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Developing genetic resources and genetic analysis of plant architecture-related traits in teosinte-introgressed maize populations

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Research Article

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Abstract

Teosinte, the wild progenitor of maize, has immense potential for providing unique traits and is more divergent compared to inbred lines and landraces. One hundred and sixty-nine teosinte-introgressed maize backcross inbred lines were developed to widen the genetic base of maize with predomestication alleles. The population was evaluated phenotypically and genotypic data of 76 SSR markers were used to map quantitative trait loci (QTLs) governing the targeted traits. Sixty-six QTLs were detected for eight plant architect-related traits that are spread over 10 different chromosomes with phenotypic variation ranging from 2.29 to 13.97%. Maximum three stable QTLs were recorded for days to anthesis (DA) followed by two for days to silking (DS), plant height (PH) and node bearing first ear (NBE). For rest of three traits namely flag leaf length (FLL), flag leaf width (FLW) and ears per plant (E/P) only one stable QTL was detected. Among the 16 common QTLs, the marker *phi328178*-linked QTL governed four characters (DA, DS, FLL, FLW) simultaneously, followed by *umc1622*-linked (ASI, FLW, E/P), *umc2341*-linked (DA, DS, NBE) and *phi075*-linked QTLs (ASI, PH, NBE) controlling three traits each. Remaining 12 QTLs controlled two characters. Molecular association between co-localized QTLs for different traits was also validated at the phenotypic level by significant correlation estimates. For eight studied traits, 53 superior lines were identified which along with parents (teosinte and maize inbred DI-103) were grouped into 12 clusters. Therefore, lines clustered independently can be combined to accumulate desirable traits for the improvement of maize.

Introduction

Maize (*Zea mays* L.) is a versatile crop and has greatly contributed to the world's economy. Being a C_4 plant with very high yield potential, it is also called the '*queen of cereals*' (Saritha *et al.*, 2020). It is the third most important cereal crop after wheat and rice in the world's agricultural economy (FAO, 2020). Maize is required by various sectors from poultry, animal husbandry to value-added products for human consumption and also is a major source of industrial raw materials for starches, acids and alcohol production (Galani *et al.*, 2020). Thus, to meet the increasing demand, improvement in yield and resiliency to climatic fluctuation is the priority area of maize improvement programme (Keimeso *et al.*, 2020). Grain yield is a complex trait, highly dependent and determined by various independent component characters. Optimizing the expression of yield contributing traits through genetic manipulation is probably the best way to enhance maize yield. Therefore, there is a need for integrated approach for improvement of the traits related to plant morphology (Wang *et al.*, 2008*a*, 2008*b*; Ramstein *et al.*, 2020).

Morphological traits related to flowering behaviour (i.e. days to anthesis, days to silking, anthesis-silking interval) are considered important yield traits under stress conditions particularly under drought (Westgate and Bassetti, 1990; Edmeades *et al.*, 1997; Sah *et al.*, 2020) and are also important for the development of varieties with variable maturity duration suited for growing under variable climatic conditions. Traits related to leaf morphology namely, flag leaf length and flag leaf width are considered important from the yield point of view. Leaf size is dependent on leaf width, leaf length and leaf area, can significantly influence canopy morphology, lead to higher photosynthetic activity and may result in higher grain yield (Zhang *et al.*, 2020). Similarly, plant height, node bearing first ear and ears per plant are important traits concerning lodging tolerance (Li *et al.*, 2007), as well as yield (Motto and Moll, 1983). Therefore, understanding the genetics of these traits is essential to breed varieties with good yield potential as well as resilient to climatic changes.

Yield and various yield-associated traits are polygenic in nature and their effective utilization in breeding depends on their dissection into simply inherited traits using the approach of quantitative trait locus (QTL) analysis. QTLs have been analysed in many investigations using population derived from biparental crosses between the contrasting maize inbred lines for flowering behaviour (Guo *et al.*, 2008; Liu *et al.*, 2016*a*), leaf morphology (Agrama *et al.*, 1999; Liu *et al.*, 2010; Ku *et al.*, 2012; Zheng and Liu, 2013; Yang *et al.*, 2015), plant height (Guo *et al.*, 2008; Yang *et al.*, 2008; Nikolic *et al.*, 2011), ear per plant (Veldboom and Lee, 1994) and node bearing ear (Nikolic *et al.*, 2011; Zhu *et al.*, 2013; Li *et al.*, 2014*a*, 2014*b*). Large-scale QTL mapping performed by Tian *et al.* (2011) for leaf length and leaf width concluded that the genetic architecture of the leaf traits was governed by numerous minor QTLs. Such QTL studies in teosinte (*Z. mays* subsp. *parviglumis* Iltis and Doebley) derived maize population are limited. In fact, cultivated modern maize is a finished product that may not contain numerous alleles, which have been lost during the course of domestication followed by selection and trait-specific breeding (Vigouroux *et al.*, 2005; Tenaillon *et al.*, 2004; Warburton *et al.*, 2008; Singh *et al.*, 2017; Tian *et al.*, 2019). Hence, many desirable alleles required for improvement in yield as well as climatic resiliency are limited in elite maize germplasm (Tarter *et al.*, 2004; Le Clerc *et al.*, 2005). However, wild relatives are expected to still possess the desirable and diverse allelic variants that were lost during the process of domestication (Liu *et al.*, 2016*a*; Mammadov *et al.*, 2018; Tian *et al.*, 2019; Joshi *et al.*, 2021; Sahoo *et al.*, 2021) and such wild alleles can be re-domesticated through introgression or pre-breeding approaches. Teosinte, the wild progenitor of maize, is distinct from maize in several aspects; namely, flowering behaviour, plant morphology, ear traits and yield (Smith and Lester, 1980; Doebley, 2004; Singh *et al.*, 2017; Adhikari *et al.*, 2019, 2020; Fu *et al.*, 2019; Yang *et al.*, 2019). Despite dramatic morphological differences, the sexual compatibility between maize and teosinte is well documented (Singh *et al.*, 2017; Kumar *et al.*, 2019). Therefore, lines with desirable traits can be generated and multiple traits can be mapped together by targeting a single mapping population. Many investigators have used teosinte-introgressed populations for QTL analysis of different traits (Calderón *et al.*, 2016; Liu *et al.*, 2016*a*, 2016*b*; Karn *et al.*, 2017; Fu *et al.*, 2019). Our research is focused on diversification of maize germplasm resources as the variability in maize inbreds and landraces is less in comparison to the wild subspecies teosinte. Therefore, in the present investigation, we intended to analyse a population comprising 169 backcross inbred lines (BILs) derived from maize and teosinte hybridization to elucidate the genetic basis of plant architecture-related traits, to assess genetic variability in the population, and to identify superior teosinte-introgressed maize inbred lines with desirable traits to be used in maize breeding programmes.

Materials and methods

Generation of materials

Materials for the investigation consisted of 169 BC_1F_5 lines derived from crossing wild progenitor teosinte (*Z. mays* ssp. *parviglumis* Iltis and Doebley) and a maize inbred line DI-103. Teosinte *parviglumis* was locally collected and maintained by controlled pollination for five successive generations. The maize inbred line used as seed parent was crossed with teosinte used as pollen parent to produce F_1s. The maize line was crossed with F_1 individuals to generate backcross, BC_1F_1 seeds. The BC_1F_1 seeds were sown in the next season and selfed to generate BC_1F_2 seeds. Subsequently, selfing was continued for three more generations to produce BC_1F_5 seeds. Thus, the 169 BC_1F_5 BILs constituted the population for the present investigation.

Experimental layout and trait evaluation

The 169 BILs of maize were sown in a randomized complete block design with two replications in two different environments (artificially inoculated with banded leaf and sheath blight causing fungal pathogen *Rhizoctonia solani* (E_1) and uninoculated (E_2)) in the *Kharif* season (June to October) 2018. Each line was planted in a 2 m long row separated at 75 cm from other rows. The 169 BILs were evaluated for eight plant architecture traits, namely, days to 50% anthesis (DA), days to 50% silking (DS), anthesis-silking interval (ASI), flag leaf length (FLL), flag leaf width (FLW), plant height (PH), ear per plant (E/P) and node bearing first ear (NBE). For recording observations, three plants were randomly selected under both the environments, scores to different characters were assigned and finally average of three plants was calculated and used for statistical analysis.

DNA extraction, SSR assay and scoring of genotyping data

For genomic DNA isolation, leaves were collected from 30 days old seeding and DNA was extracted by CTAB (cetyl trimethyl ammonium bromide) method (Doyle and Doyle, 1990) with some modification. The quality and quantity of DNA were assessed using electrophoresis (0.8% agarose gel) and spectrophotometer (Systronics PC Based Double Beam Spectrophotometer 2202), respectively. Then stock DNA was diluted to obtain working concentrations of 200 ng/µl. Polymorphism between maize inbred line DI-103 and teosinte-*parviglumis* was investigated using 168 microsatellite markers that were distributed throughout the maize genome. PCR reactions were performed in 13.8 µl reaction mixture containing 1.5 µl reaction buffer with 15 mM $MgCl_2$ (10 ×), 3 µl (200 ng/µl) genomic DNA, 0.35 µl dNTPs mix (2.5 mM each), 1.5 µl each forward and reverse primer (40 ng/µl), 0.25 µl Taq DNA polymerase (3 U/µl) and 7.2 µl deionized water. The PCR cycle was performed as: the flow for the first cycle was initial denaturation (94°C for 5 min), denaturation (94°C for 40 s), annealing (55–68°C for 40 s) and elongation (72°C for 1 min). The cycle from denaturation to elongation was repeated 35 times, followed by a final elongation (10 min at 72°C). The amplified PCR profile of each BIL with each marker was resolved on 3% agarose gel and visualized and captured using PC-based gel documentation system (Alpha Innotech Corporation, San Leandro, CA USA). The amplification profile of each marker was compared with a standard DNA ladder of 100 bp and allelic size was determined. The SSR data of each line were scored separately by using the following coding symbols.

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

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Statistical analysis and QTL mapping

R statistical software was used to perform analysis of variance (ANOVA). Ten superior lines that showed higher average estimates among the environments for all the studied traits were identified and they were classified based on the UPGMA (unweighted pair group method with arithmetic averages) method of PAST (PAleontological STatistics) software (Hammer *et al.*, 2001) and a dendrogram was generated by using Jaccard dissimilarity matrix. Polymorphism information content (PIC) value of each marker was calculated by using the formula given by Smith *et al.* (1997) $PIC = 1 - \sum_i^n fi^2$ where *fi* is the frequency of the *i*th allele. The PIC calculation was performed using Microsoft Excel. The single-marker analysis method of Win QTL Cart 2.5 software was used to perform QTL analysis (Wang *et al.*, 2012). It is the quickest method that scans linkage between the trait of interest and a single marker at a time. Based on genotyping data of each marker, individuals were grouped in different genotypic classes, and the mean value of targeted traits for each class was estimated by summing up the estimates of each individual of that particular class. Further *t*-tests were performed to compare the mean value of each genotypic class if the difference is significant then the marker based on which individuals were classified in different genotypic classes is likely to be linked with the trait of interest.

Results

Phenotyping of plant architecture traits and identification of superior lines

ANOVA revealed significant variation among 169 lines for all the traits which reflect differential allelic introgression from teosinte leading to variation in BC_1F_5 maize lines (Table 1). The range of different plant architecture-related traits in both control (E_2) and banded leaf and sheath blight disease stress (E_1) environments is presented in online Supplementary Table S1 and Figs S1–S8. Days to anthesis and days to silking of the maize inbred line DI-103 were 52.50 and 55.00 days in E_1, and 54.50 and 56.50 days in E_2, respectively. However, teosinte did not differ for days to anthesis (81.50 days, E_1) and days to silking (78.00–78.50 days, E_2) in both the environments. In BC_1F_5 maize lines, the DA and DS ranged from 45.00 to 67.00 days and 43.00 to 66.00 days in E_1, and 47.00 to 68.00 days and 44.00 to 67.00 days in E_2, respectively. In the case of teosinte and maize inbred line DI-103, ASI was nearly the same (under both the environments +3.00 to +3.50 and −2.00 to −2.50 days). However, in BC_1F_5 lines ASI varied from −5.00 to +4.00 days and −4.00 to +5.00 days under E_1 and E_2, respectively. Maize inbred line DI-103 showed FLL and FLW of 29.51 and 4.53 cm in E_1, and 30.78 and 4.66 cm in E_2, respectively. The observations noted on FLL and FLW were 26.00 and 3.88 cm, and 23.75 and 3.50 cm when teosinte was investigated in E_1 and E_2, respectively. The data on FLL in BC_1F_5 lines varied from 9.90 to 59.45 cm and for FLW from 1.01 to 6.56 cm under E_1 whereas under E_2 FLL and FLW varied from 9.40 to 60.88 cm and 2.80 to 7.60 cm, respectively. The average PH noted for maize inbred line DI-103 and teosinte was 97.20 and 241.00 cm in E_1, and 97.39 and 242.00 cm in E_2, respectively. In BC_1F_5 lines variation for PH ranged from 90.60 to 248.00 cm in E_1 and 88.00 to 229.33 cm in E_2. Among BILs, the average E/P and NBE varied from 1.00 to 4.00 and 3.00 to 7.60 in E_1 and 1.00 to 5.50 and 2.60 to 7.60 in E_2, respectively. In the case of maize inbred line DI-103,

Table 1. Analysis of variance (ANOVA) for different characters in teosinte-introgressed BILs of maize

		DA		DS		ASI		FLL		FLW		PH		E/P		NBE	
S.V.	df	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2
Replication	1	39.30	51.77	41.38	48.59	0.14	0.00	1.96	81.66	0.10	0.90	37.81	740.70	0.22	0.49	4.10	0.69
Treatment	170	35.29**	35.60**	45.31**	46.62**	2.39**	2.79**	174.58**	167.11**	2.13**	2.62**	1907.05**	1693.553**	799.87**	674.44**	1.83**	1.92**
Error	170	5.55	4.79	5.34	4.75	0.07	0.08	2.39	6.30	0.05	0.17	91.97	59.92	0.09	0.20	0.43	0.20
SEm ±		1.67	1.55	1.63	1.54	0.19	0.20	1.09	1.78	0.16	0.29	6.78	5.47	0.21	0.32	0.47	0.32
CD (at 1%)		6.13	5.70	6.02	5.68	0.70	0.07	4.03	6.54	0.57	1.09	24.98	20.17	0.77	1.16	1.71	1.17
CD (at 5%)		4.65	4.32	4.56	4.30	0.53	0.55	3.05	6.54	0.43	0.82	18.93	15.28	0.59	0.88	1.30	0.89
CV (%)		4.14	3.71	4.04	3.73	11.34	11.21	5.02	7.46	5.60	9.19	5.72	4.71	8.14	11.83	12.09	7.71

DA, days to 50% anthesis; DS, days to 50% silking; ASI, anthesis-silking interval; FLL, flag leaf length; FLW, flag leaf width; PH, plant height; E/P, ears per plant; NBE, node bearing first ear.
**1% level of significance.

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Table 2. Trait-wise list of top 10 superior BILs of maize

S. No.	Characters	BILs of maize
1.	DA	MT-17, MT-32, MT-33, MT-40, MT-88, MT-95, MT143, MT152, MT-155, MT-169
2.	DS	MT-17, MT-20, MT-32, MT-33, MT-40, MT-88, MT-95, MT-152, MT-156, MT-164
3.	ASI	MT-1, MT-3, MT-10, MT-11, MT-26, MT-29, MT-46, MT-49, MT-132, MT-167
4.	FLL (cm)	MT-15, MT-18, MT-28, MT-32, MT-35, MT-40, MT-57, MT-113, MT-142, MT-159
5.	FLW (cm)	MT-17, MT-26, MT-41, MT-43, MT-56, MT-113, MT122, MT-141, MT-142, MT-169
6.	PH (cm)	MT-5, MT-11, MT-28, MT-29, MT-32, MT-87, MT-95, MT-101, MT-150, MT-169
7.	E/P	MT-10, MT-51, MT-76, MT-78, MT-88, MT-103, MT-142, MT-158, MT-165, MT-167
8.	NBE	MT-38, MT-98, MT-103, MT-104, MT-111, MT-117, MT-127, MT-140, MT-141, MT-142

DA, days to 50% anthesis; DS, days to 50% silking; ASI, anthesis-silking interval; FLL, flag leaf length; FLW, flag leaf width; PH, plant height; E/P, ears per plant; NBE, node bearing first ear.

E/P and NBE were 1.10 and 4.50 in E_1, and 1.16 and 4.16 in E_2, respectively. However, in the case of teosinte, E/P was 242.33 and 263.50, and NBE was 6.16 and 5.83 in E_1 and E_2, respectively.

The data on the different parameters across both the environments were averaged and the top 10 superior lines were selected (Table 2). Of the total of 53 selected lines, two lines namely MT-32 and MT-142 were found superior for four different traits followed by five lines namely MT-17, MT-40, MT-88, MT-95 and MT-169 which were superior for three traits. Ten lines namely MT-10, MT-11, MT-26, MT-28, MT-29, MT-33, MT-103, MT-141, MT-152 and MT-167 were found superior for two traits whereas the remaining lines were superior for one trait only. Further clustering of these selected lines was performed by UPGMA to know the extent of diversity among lines and select diverse parents for desirable trait accumulation. The cluster analysis with UPGMA using Jaccard's similarity coefficients grouped the 55 lines (53 BILs plus maize inbred parent (DI-103) and teosinte) into 12 clusters based on molecular data with 76 SSR markers (Fig. 1, Table 3). The pair-wise genetic dissimilarity between the lines varied from 0.326 to 0.768. Teosinte clustered independently in cluster I and showed maximum divergence from MT-26 with dissimilarity value 0.768 and from MT-49 with dissimilarity value 0.766 of cluster V. With 0.675 dissimilarity value, teosinte showed divergence with MT-159 that was clustered independently in cluster III. But MT-18 and MT-32 were quite similar (67.33%) with the minimum dissimilarity value of 0.326 and both belonged to cluster 12, followed by MT-156 and MT-165 with dissimilarity value 0.328, grouped in cluster 9. Distribution patterns of lines among clusters were not uniform. Maximum 19 lines were grouped in cluster IX followed by 16 in cluster XII. Clusters V and XI consist of four lines each. In cluster VIII, three lines were present and both clusters VII and X were composed of two lines each, whereas a minimum of one line was present in clusters II, III, IV and VI.

Genotyping of mapping population

A total of 76 SSR markers that were identified as polymorphic between parents were utilized for genotyping of 169 BILs. The details of primers including the number of alleles, product length and PIC value were presented in online Supplementary Table S2. Genotyping of mapping population with 76 polymorphic SSR primers led to the identification of 207 alleles with an average of 2.7 alleles per locus. Allele size ranged from 80 bp (*umc1622*, *bnlg197*, *bnlg389*, *umc1215*, *umc1546*, *umc1428*, *umc2635* and *umc1673*) to 600 bp (*umc2392*). With an average of 0.64 PIC, primer *bnlg197* portrayed a minimum PIC value of 0.29 whereas the maximum value of 0.86 was recorded in the case of *bnlg615* and *umc1726*.

Identification of genomic regions for plant architecture-related traits

The single-marker ANOVA revealed a total of 66 QTLs for eight plant architecture-related traits spread over 10 different chromosomes. The observed phenotypic variation explained by each QTL varied from 2.29 to 13.97%. Of the total 66, 16 QTLs accounted for more than 10% of phenotypic variation in the trait whereas the remaining 50 QTLs had <10% contribution in the respective trait. Out of 66 QTLs, 29 QTLs were identified in the artificially inoculated environment (E_1) whereas 37 QTLs were identified in the un-inoculated environment (E_2). Eleven common QTLs were identified across the two environments. Trait, environment and chromosome-wise number of QTLs identified in the BC_1F_5 population are presented in online Supplementary Table S3 and Fig. 2 whereas stable QTLs along with linked markers and phenotypic value are presented in Table 4. For days to anthesis, two major QTLs, *qDA-1* and *qDA-2* were identified on chromosome 9 that were linked with markers *umc2341* and *umc1279* accounted for 12.84 and 12.81% phenotypic variation in E_1 and 11.79 and 13.97% in E_2, respectively. In addition, one minor QTL in E_1 and two minor QTLs in E_2 were also identified for days to anthesis. *qDA-1* and *qDA-2* and a minor QTL *qDA-3* were considered common and stable QTL in the two environments. One major QTL *qDS-1* linked to *umc1720* and located on chromosome 4 was responsible for 13.91% (E_1) and 12.88% (E_2) phenotypic variation for days to silk emergence. In addition, three QTLs (on chromosomes 3, 7, 9) in E_1 and two QTLs (on chromosomes 7 and 9) in E_2 having less than 10% phenotypic effect on days to silk emergence were also identified. For anthesis-silking interval (ASI) two minor QTLs linked with markers *phi075* and *umc1215* were identified on chromosome 6 in E_1 which jointly explained 5.63% phenotypic variation. Another two minor QTLs linked with *umc1622* (6.06%) and *umc1538* (2.60%) were identified on chromosomes 2 and 1, respectively, in E_2. None of the QTLs was found common across the environments for ASI. A total of four QTLs linked with markers *umc1662*, *bnlg389*, *phi328175* and *phi054* on chromosomes 4, 5, 7 and 10, respectively, in E_1 were identified for flag leaf length (FLL). Among these, *phi328175*-linked *qFLL-1* contributing 12.74% of the

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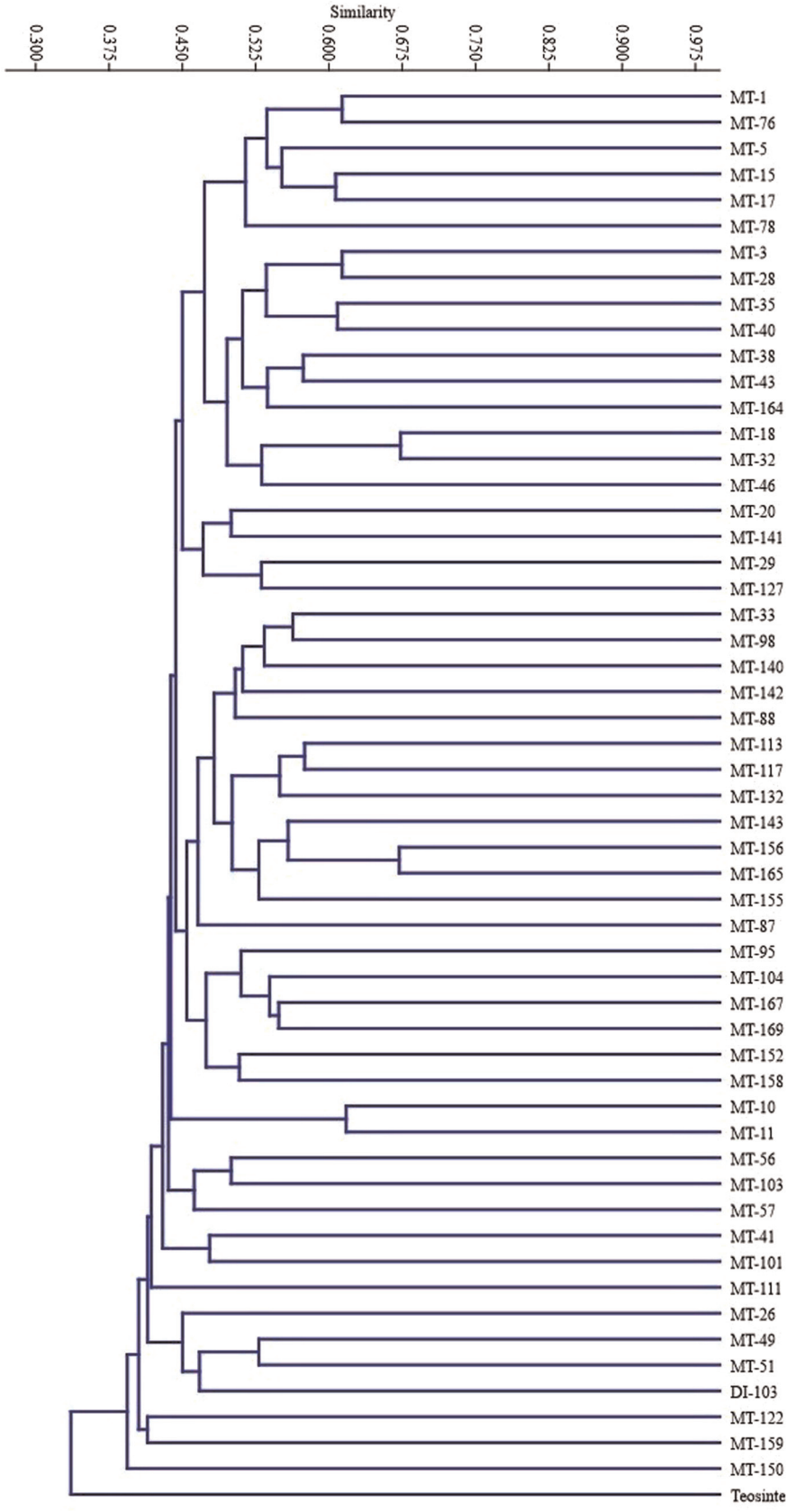

Fig. 1. Clustering pattern of 55 promising BILs of maize (including parents) based on 76 SSR markers data.

phenotypic variation for flag leaf length was considered to be a major QTL. In E_2, four QTLs linked with markers *phi104127* (2.76%) on chromosome 3, *umc2307* (2.74%) on chromosome 5, *umc1428* (2.34%) and *phi328175* (12.29%) on chromosome 7 were detected. The *qFLL-1* was the QTL identified for flag leaf length across both environments.

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Table 3. Clustering patterns of 55[a] superior BILs of maize

Cluster	No. of genotypes	Genotypes
1	1	Teosinte
2	1	MT-150
3	1	MT-159
4	1	MT-122
5	4	MT-26, MT-49, MT-51, DI-103
6	1	MT-111
7	2	MT-41, MT-101
8	3	MT-56, MT-57, MT-103
9	19	MT-33, MT-87, MT-88, MT-95, MT-98, MT-104, MT-113, MT-117, MT-132, MT-140, MT-142, MT-143, MT-152, MT-155, MT-156, MT-158, MT-165, MT-167, MT-169
10	2	MT-10, MT-11
11	4	MT-20, MT-29, MT-127, MT-141
12	16	MT-1, MT-3, MT-5, MT-15, MT-17, MT-18, MT-28, MT-32, MT-35, MT-38, MT-40, MT-43, MT-46, MT-76, MT-78, MT-164

[a]Includes parental lines DI-103 and teosinte.

A QTL, *qFLW-1*, linked with marker *umc2143* contributing 13.10% variation in flag leaf width was identified on chromosome 5 along with a minor QTL on chromosome 6 (*umc1215*-linked, 3.88%) in E_1. A total of four QTLs were detected, one each on chromosomes 2 and 5 and two on chromosome 7 for FLW in E_2. Maximum phenotypic variation of 12.62% was explained by *umc2143*-linked QTL, *FLW-1* whereas the remaining QTLs together explained 11.41% variation for the trait. For plant height under E_1, one major *phi075*-linked QTL *qPH-1* on chromosome 6 accounting for 12.98% variation was identified. Apart from the above, three minor *phi420701*, *bnlg1065* and *umc1279*-linked QTLs were also observed on chromosomes 8 and 9, respectively. Nine QTLs distributed over chromosomes 2, 3, 4, 5, 6, 9 and 10 were detected for plant height in E_2. The QTLs linked with *bnlg1520*, *dupssr5*, *umc1869*, *umc1939*, *bnlg389*, *umc1279*, *phi054* and *umc1053* accounted for phenotypic variation from 2.50 to 4.99% whereas 12.60% variation in plant height was explained by *qPH-1*. Two QTLs linked with markers *phi075* and *umc1279* were detected under both the environments. For ear per plant, four QTLs linked with marker *umc1622*, *bnlg1662* on chromosome 2, *umc2143* on chromosome 5 and *umc1304* on chromosomes 8, were detected in E_1 explaining phenotypic variation ranging from 3.71 to 12.90%. Under E_2, seven QTLs were identified that were linked with markers *bnlg615*, *umc1622*, *dupssr5*, *umc2000*, *umc1393*, *umc1053* and *bnlg1250*. Out of these seven QTL_S, two QTLs were detected on chromosomes 3 and 10 and the rest three QTLs were mapped on chromosomes 1, 2 and 7. These QTLs accounted for phenotypic variation from 2.32 to 13.66%. One of these QTLs, *qEP-1* linked with marker *umc1622* was consistent under both the environments. On chromosomes 1, 4, 6, 7, 8 and 10, a total of six QTLs were identified for node bearing first ear in E_1. The QTL linked with *umc1428*, *qNBE-1* had maximum contribution of 13.83% in phenotypic variation of node bearing first ear followed by QTLs linked with markers *umc1726* (3.81%), *umc2635* (3.49%), *umc1939* (3.21%), *umc1152* (3.12%) and *phi075* (2.49%). In E_2, one major QTL, *qNBE-1* (11.69%) along with three minor QTLs were identified for node bearing first ear on chromosomes 7 and 9. Two QTLs that were linked with markers *umc1428* and *umc2635* on chromosome 7 were found common across both the environments.

Overlapping QTLs among traits

Of the total 66 QTLs identified for different characters, 16 were noted to influence two or more than two traits (online Supplementary Table S4). Among 16 common QTLs, *phi328178*-linked QTL was simultaneously affecting four traits namely DA, DS, FLL and FLW. Three QTLs linked with markers *umc1622* (ASI, FLW and E/P), *umc2341* (DA, DS and NBE) and *phi075* (ASI, PH and NBE) influenced three characters each. Remaining 12 QTLs, *bnlg1662*-linked (FLL, EP), *dupssr5* and *umc1939*-linked (PH, NBE), *umc1720*-linked (DA, DS), *umc2143* and *umc1393*-linked (FLW, E/P), *bnlg389* and *phi054*-linked (FLL, PH), *umc1215*-linked (ASI, FLW), *umc1428*-linked (FLL, NBE), *umc1279*-linked (DA, PH) and *umc1053*-linked (PH, E/P) simultaneously affected two characters each. In addition, these traits showed a significant correlation with each other either in the positive or negative direction as mentioned in online Supplementary Tables S5 and S6. DA was significantly correlated with DS, ASI, FLW, FLW and E/P whereas DS showed a significant correlation with ASI, PH and E/P. Likewise, ASI was correlated with FLL, FLW, PH and E/P and FLL showed a significant association with FLW, PH, E/P and NBE. Similarly, FLW and PH showed significant association with PH, E/P and NBE respectively. Hence, the molecular association among traits was also validated at the morphological level through correlation studies.

Discussion

In several studies, teosinte was investigated/identified as a source of resistance to biotic and abiotic stresses (Mano and Omori, 2013; Kumar *et al.*, 2019; Stanley *et al.*, 2020; Joshi *et al.*, 2021; Shaibu *et al.*, 2021; Adhikari *et al.*, 2021*a*). However, integration of teosinte in maize breeding programme is limited and only a few reports are available on utilization of teosinte in maize germplasm diversification and yield improvement (Cohen and Galinat, 1984; Wang *et al.*, 2008*a*, 2008*b*; Liu *et al.*, 2016*a*; Singh *et al.*, 2017; Akaogu *et al.*, 2020; Adhikari *et al.*, 2021*b*). In the present study, the teosinte-introgressed BC_1F_5 population consisting of 169 BILs were phenotyped for eight plant architecture traits and significant variation was observed for all the traits. The bewildering array of variation for several morphological traits in the teosinte-introgressed maize population was also observed by Singh *et al.* (2017), Kumar *et al.* (2019), Adhikari *et al.* (2020), Wang *et al.* (2020), Adhikari *et al.* (2021*c*). The observations noted by us in the investigation are in close agreement with the work of Magoja (1991), who evaluated progeny of teosinte-introgressed BC_1F_5 maize population and reported a range for anthesis (47–67 days) and silking (46–63) duration as well as an average of three days anthesis-silking interval. In a recent experiment based on two RIL populations derived from a cross between maize and two subspecies of teosinte (*Z. mays* subsp. *nicaraguensis* Iltis and Benz and *Z. mays* subsp. *parviglumis* Iltis and Doebley), a wide range of variation for 31 morphological traits was recorded (Wang *et al.*, 2020). Similarly, in *Zea diploperennis* Iltis, Doebley and Guzman introgressed maize inbred lines,

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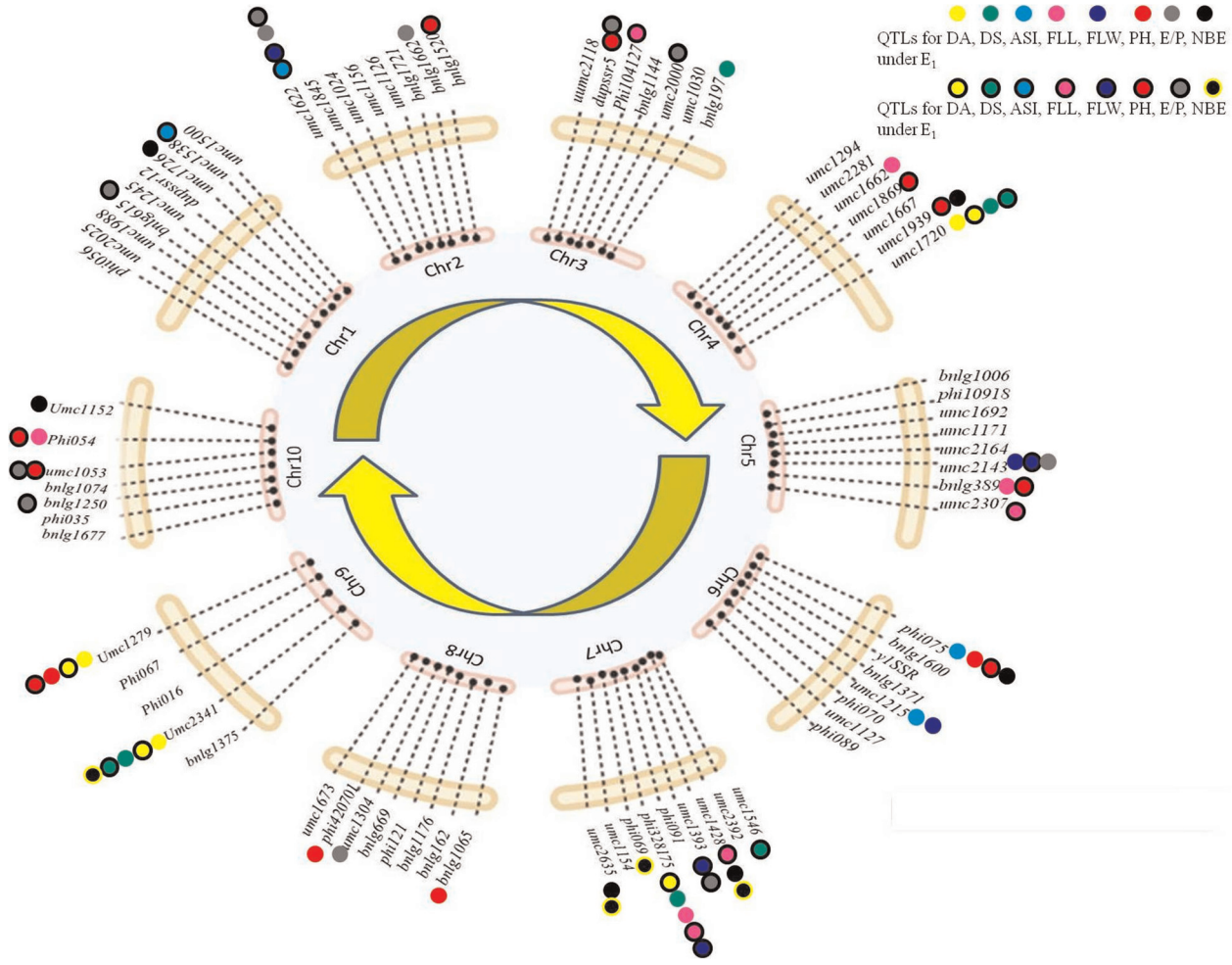

Fig. 2. Environment-wise chromosomal location of QTLs identified using 169 BILs of maize.

Table 4. Consistent QTLs identified across the environments in BILs of maize

S. no.	Traits	Stable QTL	Phenotypic value		Linked markers
			E_1	E_2	
1	DA	*qDA-1*	12.84	12.81	*umc2341*
		qDA-2	11.79	13.97	*umc1279*
		qDA-3	2.81	2.35	*umc1720*
2	DS	*qDS-1*	13.91	12.88	*umc1720*
		qDS-2	3.20	2.90	*umc2341*
3	FLL (cm)	*qFLL-1*	12.74	12.29	*phi328175*
4	FLW (cm)	*qFLW-1*	13.10	12.62	*umc2143*
5	PH (cm)	*qPH-1*	12.98	12.60	*phi075*
		qPH-2	2.41	3.33	*umc1279*
6	E/P	*qEP-1*	12.90	13.66	*umc1622*
7	NBE	*qNBE-1*	13.83	11.69	*umc1428*
		qNBE-2	3.50	2.80	*umc2635*

E_1 = artificially inoculated environment; E_2 = control environment; DA, days to 50% anthesis; DS, days to 50% silking; FLL, flag leaf length; FLW, flag leaf width; PH, plant height; E/P, ears per plant; NBE, node bearing first ear.

significant variation for yield traits was recorded (Akaogu *et al.*, 2020). For getting optimum grain yield under abiotic stresses, flowering traits particularly anthesis, silking duration and ASI are considered to be the most critical parameters. Teosinte-introgressed maize BILs possessed unique blend of earlier silking and shorter ASI and therefore may serve as potential genetic resources in maize yield maximization under abiotic stress conditions (Bänziger *et al.*, 2000; Ngugi *et al.*, 2013). Increasing plant density is now a proven concept for increasing the productivity of maize. Reduced leaf area is considered an important parameter for high-density planting due to more light penetration particularly in the ear region that facilitates translocation of photosynthetic assimilates in the ear (Huang *et al.*, 2017). Since the wide range of variation for leaf parameters is observed in 169 BILs, the possibility exists to utilize differential leaf morphology in the development of lines that are expected to perform better in different targeted environments. Prolificacy is the typical feature of teosinte and teosinte-introgressed maize populations derived by limited backcrossing (1–2 generations) with maize (Singh *et al.*, 2017; Kumar *et al.*, 2019; Adhikari *et al.*, 2020). Magoja (1991) noted variation for E/P from 1.91 to 4.4 with an average of 3.05 E/P in teosinte-introgressed BILs whereas in modern maize lines E/P is normally one but may vary from 1 to 2 E/P. Prolificacy significantly contributes towards yield enhancement and therefore, is a desired trait in maize (Motto and Moll,

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1983). Prolificacy is considered important for baby corn breeding as more ears help in earning more economic returns. These prolific lines could be targeted for the development of baby corn or prolific maize varieties. Clustering analysis based on molecular profile resulted in 12 groups that depicted wider variation among selected superior lines. Clustering of derived lines in more groups in contrast to previous studies based on maize germplasm (Enoki *et al.*, 2002; Patto *et al.*, 2004; Adu *et al.*, 2019) demonstrated diversification of maize germplasm through teosinte allelic introgression. In the present study, 12 groups were reported which were more than earlier reports as given by Akaogu *et al.* (2020) who observed four groups by targeting teosinte-*diploperennis* introgressed maize inbred lines. The possible reason may be the difference in teosinte species used. Also they performed two generations of backcrossing with maize parent which may have resulted in more allelic contribution from maize parent. In addition, they made selection for striga resistance, low soil nitrogen and drought condition during population development and as selection increases the frequency of targeted allele thereby fitness of individual but reduces overall genetic diversity (Alachiotis and Pavlidis, 2016) which might be the probable reasons for less number of clusters. Teosinte grouped independently in cluster I and differed from maize as well as the introgressed lines due to distinct morphological features. The lines from different clusters can be selected for accumulation of desirable traits. If superior lines for different traits are hybridized to accumulate desirable traits by considering their molecular diversity, chances of recovery of desirable recombinants are more as opposed to random selection of parents based on morphological estimates only.

The number of alleles per marker varied from 2 to 6 and a similar range was also recorded by Nikhou and Ebrahimi (2013). The average alleles detected in the present experiment (2.7) are in close agreement with the work of Wietholer (2008) but varied from the findings of Legesse *et al.* (2007), Wasala and Prasanna (2013), Li *et al.* (2014*a*, 2014*b*) and Abdel-Rahman *et al.* (2016) who have observed average alleles per locus of 3.85, 3.85, 2.45 and 2.3, respectively. The total alleles detected in the present study are much larger than the alleles detected by Legesse *et al.* (2007), Xiao *et al.* (2017), Maniruzzaman *et al.* (2018) and Shayanowako *et al.* (2018) who reported 104, 145, 48 and 191 alleles, respectively. Differential allelic numbers observed in the present investigation as compared to the previous studies may probably be due to the genetic constitution of experimental materials and the number of markers used. The markers used in the present experiment had a wider range of PIC (0.29–0.86), therefore, it can be interpreted that there is wider distribution of alleles in the introgressed maize population and ample allelic variation among marker loci. While working with SSR primers in maize, similar range of PIC estimates were also observed by Sserumaga *et al.* (2014), Gazal *et al.* (2016) and Adu *et al.* (2019). PIC is also known as the power of discrimination and according to Botstein *et al.* (1980) markers with >0.5 PIC are considered more informative. All the markers except three (*umc1988*, *umc1245*, *bnlg197*) used in the investigation had PIC >0.5 and therefore, these markers were assumed to have strong discriminatory power.

QTL analysis using 76 SSR markers enabled the identification of 66 QTLs. Maximum QTLs were localized on chromosomes 7 and 9 followed by chromosomes 4, 10, 5, 6, 2, 3, 1 and 8. Guo *et al.* (2008) also observed maximum QTLs for morphological traits on chromosomes 9 and 1. They also identified eight QTLs for DA on chromosomes 2, 4, 5, 6 and 8. While working with a teosinte-introgressed maize population consisting of 928 NILs, Liu *et al.* (2016*a*) detected three QTLs for DA on chromosomes 8 and 9. We have identified genomic regions governing silk emergence duration on chromosomes 3, 4, 7 and 9 whereas Guo *et al.* (2008) noted QTLs on chromosomes 2, 4, 5, 6 and 10 while working with RILs derived from the cross 5003 × p138. The QTLs linked with the *umc1538* marker for ASI in present investigations are in close agreement with the results of Szalma *et al.* (2007) who have detected one QTL for ASI linked with marker loci *umc1538*. Similarly, many QTLs, accounting phenotypic variation from 1.69 to 9.07% in ASI, were also observed earlier by Guo *et al.* (2008). Semagn *et al.* (2013) noted a large number of QTLs, distributed over all 10 chromosomes without any specific pattern. In our experiment, the genomic regions influencing ASI are located on chromosomes 1, 2 and 6, whereas Zhao *et al.* (2018) reported QTLs for ASI on chromosomes 4 and 7 and Ribeiro *et al.* (2018) on chromosomes 6 and 10. Observations of the present investigation indicate that genomic regions for leaf traits were dispersed over seven chromosomes (2, 3, 4, 5, 6, 7 and 10). Earlier observations also indicated localization of QTLs for leaf morphology over all the 10 chromosomes (Agrama *et al.*, 1999; Liu *et al.*, 2010; Nikolic *et al.*, 2011; Ku *et al.*, 2012; Zheng and Liu, 2013; Guo *et al.*, 2015). Fu *et al.* (2019) carried out QTL analysis for FLL in teosinte-introgressed maize population and detected 17 minor QTLs distributed over nine chromosomes 1, 2, 3, 4, 5, 6, 7, 8 and 9 with phenotypic variation ranging from 1.2 to 6%. The results of Fu *et al.* (2019) are in agreement with the outcome of our investigations as four QTLs in E_1 and four in E_2 were identified on five chromosomes (3, 4, 5, 7 and 10) for FLL. Liu *et al.* (2017) detected 17 QTLs for leaf width across chromosomes 1, 2, 5, 7, 8 and 9.

By meta-analysis (statistical analysis of independent studies of QTL mapping) Wang *et al.* (2016) identified several QTLs for PH distributed over all the 10 chromosomes of maize. In our investigation, we noted QTLs on chromosomes 2, 3, 4, 5, 6, 8, 9 and 10 influencing plant height. Many earlier reports also indicate similar localization of QTLs for PH on chromosome 1, 2, 3 4, 5 and 6 (Lima *et al.*, 2006; Yang *et al.*, 2008; Nikolic *et al.*, 2011; Wassom, 2013; Zhu *et al.*, 2013; Zhao *et al.*, 2018). Our observations on QTLs for ear numbers are supported by Veldboom and Lee (1994) who have detected two QTLs on chromosomes 3 and 6 each explaining 5.4% phenotypic variation. Lima *et al.* (2006) identified eight QTLs for ear numbers distributed on chromosomes 1, 2, 5, 6 and 8. The presence of genomic region controlling ear number on chromosome 1 was supported by Ribeiro *et al.* (2018) and on chromosome 8 by Mendes-Moreira *et al.* (2015). Previously Wills *et al.* (2013) carried out an experiment targeting teosinte for mapping of QTLs for ear numbers by developing maize-teosinte BC_2S_3 RILs and identified eight QTLs on the first chromosome with phenotypic variation 0.86–6.05%.

In this investigation, QTLs for ear position were identified on chromosomes 1, 4, 6, 7, 8, 9 and 10. QTLs for ear position have also been noted earlier on chromosome 6 by Nikolic *et al.* (2011), on chromosomes 1, 8 and 10 by Zhu *et al.* (2013), on chromosome 1 by Li *et al.* (2014*a*, 2014*b*), and on chromosomes 4, 6 and 7 by Zhao *et al.* (2018). Lima *et al.* (2006) identified nine minor QTLs with phenotypic variation ranging from 1.02 to 8.92% distributed over chromosomes 2, 3, 4, 7, 9 and 10 whereas the similar distribution of 23 QTLs was recorded by Dong *et al.* (2015). In the present mapping experiment, some genomic regions are common with previously mapped regions but the

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majority of the regions regulating targeted traits are novel. The genome size significantly varied among cultivated maize and teosinte. The average genome of maize (1.095) was significantly smaller ($P < 0.001$, Kruskal–Wallis test) than the average genome size of teosintes (1.129). Several scientific reports reveal that the difference in genome size of maize and teosinte is associated with both gene content (Swanson-Wagner *et al*., 2010) and transposable element (TE) (Wang and Dooner, 2006). Illegitimate recombination, transposon-derived unequal homologous recombination and double-strand break repair are the most leading causes of genome shrinkage in cultivated maize (Schubert and Vu, 2016). The bigger genomes of teosinte tend to have more genes, more and longer introns and more transposable elements than modern maize with smaller genomes. The difference in genome size and allelic state may be the probable cause for mapping of the more novel genomic regions. Among detected QTLs, 16 QTLs were co-associated with various traits. Previous researchers also identified co-localized QTLs for PH and NBE (Lima *et al*., 2006; Wang *et al*., 2018), PH and flowering time (Durand *et al*., 2012), ASI and E/P (Ribaut *et al*., 2007; Wang *et al*., 2016), PH, NBE and leaf parameters (Yi *et al*., 2019). The molecular associations among traits are also consistent at the morphological level as significant correlation among the traits was also reported during due course of investigation. Hence the probable reason for co-localization of QTLs could be either tight linkage or pleiotropy (Lima *et al*., 2006; Durand *et al*., 2012; Hu *et al*., 2012). These genomic regions could be introgressed in different combinations for modelling maize plants that produce optimum yield under a targeted environment. Such as *umc1720*-linked (DA, DS) and *umc1622*-linked (ASI, FLW, and E/P) regions could be utilized for designing maize plants suited for drought-prone areas because anthesis, silking, anthesis-silking interval and ear per plants are important drought-adoptable traits (Ngugi *et al*., 2013; Ross *et al*., 2020). Plant height and leaf parameters are important under high-density planting (Lambert *et al*., 2014; Huang *et al*., 2017). Hence *phi054*-linked (FLL, PH) region could be utilized for the development of maize plants that would be suited well under high-density planting. For lodging tolerance and mechanical harvesting, PH and ear position are important parameters (Josephson and Kince, 1977; Li *et al*., 2007) and *umc1939*-linked (PH, NBE) and *phi075*-linked (ASI, PH, and NBE) regions could be introgressed for simultaneous improvement of these traits. Therefore, it could be possible to incorporate and improve several traits together by harnessing co-localized QTLs either through marker-assisted selection or map-based cloning strategy.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1479262122000223.

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