* 2019), a larger number of clusters in the present experiment indicates the possible role of teosinte allele introgression in maize diversification. The genetic similarity among lines ranges from 0.207 to 0.649. Teosinte being clustered independently in cluster I showed maximum dissimilarity value 0.793 with MT-147 followed by 0.784 with MT-36 that clustered together in cluster III. The clustering of teosinte independent of maize and derived lines correspondent to its distinct morphological feature (DOEBLEY, 2004; YANG *et al.,* 2019; ADHIKARI *et al.,* 2021). However, MT-87 and MT-89 were the most similar lines (64.9%) due to minimum dissimilarity value 0.351 followed by MT-130 and MT-136 (61.7%) with dissimilarity value 0.383. These four lines (MT-87, MT-89, MT-130, and MT-136) belonged to cluster V. Distribution patterns of 46 lines among 11 clusters were not uniform. Maximum 11 lines were grouped in cluster IX followed by 8 in clusters III and X each. Cluster V consists of 7 lines whereas clusters II, IV, VI, VIII, and XI are composed of 2 lines each. Minimum 1 line was present in clusters I and VII. For achieving a higher yield there is a need for the accumulation of contributing traits together which can be achieved by selecting lines from different clusters. Chances of getting desirable recombinant are more if the parental selection is based on molecular diversity than random selection of parents based on morphology only.

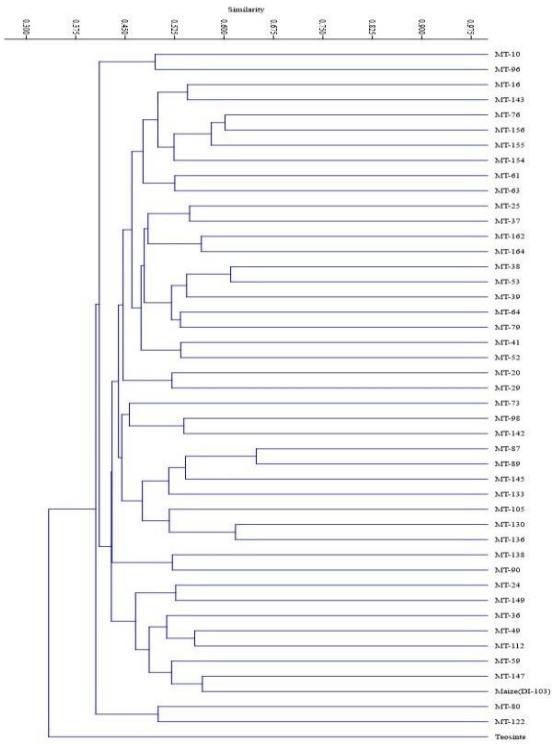

Figure.7 Clustering pattern of 46 (including parents) promising teosinte derived maize lines using 76 polymorphic SSR markers data

Heritability and correlation analysis

ROBINSON (1966) have classified heritability (h^2b) into three groups namely low (<50%), medium (50-75%) and high (> 75%) depending upon heritability percentage. High heritability was recorded in the case of EL, ED, K/R, and TW under both the environment whereas KR/E depicted high heritability under E_1 and moderate under E_2 (Table 3). Likewise, previous researchers also detected high heritability for EL (KABDAL *et al.*, 2003; NOOR *et al.*, 2010; YANG *et al.*, 2020), ED (ANSHUMAN *et al*., 2013), KR/E (BARTAULA *et al.*, 2019; YANG *et al*., 2020), K/R (NATARAJ *et al.*, 2014), TW (NOOR *et al.*, 2010; SESAY *et al.*, 2018; YANG *et al*., 2020). These high heritability estimates indicate minimal environmental influence on the expression of these traits therefore greater correspondence between phenotype and breeding values. However low heritability was recorded in the case of GY/P in both E_1 and E_2 which related to its polygenetic nature. It is well known that yield contributing traits exhibited high heritability than yield itself (PENG *et al.*, 2011).

EL, ED, KR/E, K/R, and GY/P were highly positively correlated with each other under both the environment. However, TW was positively correlated with each trait in E_1 but showed a non-significant association under E_2 (Table 6 and 7). Similarly in previous studies yield traits (TW, KR/E, and K/R) are reported to demonstrate a significant positive correlation with grain yield (SABADIN *et al.*, 2008; LI *et al*., 2010; BARTAULA *et al*., 2019).

Table 6. Pearson Correlation Matrix under E_1

	EL	ED	KR/E	K/R	TW	GY/P
EL	+1.000	++0.970**	+0.973**	+0.952**	+0.964**	+0.829**
ED	+0.970**	1.000	+0.982**	+0.944**	+0.968**	+0.810**
KR/E	+0.973**	+0.982**	1.000	+0.957**	+0.962**	+0.831**
K/R	+0.952**	+0.944**	+0.957**	1.000	+0.937**	+0.823**
TW	+0.964**	+0.968**	+0.962**	+0.937**	1.000	+0.805**
GY/P	+0.829**	+0.810**	+0.831**	+0.823**	+0.805**	1.000

** 1% level of significance

EL-Ear length, ED-Ear diameter, KR/E- Kernel rows per ear, K/R- Kernels per row, TW- Test weight, GY/P-Grain yield per plant

Table 7. Pearson Correlation Matrix under E_2

	EL	ED	KR/E	K/R	TW	GY/P
EL	1.000	+0.381**	+0.353**	+0.669**	0.063NS	+0.171*
ED	+0.381**	1.000	+0.421**	+0.312**	0.112NS	+0.182*
KR/E	+0.353**	+0.421**	1.000	+0.480**	0.002NS	+0.165*
K/R	+0.669**	+0.312**	+0.480**	1.000	0.030NS	+0.295**
TW	0.063NS	0.112NS	0.002NS	0.030NS	1.000	0.071NS
GY/P	+0.171*	+0.182*	+0.165*	+0.295**	0.071NS	1.000

** 1% level of significance, *5% level of significance, NS non significant

EL-Ear length, ED-Ear diameter, KR/E- Kernel rows per ear, K/R- Kernels per row, TW- Test weight, GY/P-Grain yield per plant

Genotyping of mapping population

Seventy-six polymorphic SSR markers between parents were utilized for genotyping of 169 teosinte-derived BC_1F_5 maize lines (Table 8).

Table 8. List of polymorphic markers and their PIC value, product length and number of alleles

S. No.	Primer Name	No. of allele	Product length (bp)	PIC
1	*phi056*	2	250-270	0.78
2	*umc2025*	3	140-180	0.79
3	*umc1988*	6	120-300	0.47
4	*bnlg615*	4	250-270	0.86
5	*umc1245*	2	150-190	0.44
6	*dupssr12*	3	140-190	0.72
7	*umc1726*	4	110-250	0.86
8	*umc1538*	4	150-210	0.64
9	*umc1500*	2	150-180	0.61
10	*umc1622*	2	80-90	0.62
11	*umc1845*	3	150-180	0.62
12	*umc1024*	2	180-200	0.67
13	*umc1156*	3	110-130	0.68
14	*umc1126*	3	150-170	0.64
15	*bnlg1721*	4	100-220	0.59
16	*bnlg1662*	2	150-190	0.63
17	*bnlg1520*	3	180-210	0.60
18	*umc2118*	2	130-150	0.62
19	*dupssr5*	4	120-250	0.58
20	*phi104127*	2	210-240	0.60
21	*bnlg1144*	2	150-200	0.62
22	*umc2000*	3	180-290	0.69
23	*umc1030*	3	100-150	0.56
24	*bnlg197*	3	80-120	0.29
25	*umc1294*	3	200-300	0.51
26	*umc2281*	3	180-200	0.61
27	*umc1662*	2	100-120	0.62
28	*umc1869*	3	130-250	0.74
29	*umc1667*	3	140-170	0.63
30	*umc1939*	4	170-280	0.64
31	*umc1720*	3	150-190	0.58
32	*bnlg1006*	4	190-250	0.62
33	*phi10918*	2	180-350	0.66
34	*umc1692*	2	110-200	0.60
35	*umc1171*	3	150-400	0.74
36	*umc2164*	3	120-150	0.69
37	*umc2143*	2	150-170	0.65

38	*bnlg389*	2	80-100	0.65
39	*umc2307*	3	150-350	0.68
40	*phi075*	3	220-250	0.58
41	*bnlg1600*	3	150-190	0.72
42	*y1SSR*	2	200-210	0.54
43	*bnlg1371*	2	90-150	0.65
44	*umc1215*	2	80-90	0.62
45	*phi070*	2	90-100	0.66
46	*umc1127*	3	180-200	0.74
47	*phi089*	2	90-100	0.62
48	*umc1546*	2	80-150	0.63
49	*umc2392*	5	200-600	0.61
50	*umc1428*	2	80-100	0.56
51	*umc1393*	2	100-120	0.76
52	*phi091*	2	110-130	0.56
53	*phi328175*	4	140-300	0.58
54	*phi069*	4	200-500	0.59
55	*umc1154*	2	150-190	0.63
56	*umc2635*	2	80-90	0.60
57	*phi420701*	2	300-320	0.55
58	*umc1304*	3	150-175	0.74
59	*bnlg669*	4	110-250	0.62
60	*phi121*	2	90-100	0.62
61	*bnlg1176*	3	190-260	0.58
62	*bnlg162*	2	250-290	0.65
63	*bnlg1065*	3	220-250	0.62
64	*umc1673*	2	80-100	0.76
65	*umc1279*	2	90-100	0.69
66	*phi067*	2	200-210	0.67
67	*phi016*	2	150-170	0.58
68	*umc2341*	2	140-170	0.67
69	*bnlg1375*	3	120-150	0.60
70	*umc1152*	2	190-200	0.64
71	*phi054*	2	100-110	0.65
72	*umc1053*	3	100-150	0.67
73	*bnlg1074*	3	190-400	0.65
74	*bnlg1250*	3	100-290	0.63
75	*phi035*	2	100-150	0.68
76	*bnlg1677*	3	180-200	0.69
Total	207			
Average	2.70			0.64

The analysis of the results of polymorphic markers microsatellites loci enabled the identification of 207 alleles with a mean of 2.7 alleles per locus. These findings are in close correspondence with the earlier observation of WIETHOLTER (2008) who observed an average of 2.7 alleles per locus in 23 SSR loci. LI *et al*. (2014) observed 2.45 average numbers of alleles based on 11 SSR loci. Similarly, molecular diversity analysis of 27 maize inbred lines based on 10 SSR markers resulted in 23 polymorphic alleles with an average of 2.3 alleles per locus (ABDEL-RAHMAN *et al*.**,** 2016). The minimum length of the amplified product was 80bp that was obtained in the case of primers umc1622, bnlg197, bnlg389, umc1215, umc1546, umc1428, umc2635 and umc1673 whereas maximum product length 600 bp was obtained in the case of umc2392 followed by 500 bp in the case of phi069. WANG *et al*. (2013) detected fragment sizes of 206-299 bp in the case of SSR markers. The number of alleles ranged from 2 to 6 per locus. Out of 76 markers, 36 had 2 alleles per locus, 28 primers had 3, 10 had 4 alleles per locus, and markers umc2392 and umc1988 had 5 and 6 alleles, respectively. NIKHOU and EBRAHIMI (2013) reported 2-6 alleles per locus based on the experiment conducted with SSR markers.

Polymorphism information content (PIC) value also named as power of discrimination of markers varied from 0.29 (bnlg197) with product length of 80-120 bp to 0.86 for bnlg615 and umc1726 with product length of 250-270 bp and 110-250 bp, respectively. WANG and CHUANG (2013) reported the range of PIC from 0.86 to 0.93. In our experiment, the average PIC value was 0.64. The PIC value from 0.56 and 0.89 with a mean of 0.78 was observed in an investigation conducted by GAZAL et al. (2016). SHEHATA *et al.* (2009) noted PIC value between 0.42 and 0.88 with an average of 0.58 when they used six SSR markers with eight maize inbred lines. PIC and alleles per locus of a marker indicating that the primer is highly polymorphic and the degree of polymorphism at the locus among the lines is high. As PIC value indicates discriminatory power of marker, most of the makers that were utilized in the present study were strong enough to discriminate different lines of mapping population, as the PIC value of most of the marker's ranges from 0.60 to 0.86 with a mean value of 0.64. Due to the PIC value > 0.5, all the studied markers except umc1988, umc1245, bnlg197 were highly informative (BOTSTEIN *et al*., 1980).

Identification of QTLs

The single-marker ANOVA revealed a total of 59 QTLs for six yield traits that spread over 10 different chromosomes with phenotypic variation ranges from 2.29 to 6.04%. Out of 59 QTLs, under the artificially inoculated environment (E_1) 35 QTLs and under un-inoculated environment (E_2) 24 QTLs were detected. Out of 59 QTLs, 9 QTLs were detected in both environments. Maximum numbers of QTLs were localized on chromosomes 4 and 9 and 6 followed by 10, 5, 1, 8, 2, 3, and 7. GUO *et al.* (2008) reported that the maximum number of QTLs for morphological traits was clustered on chromosomes 9 and 1 whereas, on chromosomes 2, 5, and 6 maximum QTLs were detected by YANG *et al*. (2020). Likewise for yield component traits, maximum QTLs on chromosome 4 were reported by LI *et al*. (2011). The trait, environment, and chromosome-wise number of QTLs identified in the BC_1F_5 population are presented in Table 9.

For EL, 7 QTLs on chromosomes 1, 4, 5, 6, 9, and 10 have been noted in E_1. These QTLs were linked with markers bnlg615, umc1720, umc2143, phi070, phi016, bnlg1375**,**

and umc1152 and collectively explained 15.85% phenotypic variation. In total five QTLs for ear length were identified in E_2 that were located on 3 chromosomes i.e. 3, 5, and 6. These QTLs were linked with markers umc1030, phi10918, umc1171, umc2143, and phi070**,** with phenotypic variation of 2.68, 2.53, 3.2, 2.61 and 2.81% respectively. Of these QTLs, two QTLs i.e. umc2143-linked and phi070-linked were common under both the environments. VELDBOOM and LEE (1994) identified five QTLs on chromosomes 1, 3, 5, 6, and 8 with phenotypic variation 2.1 to 8%. Six QTLs with phenotypic variation from 2.1 to 5.6% over five chromosomes (1, 2, 4, 6, and 8) were identified by AUSTIN and LEE (1996). Likewise, two minor QTLs with phenotypic variation 2.29 to 2.78% were also identified by GOU *et al***.** (2008). Several QTLs affecting EL distributed over all 10 maize chromosomes were identified by various researchers: on chromosomes 3 and 5 (MENDES-MOREIRA *et al***.,** 2015), on chromosomes 5 and 7 (CHEN *et al.*, 2016), on chromosomes 1, 6, and 10 (SU *et al***.,** 2017) and chromosomes 2, 4 and 9 (ZHAO *et al.*, 2018).

Table 9. Trait, environment and chromosome wise number of QTLs identified in BC_1F_5 population

S. No.	Traits	Environment	No. of QTLs	Chromosome No.									
				1	2	3	4	5	6	7	8	9	10
1	EL	E_1	7	*			*	*	*			**	*
		E_2	5			*		***	*				
2	ED	E_1	6				*		*		*	*	**
		E_2	2								*	*	
3	KR/E	E_1	7	*			*		*		*	*	**
		E_2	5	*		*	*	*	*				
4	K/R	E_1	4				**		*			*	
		E_2	4		**			*		*			
5	TW	E_1	6				*		*		*	**	*
		E_2	5	*	*		**			*			
6	GY/P	E_1	5	*			*	*	*				*
		E_2	3						*			*	*
Total		E_1	35	3	0	0	7	2	6	0	3	7	7
		E_2	24	2	3	2	3	5	3	2	1	2	1
Grand Total			59	5	3	2	10	7	9	2	4	9	8

Markers umc1720, umc1215, bnlg162, phi016, umc1152, and bnlg1250 linked with ED QTLs were located on chromosomes 4, 6, 8, 9, and 10 in E_1. These six QTLs explained 3.34, 2.47, 2.83, 2.87, 2.5 and 2.74% phenotypic variation, respectively. In E_2 two QTLs with a phenotypic variation of 3.68 and 2.33% were found on chromosomes 8 and 9 and were linked with markers bnlg162 and umc1279. A common QTL linked with marker bnlg162 on chromosome 8 was consistent in both the environments and was considered stable QTL. VELDBOOM and LEE (1994) identified QTLs governing ED distributed over chromosomes 1, 2, 3,

6, 7, and 8 explaining 2.6 to 6.1% phenotypic variation. Several QTLs for ED on chromosomes 1, 3, 7, 4, and 8 have been reported earlier by MENDES-MOREIRA *et al***.** (2015). Similarly, CHEN *et al*. (2016) detected numerous QTLs for ED over chromosomes 1, 2, 3, 4, 5, and 8. Five QTLs affecting ED on chromosomes 1, 4, and 7 were also reported by SU *et al.* (2017).

In E_1, seven putative QTLs for KR/E were identified on chromosomes 1, 4, 6, 8, 9, and 10. These QTLs were linked with markers umc1988, umc1720, umc1215, phi016, umc1152 and bnlg125**0** and accounted 19.52% phenotypic variation collectively. For KR/E five QTLs on chromosomes 1, 3, 4, 5, and 6 were identified in E_2. The umc1988-linked QTL, bnlg1144-linked QTL, umc1720-linked QTL, phi10918-linked QTL, and umc1215-linked QTL had coefficients of determination of 3.38, 3.13, 3.54, 2.31, and 2.89% for KR/E, respectively. Three QTLs were noted to be common in both the environments that were linked with markers umc1988, umc1720, and umc121. Four QTLs governing KR/E explaining 2.1 to 11.2% phenotypic variation on chromosomes 1, 2, and 4 have been reported by VELDBOOM and LEE (1994). AUSTIN and LEE (1996) reported ten minor QTLs for KR/E on chromosomes 1, 3, 4, 5, 6, 9, and 10 with phenotypic variation ranges from 2.4 to 7.3%. Likewise, GUO *et al***.** (2008) detected three minor QTLs on chromosomes 6, 7, and 10 with 1.68 to 2.08% phenotypic variation. MENDES-MOREIRA *et al.* (2015) have detected three QTLs on chromosomes 1, 2, and 3, and one QTL on chromosome 1 was identified by CLAUDIA *et al*. (2016) using BC2S3 maize-teosinte recombinant inbred lines (RILs). CHEN *et al.* (2016) detected QTLs on chromosomes 4 and 5 by using the RILs population. LIU *et al***.,** (2016) identified four QTLs on chromosomes 1, 2, 4, and 5 in the maize-teosinte introgression population. On chromosome 8, QTLs for KR/E were also reported by SU *et al.* (2017).

Four QTLs for K/R were detected that were located on chromosomes 4, 6, and 9 in E_1. Maximum phenotypic variation was explained by QTL linked with marker umc1939 (3.34%) followed by QTL linked with umc1215 (3.03%), phi016 (2.6%) and umc1720 (2.43%). In E_2, four QTLs were detected on chromosomes 2, 5, and 7 and were linked with markers umc1156, bnlg1662, phi10918, and umc2392 with phenotypic variation from 2.59 to 4.28%. None of the QTLs were found common under both environments. Using different mapping populations more than 23 QTLs for K/R have been identified on 9 of the 10 chromosomes of maize. CHEN *et al***.** (2016) and SU *et al***.** (2017) have identified several QTLs for K/R on chromosomes 4, 5, and 6.

For TW, six QTLs on chromosomes 4, 6, 8, 9, and 10 were detected in E_1. These QTLs were explaining phenotypic variation from 2.32 to 3.5% and were linked with markers umc1939, umc1215, bnlg162, umc1279, phi016**,** and umc1152**.** Five QTLs that were linked with markers umc1500, umc1156, umc1667, umc1939 and phi069 were found in E_2 and located on chromosomes 1, 2, 4, and 7 with phenotypic variation from 2.33 to 4.19%. One umc1939-linked QTL was found common between both environments. AUSTIN and LEE (1996) detected several QTLs distributed over all 10 chromosomes of maize except chromosome 6 with 2.5 to 7.8% phenotypic variation. In the present investigation, we have reported QTLs for test weight on 7 different chromosomes. Similarly, various researchers reported TW QTLs that were distributed on chromosomes 1 and 4 (GUO *et al.*, 2008), on chromosome 1 (YANG *et al.*, 2016), on chromosomes 3, 4, 5, 6 and10 (ABDEL-RAHMAN *et al***.,** 2016), on chromosomes 4 and 7 (CHEN *et al.*, 2016), on chromosomes 2, 4, 7, 8 and 9 (PAN *et al.*, 2017), on chromosomes 3, 4, 6 and 8

(SU *et al.*, 2017), on chromosomes 1 and 4 (ZHAO *et al*., 2018). LIU *et al*. (2016) identified eight QTLs over chromosomes 1, 2, 3, 5, and 8 in the maize-teosinte introgression population.

Five QTLs that were located on five chromosomes, 1, 4, 5, 6, and 10 were detected in E_1 for GY/P. These QTLs were linked with markers bnlg615, umc1720, bnlg1006, umc1215, and umc1279 and responsible for phenotypic variation from 2.32 to 6.04%. In E_2, three QTLs were detected on chromosomes 6, 9, and 10. These phi089-linked QTL, umc1279-linked QTL, and phi054-linked QTL together accounted for 9.08% phenotypic variation. One QTL that was linked with marker umc1279 was detected under both environments. In a previous study, VELDBOOM and LEE (1994) detected one QTL for yield on chromosome 6. AUSTIN and LEE (1996) noted six QTLs distributed over 5 chromosomes, 1, 5, 6, 7, and 8 with phenotypic variation ranges from 2.5 to 7.6%. LIMA *et al.* (2006) identified 16 minor QTLs over seven maize chromosomes, namely 1, 2, 3, 4, 5, 6, and 7 with phenotypic variation ranging from 1.02 to 4.66%. Four regions for grain yield and related traits on chromosome 10 were also observed by LI *et al.* (2010). Similarly, in the present study, a stable QTL that was linked with marker umc1279 on chromosome 10 was detected under both environments. SEMAGN *et al*. (2013) identified several QTLs overall 10 chromosomes. Likewise, on chromosomes 1, 4, 5, 6, 8, and 10, several QTLs have been observed earlier by VEIGA *et al.* (2012); YANG *et al*. (2016); CHEN *et al*. (2016); SU *et al.* (2017); NIKOLIC *et al.* (2018); RIBEIRO *et al.* (2018).

QTLs overlapping among yield and contributing traits

In the present study, ten QTLs regulating multiple traits were identified (Table 10). Among them, four regions namely phi016-linked (EL, ED, KR/E, K/R, TW), umc1720-linked (EL, ED, KR/E, K/R, GY/P), umc1215-linked (ED, KR/E, K/R, TW, GY/P), and umc1152-linked (EL, ED, KR/E, K/R, TW) were simultaneously regulating five traits. Three QTLs that were linked with markers phi10918 (EL, KR/E, K/R), bnlg162 (ED, KR/E, TW), and umc1279 (ED, TW, GY/P) were controlling three characters each. The remaining Three QTLs that were linked with markers umc1988 (EL, TW), umc1939 (K/R, TW), and bnlg1250 (ED, KR/E) were responsible for four characters each. Similarly, overlapping regions for yield contributing traits were also reported by LIU and CHEN (2011) (EL, K/R, T/W, and GY/P), YANG *et al.* (2016) (GY/P and TW), MIKIC *et al.* (2016) (ED, K/R, EL, and GY/P), HUO *et al.* (2016) (EL and K/R) and YANG *et al.* (2020) (ED, KR/E, K/R, and GY/P). In the present study co-localized QTLs for yield and yield contributing traits, were consistent with significant phenotypic correlations among grain yield and its component traits. The co-localization of QTL may mean tight linkage or pleiotropy (LIMA *et al*., 2006; HU *et al*., 2012). In various fine mapping and map-based cloning studies, it has been observed that numerous QTL exhibits pleiotropic effects on yield-related traits (FAN *et al.*, 2006; XUE *et al.*, 2008). XIE *et al*. (2008) evaluated 7 co-localized yield-related QTLs in rice and concluded that that could be a single pleiotropic gene that regulates several traits simultaneously. It may be possible that co-localized regions might contain a single QTL with pleiotropic effect on multiple traits or several tightly linked QTL for individual traits. The real reason for the co-localization of QTLs can be revealed further either through fine-mapping or by single substitution lines development for the region containing QTLs.

Table 10. List of co-localized QTLs for yield and contributing traits

S. No.	Markers	Bin	Traits					
			EL	ED	KR/E	K/R	TW	GY/P
1	*umc1988*	1.06	*				*	
2	*umc1939*	4.09				*	*	
3	*umc1720*	4.10	*	*	*	*		*
4	*phi10918*	5.03	*		*	*		
5	*umc1215*	6.03		*	*	*	*	*
6	*bnlg162*	8.05		*	*		*	
7	*umc1279*	9.00		*			*	*
8	*phi016*	9.04	*	*	*	*	*	
9	*umc1152*	10.01	*	*	*		*	
10	*bnlg1250*	10.05		*	*			

*common QTLs

EL-Ear length, ED-Ear diameter, KR/E- Kernel rows per ear, K/R- Kernels per row, TW- Test weight, GY/P-Grain yield per plant

CONCLUSION

In the teosinte-introgressed maize population sufficient variation was recorded it reflects the possible role of wild progenitor in maize diversity enhancement. Based on clustering analysis of lines that found superior for one or more yield and contributing traits could be utilized in hybridization programme for accumulation of desirable traits together. EL, ED, KR/E, K/R, and TW are important yield contributing traits in maize therefore by simulations improvement of these trait targeted yield can be achieved. Among detected QTLs, some QTLs were co-associated with two or more desirable traits. Therefore by harnessing co-localized QTLs it is possible to incorporate and improve several traits together. These identified chromosomal regions could be targeted to carry out marker-assisted selection, fine-mapping as well as map-based cloning in maize.

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IDENTIFIKACIJA QTL-ova ZA PRINOS I KOMPONENTE PRINOSA KOD KUKURUZA-BILs DOBIJENIH IZ TEOZINTE U OPTIMALNIM I USLOVIMA BIOTIČKOG STRESA

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Izvod

Kod kukuruza, prinos zrna je najvažnija osobina koja ima složen obrazac nasleđivanja. Osobine koje doprinose prinosu stabilnije su i imaju veću heritabilnost od prinosa. Stoga je ovo istraživanje sprovedeno da bi se identifikovali kvantitativni lokusi svojstava (QTLs) povezani sa prinosom zrna i njegovim komponentama pomoću SSR markera. Populacija od 169 linija BC1F5 izvedena je ukrštanjem inbred linije kukuruza DI-103 i teozinte-*parviglumis* i korišćena je za genotipizaciju i fenotipizaciju. U bolesnom stanju (E1), dužina klasa (EL), prečnik klasa (ED), redovi zrna po klasu (KR/E), zrna po redu (K/R), ispitna težina (TW) i prinos zrna po biljci (GY/P) je imao 7, 6, 7, 4, 6 i 5 QTL -ova, dok je u kontrolisanom stanju (E2) 5, 2, 5, 4, 5 i 3 QTL-a otkriveno za navedene osobine, respektivno. Dosledni QTL-ovi u svim okruženjima otkriveni su za 5 od 6 ispitivanih osobina, a broj QTL-ova je bio EL (2), ED (1), KR/E (3), TW (1) i GY/P (1), dok, za K/R nijedan QTL nije bio zajednički između E1 i E2. Analizom mapiranja identifikovali smo genomska područja povezana sa dve osobine na način koji je u skladu sa fenotipskim korelacijama među osobinama, podržavajući ili pleiotropiju ili tesnu vezu između QTL-ova. Identifikovana su tri ko-lokalizovana QTL-a između prinosa zrna i osobina koje doprinose prinosu. Posebno je *umc1720* povezan QTL u binu 4.10 istovremeno bio odgovoran za GY i EL, ED, KR/E, K/R; *umc1215*-vezani QTL u binu 6.03 bio je istovremeno odgovoran za GY i ED, KR/E, K/R, TW; *umc1279*-QTL je bio odgovoran za GY i ED, TW. Rezultati sugerišu da su hromozomske regije koje sadrže ko-lokalizovane QTL-ove koje upravljaju sa više osobina povezanih sa prinosom potencijalni ciljevi za selekciju. Osim toga, za 6 proučavanih osobina identifikovane su 44 superiorne linije, a zajedno sa oba roditelja, odnosno kukuruzom (DI-103) i teosintetom, grupisane su u 11 grupa. Stoga se linije grupisane nezavisno mogu koristiti u programu hibridizacije za akumulaciju osobina koje doprinose prinosu za njegovo povećanje.

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