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Morphological and molecular characterization of teosinte derived maize population

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Abstract

Germplasm enhancement seems to be an essential part of a breeding programme to improve resiliency, adaptability and productivity of the crops. To develop and diversify the maize germplasm, teosinte a wild relative of maize was integrated in crossing programme and BC_1F_4 lines were developed. Five BC_1F_4 lines along with teosinte and maize inbred DI-103 were characterised using quantitative characters and molecular markers. Morphological characterization was done with the help of visual parameters and quantitative traits and for molecular characterization fifty six SSR markers were used. SSR data were analysed with the help of software Mapmaker and twelve linkage groups were generated. Maximum allelic contributions from parent teosinte were found in the introgressed line AM-5 (53.4%) followed by AM-12 (48.9 %), whereas, least contribution of 34.1 % was found in AM-7. The maximum genetic distance among the introgressed lines was observed between AM-2 and AM-9 (0.75) followed by AM-2 and AM-7 (0.70), AM-7 and AM-9 (0.70). The maximum number of cob was found in AM-5 (5.00) followed by AM-2 (4.00). Grain yield per plant was found highest for AM-2 (100.00 g) followed by AM-12 (80.00 g), while, least value was observed for AM-7 (42.00 g). The results indicated differential parental contributions which leads to diversification in the progenies derived from diverse crosses in maize and further opined that such crosses seems to be essential for creating adapted germplasm to whom breeders are looking for.

Key words: Introgression, wild species, characterization, teosinte, SSR

Introduction

Agriculture is vulnerable to climate change and at global level climate change is the major concern. Climate changes have adverse impacts on food production, quality and food security. According to USDA database, global maize production was 1070.51 million tonnes during 2016-17, from an area of 183.76 million hectare with productivity of 5.83 tonnes per hectare (USDA database). According to Department of Agriculture, Cooperation & Farmers Welfare, Ministry of Agriculture & Farmers Welfare, maize production of India was 27.1 million tonnes during 2017-18, from an area of 9.3 million hectare. Inadequate soil moisture availability particularly during reproductive stage is one of the major limiting factors for maize production and productivity. Investigation on complex trait like drought tolerance have been focused on identifying the genetic basis of yield and its components and secondary traits viz., including anthesis-silking interval (ASI), root architecture, thousand kernel weight, leaf rolling and stay green. Conventional breeding has contributed a lot in the development of tolerant genotypes but abiotic stress tolerance breeding is limited by the complex nature of stress intensity, frequency, duration and timing, linkage drag of undesirable traits/genes with desirable traits. Therefore, transfer of desirable genes/alleles or genomic regions from diverse plant genetic resources limited by gene pool barriers giving molecular breeding a good option for breeding plant genotypes that can thrive in stress environments.

Zea mexicana or *Tripsacum floridanum*, wild relatives of maize, are source of novel genes for improvement of drought tolerance and other stresses. Teosinte is a wild progenitor of domesticated maize and has greater genetic diversity. However, limited genetic resources have been efficiently examined to tap the desirable allelic diversity. Exploitation of teosinte as a genetic resource for biotic and abiotic stresses becoming important area of research in the recent years through wide hybridization. QTL

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controlling root aerenchyma formation in a maize × teosinte F_2 population have been identified (Mano et al. 2005a; 2007a,b). Teosinte was the donor of several QTLs associated with the increased capacity to form aerenchyma confirming the potential of teosinte genes to develop improved maize germplasm (Qing et al. 2011). Wild crop relatives have been playing enormously important roles both in the depiction of plant genomes and the genetic improvement of their cultivated counterparts (Canci and Toker 2009). Molecular markers offer a powerful tool, not only for identifying the genomic regions associated with traits of interest but also in following the possible introgressions of genomic regions (Timonova et al. 2013; Mallick et al. 2015). There is a need for systematic efforts to introgress a broad range of wild relative diversity into our crop plants, with the goal of creating a genetic toolbox from which natural adaptations for traits such as disease resistance, tolerance to climatic extremes (especially temperature and moisture), and productivity in otherwise marginal soils can be identified and deployed (Warschefsky et al. 2014).Through systematic introgressions, it is feasible to quickly recover both wild relative stress tolerance from wild progenitor and cultivated agronomic traits of interest from desirable maize lines through advance generation backcrosses (Tanksley and McCouch 1997). There is a need of domestication of wild alleles so that the desirable improvement can be achieved for different biotic as well as abiotic stresses. Graphical genotyping proposed by Young and Tanksley (1989) allows visualization of introgressed regions of parental genome. To characterize the teosinte introgressed line, there is a need to know the contribution from their parents at molecular level as well as phenotypic level and to identify the amount of genome from wild progenitor teosinte. Therefore, our hypothesis was to characterize the teosinte introgressed lines with the help of SSR markers and using morphological descriptors to know the allelic composition of lines at molecular level and to understand the morphology of the teosinte derived lines.

Materials and methods

The present investigation was conducted at N. E. Borlaug Crop Research Centre for field evaluation and the lab work was carried out in Cyto-genetics and Molecular Biology Laboratory, Department of Genetics and Plant Breeding, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand.

Plant materials

The present study was undertaken with wild progenitor teosinte (*Zea mays* ssp. *parviglumis*) and a maize inbred line DI-103. To develop the uniform near to homozygous line the maize inbred parent DI-103 was crossed with teosinte as pollen parent to produce F_1s and F_1s were backcrossed with DI-103 as a recurrent parent. Subsequently selfing was done to produce BC_1F_4 population which was evaluated in the *rabi* 2017-18 for morphological traits namely stem anthocyanin coloration (SAC), flag leaf angle (FLA), anthocyanin coloration on glume tip (ACTT), anthocyanin coloration on glume base (ACTB), anthocyanin coloration on anthers (ACA), anthocyanin coloration of silk (ACS), density of spikelets (DS), tassel length (TL), days to anthesis (DA), days to silking (DS), anthesis silking interval (ASI), cobs per plant (C/P), ear length (EL), ear diameter (ED), kernel row per ear (KRE), kernel per row (KR), and grain yield per plant (GYP). Fifty six microsatellite markers were used for molecular characterization of the introgressed lines. The present study comprised of five lines, each progeny of single plant. These lines were evaluated as in three replication as single row for the phenotyping of morphological traits associated with wild as well as maize plant. Each row was 2 m long and 75 cm apart.

Genomic DNA extraction and PCR amplification

The genomic DNA from each genotype was isolated from young healthy leaves of 30 days old seedlings. DNA was extracted using CTAB (Cetyltrimethyl ammonium bromide) method (Doyle and Doyle, 1990). The quality of DNA was assessed by gel electrophoresis (0.8 % agarose) and quantity was estimated using spectrophotometer. RNAse treated DNA samples were diluted to a working concentration of 100 ng/μl and stored for further PCR amplification.

Fifty six SSR primers covering whole genome was used from the maize database: http://maize.gdb. Amplifications were performed in a 12.55μl reaction mixture containing 1.5 μl Taq buffer (1X) containing [10mM Tris-HCl (pH 8.3), 50 mMKCl, 2.5mM MgCl2], 0.8mM of dNTPs, 0.04 μM of each forward and reverse primers, 100 ng genomic DNA and 0.25 ul 3 units/μl Taq DNA polymerase. The PCR reaction was performed in an Agilent and Prima 96 plus thermal cycler. The PCR reaction conditions for SSR markers were as follows: for amplification in the first cycle, initial denaturation was conducted at 94°C for 5 min then at 94°C for 40 seconds. Then it was followed by

Table 1. Allelic contribution of parents to the BC_1F_4 teosinte derived maize lines

Genotype	– (%)	A (%)	B (%)	D (%)	H (%)	Total (cM)	Recombination (%)	H-segments
AM-2	2.3	42	40.9	0	14.8	44	18	5
AM-5	6.8	27.3	54.5	0	11.4	44	18	4
AM-7	6.8	53.4	34.1	0	5.7	44	21	3
AM-9	4.5	34.1	38.6	1.1	21.6	44	17	6
AM-12	0	33	48.9	0	18.2	44	13	5

– = Absent allel; A Allel of teosinte; B = Allele of maize allel; D = Dominant allele and H = Heterozyote

annealing at 55°C for 40 seconds and elongation at 72°C for 1 minute. The cycle was repeated 35 times followed by a final extension for 10 min at 72°C. The amplicons generated were resolved on 2.5 % agarose gel using horizontal gel electrophoresis assembly. After 75 % of the gel run, the amplicons were visualized and photographed under UV light (Alpha Innotech Corporation, USA).

Linkage detection

Parental polymorphism survey was undertaken with 115 microsatellite markers. The available microsatellite markers are distributed throughout the maize genome in a reference map (maizegdb.com). Markers showing parental polymorphisms were selected for the genotyping of five BC_1 F_4 lines.

The 56 SSR markers found polymorphic between the two parents were used to genotype five BC_1F_4 lines. Each SSR marker was scored on the five BC_1 F_4 lines including two parents i.e. parent 1 (DI-103) and parent 2 (teosinte). These five teosinte derived BC_1 F_4 maize lines were selected on the basis of different morphological traits which is the characteristics features of teosinte, so that the introgression of wild alleles can be recognized.

Mapmaker 3.0 software was used to create the linkage group for polymorphic SSR markers between two parents. The genotypic data for polymorphic SSR markers on five BC_1 F_4 lines were fed in the software and with the help of group command at default setting of LOD score of 3.0 and 12 linkage groups were created. To find the correct order of the markers on linkage group, sequence, compare, try and ripple commands were used. The generated linkage order and position (cM) data were used to feed in the GGT2.5 software to analyze the allelic contribution in the advance lines with the help of graphical representation of chromosomes of linkage groups.

Results and discussion

Molecular characterization

The SSR markers found polymorphic between parents were used to characterize teosinte derived BC_1F_4 maize lines followed by determination of contribution of each parent in the progenies. Of the five BC_1F_4 maize lines analyzed, the maximum allelic introgression from teosinte was noted in AM-5 (54.5%) followed by AM-12 (48.9%), whereas, least contribution of 34.1 % was found in AM-7 (Table 1). However, the maximum contribution of 53.4% from parent maize was observed in introgressed line AM-7 followed by AM-2 (42%) and the least contribution was observed in AM-5 (27.3%) followed by 33% in the AM-12 (Table 1). Among the introgressed lines, maximum heterozygosity was exhibited by AM-9 (21.6%) having 6 heterozygous segments followed by 18.2% by AM-12 with 5 heterozygous segments, whereas, least heterozygosity and heterozygous segment was found in AM-7 (5.7%, 3) followed by AM-5 (11.4%, 4). The maximum recombination among the introgressed line was exhibited by AM-7 (21%) followed by AM-2 and AM-5 with 18% each. However, the least recombination was observed by AM-12 (13%) followed by AM-9 (17%). The maximum genetic distance among the introgressed lines was observed between AM-2 and AM-9 (0.75) followed by AM-2 and AM-7 (0.70), AM-7 and AM-9 (0.70). However, the least distance was found between AM-9 and AM-12 (0.18) trailed by AM-5 with AM-9 and AM-12 (0.55) (Table 2).

Linkage analysis using the SSR data revealed twelve linkage groups rather than ten linkage groups in maize (Table 3, Fig. 1). Stam (2012) opined that a conservative threshold of the LOD score may lead to more linkage groups than the haploid chromosome number. Canady et al. (2006) reported about recombination frequency for *Solanum lycopersicoides* × *Solanum lycopersicum* hybrids, the inter-species

Table 2. Genotypic distance of teosinte derived maize lines

	AM-2	AM-5	AM-7	AM-9	AM-12
AM-2	0.0				
AM-5	0.64	0.0			
AM-7	0.70	0.61	0.0		
AM-9	0.75	0.55	0.70	0.0	
AM-12	0.68	0.55	0.64	0.18	0.0

Table 3. Allelic status of the BC_1F_4 teosinte derived maize lines

Linkage groups	Alleles of DI-103	Alleles of Teosinte	Alleles of both DI-103 and Teosinte
Group1		*umc1530, umc1538, umc1845, umc 2351, umc1988, umc1551*	
Group2		*Umc1823, umc1939, phi2817*	
Group3	*umc1299, phi070*		
Group4		*bnlg1890, bnlg1065*	
Group5	*umc1127*	*umc1726*	
Group6		*bnlg1446, umc1294, umc1546*	
Group7		*umc1131, phi089*	
Group8	*bnlg1429, bnlg1458, umc1651, umc1720, phi0113, umc2143, phi022, umc1500*	*umc1303*	
Group9	*Umc1024*		*Phi035*
Group10	*umc1171, umc2099, bnlg1031*		
Group11		*bnlg609, nc013, umc1227*	
Group12	*umc1444, umc1291, umc1279, umc2025, umc1126, umc1073, umc1667, umc2182, umc2255, umc1154*	*Phi056, bnlg1447, umc1537, umc 1673, phi10412*	*umc1156, umc2152, umc2341, umc1304*

recombination rate was found to be 10% of the intra-species recombination frequency. Alien introgression is considered to be a useful tool for enriching the genetic diversity by introducing new traits and for germplasm development. Present investigation is in parity with the work of Srdiæ et al. (2007) who characterized inbred lines with RAPD markers and showed that the estimation of the genetic distance between maize inbred lines by different markers. The QTL controlling prolificacy was fine mapped to *grassy tillers 1*, a home domain leucine zipper transcription factor that was previously demonstrated to control tillering (Whipple et al. 2011). The QTL on chromosome 5 originally thought to be a master controller of a number of ear-related traits (kernel row number, ear diameter, pedicellate spikelet length, and shattering) fractionated into multiple independent factors (Lemmon and Doebley, 2014). Similar molecular and morphological characterization was done in wheat by Todorovska et al. (2016), who characterized eight backcross lines obtained from interspecific hybridization of six Bulgarian durum wheat cultivars with eight alien species of the family Gramineae, using a backcross strategy and also Nataraj et al. (2017) did in the same crop for rust resistance introgression and graphical representation of introgressed lines.

Morphological characterization

Visual parameters : To characterize the phenotypic characters, visual traits play important role to describe the introgressed traits. Since pigmentation is important characteristic feature of teosinte, therefore, study of visual parameters provides us the insight of introgressed line (Table 4). In the present study stem anthocyanin colouration (SAC) was present in the parent teosinte, whereas, absent in maize inbred line DI-103. The presence of stem anthocyanin coloration (SAC) was not observed in any of the introgressed line, similar to the other parent DI-103 where it was absent. Flag leaf angle (FLA) was found >45 in AM-2, 4, 5 and 12 whereas, AM-7 showed <45 angle of flag

Table 4. Mean value and range of morphological traits for characterization of BC_1F_4 teosinte derived maize lines

	Characters	AM-2	AM-5	AM-7	AM-9	AM-12	Range	DI-103	Teosinte
1	Stem anthocyanin coloration (SAC)	A	A	A	A	A	A	A	P
2	Flag leaf angle (FLA)	>45	>45	<45	>45	>45	>45<	<45	>45
3	Anthocyanin coloration on glume tip (ACTT)	P	A	A	P	A	P - A	A	P
4	Anthocyanin coloration on glume base (ACTB)	A	P	A	P	P	P - A	A	P
5	Anthocyanin coloration on anthers (ACA)	A	A	P	A	A	P - A	A	P
6	Anthocyanin coloration of silk (ACS)	P	P	P	P	A	P - A	A	P
7	Density of spikelets (DS)	S	S	S	S	S	S	S	D
8	Tassel length (cm)	29.00	26.00	29.00	28.00	38.00	26.00-38.00	31.00	37.10
9	Days to anthesis	94.00	105.00	105.00	100.00	112.00	94.00-112	88.60	95
10	Days to silking	96.00	107.00	104.00	102.00	109.00	96.00-109.00	89.00	93
11	Anthesis silking interval (ASI)	2.00	2.00	1.00	2.00	3.00	1.00-3.00	0.40	4
12	Flag leaf width (cm)	4.50	3.37	3.80	3.50	4.12	3.37-4.50	2.80	4.50
13	Flag leaf length (cm)	33.25	32.00	33.00	28.40	38.00	28.40-38.00	35.60	42.80
14	Tillers per plant	1.50	2.00	2.50	3.00	1.20	1.20-3.00	1.00	5.00
15	Plant height (cm)	120.00	147.50	122.50	129.00	133.00	120.00-147.50	97.00	264.00
16	Cobs per plant	4.00	5.00	3.00	2.00	3.00	2.00-5.00	1.80	417.00
17	Ear length (cm)	11.00	13.50	8.00	9.50	12.50	8.00-13.50	12.00	4.00
18	Ear diameter (cm)	2.50	2.50	1.50	2.50	3.00	1.50-3.00	3.40	0.77
19	kernel rows per ear	10.00	13.00	10.00	11.00	12.00	10.00-13.00	12.00	1.00
20	kernels per row	22.00	24.00	10.00	18.00	27.00	10.00-27.00	26.00	6.00
21	1000 kernel weight (g)	175.60	178.95	146.35	121.65	138.60	121.65-178.95	188.95	66.2
22	Grain yield per plant (g)	100	50	42	64	80	42-100	60	158.46

A = Absent; P = Present; S = Sparsh; D = Dense

leaf. The wild parent i.e. teosinte having leaf angle >45, while, inbred line DI-103 exhibited <45. Leaf angle play important role in the stress condition. The angle of leaf is critical for the incidence of radiations, therefore, affect photosynthesis. Anthocyanin coloration on glume tip (ACGT) was observed in AM-2 and AM-9 and this trait was contributed by teosinte. Anthocyanin coloration on glume base (ACGB) was found in introgressed lines AM-5, 9, 12. Anthocyanin colouration on anther and silk was found in the introgressed lines AM-7 and AM-2, 5, 7, 9 respectively. Sparse types of spikelets were found in all the introgressed lines studied and this trait was introgressed from DI-103 inbred line. Teosinte has dense type of spikelets. Density of Spikelets is important for the amount of pollen production.

Quantitative parameters

In the present study morphological characterization of teosinte derived BC_1F_4 lines exhibited the range of tassel length (cm) to be from 26.00 to 38.00 whereas, the inbred DI-103 and teosinte had 31 cm and 37.10 cm respectively. Among the derived line, AM-12 had maximum (38.00 cm) tassel length followed by AM-2 (29.00 cm) and AM-5 (29.00 cm), while, minimum length was found in AM-5 (26.00). The days to anthesis in teosinte had 95 days, whereas, 88 days was found in inbred DI-103. The days to anthesis of introgressed lines varied from 112.00 (AM-12) to 94.00 (AM-2). Shorter days to anthesis are beneficial for the short

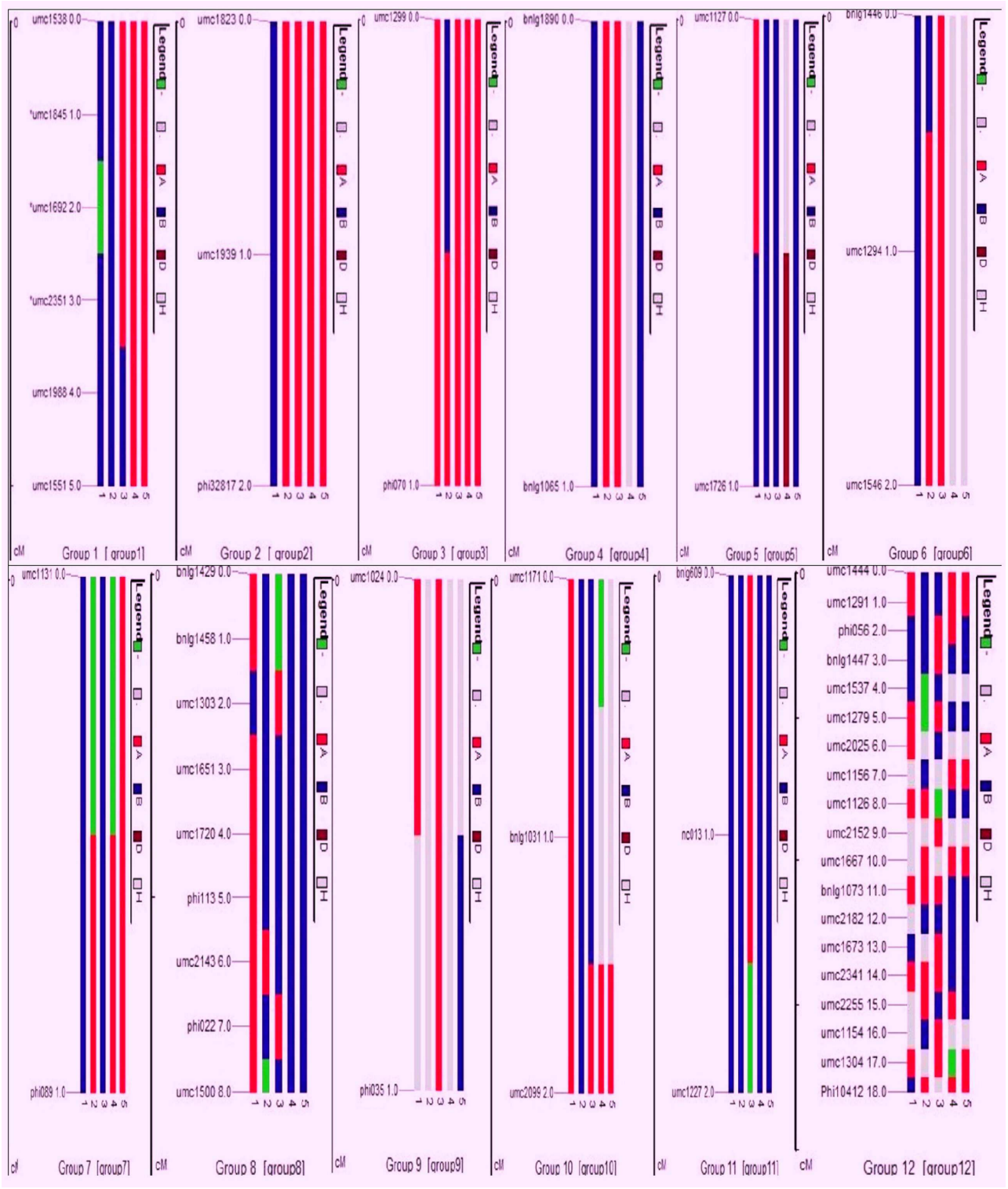

Fig. 1. Graphical representation of allelic position of linkage groups of Teosinte derived BC_1F_4 maize lines: (-) = Absent allele, (A) = Teosinte allele, (B) = Maize allele, (D) = Dominant allele, (H) = Heterozygous allele

duration maize. For forage purpose longer days to anthesis is critical. The highest days to silking was found in AM-12 (109) followed by AM-5 (107), while, the lowest value (96.00) was observed and parents DI-103 and teosinte was found 89 and 95 days respectively. ASI of introgressed lines ranged from 1

day (AM-7) to 3 days (AM-12) and their parental lines had 0.40 (DI-103) and 4 days (teosinte). ASI is the most crucial stage of reproductive system of maize. It is mainly affected by the abiotic stresses. Droughts during this stage hinder the amount of pollination and subsequently reduction in the yield.

Flag leaf length and width of teosinte introgressed lines varied from AM-9 (28.40 cm), AM-5 and (3.37 cm) to 38.00 cm (AM-12) and 4.50 cm (AM-2) respectively and their parents had DI-103 (35.60 cm and 2.80 cm) and teosinte (42.80 cm and 4.50 cm). Tillers per plant of inbred DI-103 was one and their counterpart teosinte was five. The highest value (3.00) was found in AM-9 followed by AM-7(2.50) and the least value was observed for AM-12 (1.20). Tillering is the characteristic features of wild teosinte. Therefore, this trait had been introgressed from teosinte in the derived lines. The plant height of derived lines varied from 120 cm (AM-2) to 147.50 cm (AM-5), whereas, value of 97.00 cm was observed in inbred DI-103 (Table 4).

The yield contributing traits like number of cobs ranged from 2 to 5. The maximum number of cob was found in AM-5 (5.00) followed by AM-2 (4.00). The largest variation in parents among all morphological traits was found in number cobs per plant i.e. inbred DI-103(2.00), whereas, teosinte had 417 cobs. Ear length varied from 8.00 cm (AM-7) to 13.50 cm (AM-5). Parents were diverse for this trait, the value cob length of 12.00 cm was found in DI-103 while, in teosinte the ear length was 4.00 cm. Ear diameter of teosinte was found only 0.77 cm, whereas, in DI-103, it was observed 3.40 cm and their decedents varied from 1.50 cm (AM-7) to 3.00 cm (AM-12). The number of kernel rows per ear was found highest in AM-5 (13.00) followed by AM-12 (12.00) and least was observed in AM-2 and AM-7 with the value of 10.00. Number of Kernels per row varied from the value 10.00 (AM-7) to 27.00 (AM-12). Teosinte had 6 kernels in a single row ear, while DI-103 had 26.00 kernels per row. 1000 kernel weight was found maximum (178.95g) for the introgressed line AM-5 followed by AM-2 (175.60 g) and the minimum value was observed 121.65 for AM-9. Grain yield per plant was found highest for AM-2 (100.00 g) followed by AM-12 (80.00 g), while, least value was observed for AM-7 (42.00 g). Inbred line DI-103 one of the parents had grain yield per plant 60g. Morphological characterization is critical to understand the behavior of trait along with the trait contribution from parental lines. The plant breeder would have to extend crosses to the wild relatives to introduce novel alleles and diversify the genetic base of elite breeding materials. These introgression of wild alleles and selection during domesticating the specific wild alleles controlling morphological and agronomic traits, ultimately leads in reduced genetic diversity relative to unselected genes for maize improvement. The use of maize wild relatives (teosinte and tripsacum) to improve maize performance is well established with important examples dating back more than 60 years (Doebley et al. 1990). In fact, teosintes and Tripsacum are known to possess genes conferring tolerance to several biotic and abiotic stresses including chlorotic dwarf virus, downy mildew, Fusarium, *Striga hermonthica*, rootworms, drought and flooding (Maazou et al. 2017). Maize plants were found to be more frequently infested by this important lepidopteran pest than teosinte plants (Takahashi et al. 2012). Several workers characterized the genetic materials for different environments to extract the useful trait or lines which can be used in the breeding program. The similar work performed by Anjum et al. 2011 and conducted experiments in drought stress and reported at tasseling stage reduce the yield by affecting the number of kernels per row, number of kernel rows, harvest index, number of kernels per cob and grain yield per plant. Silk is female reproductive part of maize plant and receptivity is important for proper pollination and fertilization by male counterpart i.e. pollen. Shrestha (2013) did characterization of maize inbred lines with the help of morphological traits who found 163.27 cm plant height and ear height was at 82.36 cm, tassel length was found 37.63 cm having 9.90 branch number, 52.6 days for tasseling and 55.2 days for silking were observed. The available variability in wild relatives could be exploited for improvement of cereal crops like maize, rice and wheat for biotic and abiotic stresses through introgression of desirable genetic characters to cultivated varieties, which could be aided by careful monitoring of alien gene introgression at the molecular level (Prakash et al. 2002).

Authors Contribution

Conceptualization of research (AK, NKS); Designing of the experiments (AK,SA); Contribution of experimental materials (NKS); Execution of field/lab experiments and data collection (AK, SA, AJ); Analysis of data and interpretation (AK, NKS); Preparation of the manuscript (AK, NKS).

Declaration

The authors declare no conflict of interest.

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