PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.3704

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Introgression profiling of F_4 population derived from the cross of *Zea mays* × Teosinte *spp. Mexicana* using SSR markers

Ramandeep Kaur[1], Ashmita[1], Nidhi Devi[2], Vikrant Tyagi[2], Yogesh Vikal[1] & Priti Sharma[1]*

[1]School of Agricultural Biotechnology, College of Agriculture, Punjab Agricultural University, Ludhiana141 004, Punjab, India

[2]Department of Genetics, Plant Breeding and Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib 173 101, Himachal Pradesh, India

*Email: pritisharma@pau.edu

OPEN ACCESS

ARTICLE HISTORY
Received: 13 April 2024
Accepted: 08 November 2024
Available online
Version 1.0 : 28 January 2025

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Kaur R, Ashmita, Devi N, Tyagi V, Vikal Y, Sharma P. Introgression profiling of F4 population derived from the cross of *Zea mays* × Teosinte *spp. Mexicana* using SSR markers. Plant Science Today (Early Access).https:/doi.org/10.14719/pst.3704

Abstract

To increase the production of crops together with resistance to biotic and abiotic stresses, germplasm enrichment is much more important in any breeding programme. Exploitation of Teosinte and Tripsacum, the wild relatives of maize, as the sources of novel genes to improve resiliency, adaptability and productivity in maize, has been documented. In the present study, teosinte was used in the crossing programme. The experiment material comprised 109 RILs derived from Teosinte *spp. mexicana* and popcorn. Using SSR markers, the introgression profiling of teosinte-derived maize F_4 population (109 maize-teosinte derivatives). Morphological characterization for different parameters, i.e., days to maturity, number of cobs, number of tillers, seed rows per cob and cob length, showed significant variation among all the traits studied. The highest significant positive correlation was observed between the number of rows per cob and cob length. Introgression profiles of different lines were inferred from the consensus of genotypic and morphological data, which revealed that the marker bnlg1297 was common among the lines that exhibit a higher number of tillers and cobs. Therefore, these specific genomic regions might be associated with these traits. Thus, these results showed different parental contributions, which leads to diversification in the progenies derived from diverse crosses in maize. Further, such crosses appear crucial for producing germplasm for which breeders are looking.

Keywords

Introgression profiling; Popcorn; Recombinant Inbred Lines; SSR markers; Teosinte

Introduction

Maize (*Zea mays* L.) is one of Poaceaes' most economically valuable cereal crops (1). It is an essential food, feed and biofuel resource with broad dietary and cultural importance. It was domesticated from teosinte in southern Mexico 9000 years ago (1), but both differ significantly in appearance and traits like plant height, flowering and seed architecture (2). During crop domestication, there was a reduction in genetic diversity, particularly in genes underlying traits that were targeted by the selection process (3). As per the report of FAO, nearly 1147.7 million MT of maize is being produced in over 170 countries from 193.7 million ha with an average productivity of 5.75 t/ha (4). In India, Andhra Pradesh, Rajasthan, Karnataka and Madhya Pradesh are the leading crop producers, thus playing a conducive role in maize production. The variation in admixture is a key element of modern maize genetic and phenotypic diversity, both at the level of individual loci and as a factor driving a significant component of additive genetic variation across several agronomic traits (5). Various mutagenic effects, genetic recombination and heterotic phenomena have led to evolution. The increase in heterosis in maize also

affects the introgression process, not because of alloploidy but due to various gene combinations introduced at different intervals in maize (6). The genetic material can be transferred from one species to another with the help of hybridization and repeated backcrossing, termed introgression (7). Introgression is beneficial in transferring specific QTLs from the wild ancestor and is a long-term process as it takes various generations before the backcrossing occurs. Different recurrent parents are used to avoid inbreeding. Majorly, single genes are transferred, but in some cases, more than one gene can also be assigned.

Marker-assisted selection can be used to introgress alleles and reduce the number of generations and sample size. In contrast, it is comparatively large if done with the help of conventional breeding methods (8). With the help of introgression breeding, a specific trait can be transferred from donor parent to recipient parent using hybridization and backcrossing method. A genomic description of introgression can significantly expand understanding of evolution through hybridization, showing how specific alleles, genes and genomic regions resist these processes. Moreover, analysis of introgression in crops during post-domestication development can provide insight into the genetic architecture of adaptation to encountered abiotic and biotic conditions (9).

Teosinte differs from maize in terms of kernel size and the former has minute kernels compared to the latter, enclosed within a hard fruit case, which is not present in maize inbreds and landraces (10). The kernel composition in modern maize is different from that of teosinte; the inbred kernel comprises 71.7% starch, 9.5% protein and 4.3% oil (11). On the contrary, teosinte kernels have 52.92% starch, 28.71% protein and 5.61% oil, strongly suggesting that the increase in kernel size, fruit case-less kernels and growth in kernel starch were the targets of artificial selection during maize domestication (12). Teosinte possess more than one ear at each node, whereas maize only has one ear per node. The gene responsible for this difference was the gt1 (grassy tillers 1) gene and the allele substitutions in this gene led to a lower ear number in maize (13).

Several studies have been reported. A total of 36 QTLs associated with agronomic important traits like ear weight, prolificacy, ear number, ear length and diameter, number of rows on the ear and number of kernels per row on the ear were mapped in $F_{2:3}$ tropical maize progenies developed from the cross of IG-1 and BR-106 inbred lines (14). The study identified two QTLs, Tin8 on chromosome 8 and tb1 on chromosome 1, which were associated with tiller number in maize (15). Irrespective of teosintes' resistance to various biotic and abiotic factors, traits like more tiller numbers and cobs can be used to develop maize-teosinte introgression lines. Using these introgression lines, mapping of QTLs harbouring the regions controlling these traits can be identified, followed by fine mapping of QTLs and the genes linked to these traits can be mined out and used for further breeding programmes.

Materials and Methods

Plant material

The plant material used in the study consists of 109 recombinant inbred lines (RILs). The RIL population was developed by crossing a high-popping variety of popcorn (as a female parent) with a wild cultivar, Teosinte spp. *mexicana* (as a male parent). The experiment was sown during *Kharif* 2020 in the last week of May in the experimental research farm of Eternal University, Baru Sahib, Himachal Pradesh. Each RIL was depicted by a plot of two rows of 1m length and row spacing maintained at 40 cm in a Randomized Block Design with three replications. The molecular studies were conducted in the Molecular Biology Laboratory, School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, in 2021.

Morphological data

Morphological data was recorded at Eternal University, Baru Sahib. The data was recorded for five traits: days to maturity, number of cobs, number of tillers, seed rows per cob and cob length. The days to maturity were calculated from the date of sowing to the physiological maturity of the maize crop. The total number of cobs and tillers of five randomly chosen plants was counted and the mean value was calculated for each RIL. Five randomly selected plants were marked, data for seeds per cob and cob length were recorded and the mean value was computed. The mean values of each RIL were subjected to statistical analysis. Calculations were performed following standard procedures for estimating components of genetic variation with the help of the SAS statistical software, version 9.2.SAS Institute, Inc. SAS users' guide. Version 9, 4th ed. Cary, NC. 2004 (Clark and Kempthorne 1958). The Pearson correlation coefficients and Principal Component Analysis (PCA) were determined and plotted via packages corrplot and performance analytics in R statistical software (16).

Genotyping of parental lines and RILs

The genomic DNA of RILs and parental lines was isolated using the CTAB (Cetyl Trimethyl Ammonium Bromide) method (17). DNA quantification was done on 0.8% agarose gel. SSR (simple sequence repeats) markers were taken from the Maize genome database, spanning all 10 linkage groups for complete genome coverage. A total of 250 SSR markers were screened for parental polymorphism. *In vitro* polymerase chain reaction (PCR) was carried out with a total volume of 10 µL of PCR reaction using 70 ng template DNA, 3 µL (2X Premix) Master mix, 3.5 µL DNase free water and 0.375 µM each of forward and reverse primers. The PCR amplification reaction consisted of initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 60 sec, annealing at 50-65 °C (depending on the primer) for 90 sec and extension at 72°C for 120 sec, final extension step at 72°C for 7 min and the product was kept on hold at 4 °C after the PCR completion. Further, the quantification of PCR products was done using 3% agarose gel.

Scoring of SSR marker data

To find polymorphic markers, each gel was scored twice to avoid scoring errors. SSR markers were scored as co-dominant amplicons. The polymorphic markers were genotyped on the F_4 population. Similarly, scoring was done for the population. The individual amplicons were scored as 'A' for popcorn, 'B' for Teosinte, 'H' for heterozygous plants and 'M' for the missing data.

Graphical genotyping

GGT 2.0 software was used for graphical genotyping. GGT requires input in the form of GGT data files or a spreadsheet. GGT data files were obtained from two sources of data: A locus file containing marker names and raw marker scores and a (linkage)

map file specifying marker positions on a linkage map. GGT 2.0 was used to envision data of markers with known map positions on a genetic map shown graphically by estimated lengths of genomic compositions as coloured chromosome bar segments. Then, the phenotypic data of the RIL population was compared with the introgression profiling of the RIL population.

Results and Discussion

Phenotypic performance of morphological traits

The RIL population was evaluated for different morphological traits and the results are presented in Table 1. The wild progenitor teosinte matured in 142 days, whereas the popcorn reached its maturity stage in 95 days. About 31 recombinant inbred lines that mature in 100-125 days were identified. After harvesting, it was found that in popcorn, there were two cobs; in teosinte, there were sixteen cobs per plant. Approximately 17 RILs with twelve or more than twelve cobs were identified. The teosinte possesses more tillers, i.e., 6 tillers per plant. On the contrary, popcorn contains very few tillers, i.e., 1 tiller per plant. A total of 12 RILs having tiller number 3 or more than 3 were identified. Teosinte had only 2 seed rows per cob, but popcorn had 9 rows per cob. About 13 RILs having 6 or more seed rows per cobs were identified. The cob length in popcorn was more than teosinte. It was about 15 cm in popcorn and only 6 cm in teosinte. Approximately 29 lines with cob lengths of more than 8 cm and markers linked to each trait were found in all these identified RILs.

ANOVA analysis showed significant variation within the genotypes for all the traits studied. While within the replications, non-significant variation was found for days to maturity, number of cobs per plant and number of tillers, whereas significant variation was observed for number of seed rows per cob and cob length. The maximum coefficient of variation was found for several tillers (26.36) and the minimum was observed for days to maturity (2.18). On the contrary, the least significant difference was highest for days to maturity (4.6) and lowest for the number of tillers per plant (0.61). In correlation analysis, the number of rows per cob had a negative correlation with days to maturity, number of cobs and number of tillers, while there was a significant positive correlation with cob length (0.54). A highly substantial at p<0.001 correlation was observed between several rows per cob and cob length (0.54). A positive correlation was found between days to maturity with several tillers, whereas a negative correlation was found with cob length and number of rows per cob Fig.1.

Principal component analysis (PCA) for morphological traits

Principal component analysis (PCA) is a powerful method for extracting useful information from huge amounts of data. The PCA was performed for morphological traits of the maize-teosinte RIL population. The first three principal components explain the variability of PCA concentrates. The first two PCs explained 62.39 percent of the total variation (Table 2). The

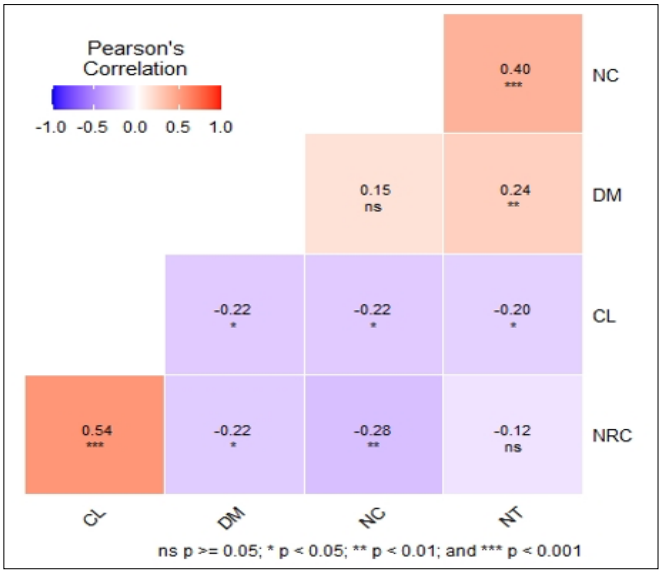

Fig. 1. Pearsons' correlation coefficients for the assessed maize RIL lines.

variance described by the three, four and five PCs is insignificant. The components were not considered in the principal components where the Eigenvalues were lower than unity. This occurred in the current study after the two principal components contributed more than 60% of the total variance in the current experimental material. Significant PCs had Eigen values ranging from 2.05 (PC1) to 1.06 (PC2) (Table 2). The total contribution of a given trait (contribution), on explaining the variations retained by two PCs (PC1 and PC2) is provided by contribution = [(C1 x Eig1) + (C2 x Eig2)] / (Eig1 + Eig2), where: C1 and C2 are the contributions of the variable on PC 1 and PC 2, respectively and Fig 1-2 are the eigenvalues of PC1 and PC2, respectively.

The GT data are approximately displayed in a GT biplot (Fig.2a and 2b), which can be used to visualize the trait associations and the trait profiles of the genotypes. Regarding the trait-standardized GT data, when two vectors are close, forming a slight angle (acute, < 90°), the two variables they represent are strongly positively correlated. If vector rays meet each other at 90°, they are not likely to be correlated. Similarly, if the rays diverge and form a large angle (close to 180°), they are negatively correlated. The first two PC values used to construct biplot graphs explained 62.39% of the variation. The first PC contributed 41.09% of the total variation; the second component accounted for 21.30% of the variation, whereas the third and fourth components showed 17.13 and 11.83%, respectively (Table 2). Based on the factor loading graph (Fig.2a and b), cob length is strongly correlated with the number of rows per cob and negatively correlated with days to maturity, shown in Fig. 2a-2b.

Table 2. Eigenvalues (latent roots) and rotated component loadings (values of principal component traits of maize RIL population)

PCA	Eigenvalue	Percentage of variance	Cumulative percentage of variance
PCA 1	2.05	41.09	41.09
PCA 2	1.06	21.30	62.39
PCA 3	0.86	17.13	79.52
PCA 4	0.59	11.83	91.36
PCA 5	0.43	8.64	100.00

Table 1. Mean performance of parental and recombinant inbred lines for different morphological traits

	Days to maturity	Number of cobs per plant	Number of tillers per plant	Seed rows per cob	Cob Length (in cm)
Popcorn (HPV)	95	2	1	9	15
Teosinte	142	16	6	2	6
RIL population (range)	118-138	2-22	1-4	3-8	4-13
Mean	130	8	1	5	7

Morphological characterization is a key factor in understanding the behaviour of traits along with the trait contribution from parental lines. The plant breeders expand crosses with the wild relatives to introduce novel alleles and diversify the genetic base of elite breeding materials. The introgression of wild alleles into inbreds and selection while domesticating the specific wild alleles controlling morphological and agronomic traits ultimately leads to reduced genetic diversity relative to unselected genes for maize improvement. The use of maize wild relatives to improve maize performance is well established, with essential examples dating back over 60 years (18).

Tillering is the distinctive feature of wild teosinte. Thus, this trait had been introgressed from teosinte in the derived lines. In the present study, the tiller number of RILs ranges from 1-4 and a total of 12 RILs having tiller number 3 or more than 3 were identified. The no. of tillers in teosinte was 6, whereas in popcorn, there were very few tillers, i.e., 1 tiller per plant. Similar studies reported one tiller per plant of inbred DI-103 ; their counterpart teosinte had five tillers per plant (19). In BC_1F_4 lines, the tillers per plant vary from 1.20-3.00. The present investigation measured cob length as 15 cm in popcorn and 6 cm in teosinte. About 20 inbred lines possess a cob length of more than 8 cm, i.e., introgression from popcorn.

In one study, in teosinte-derived lines, ear length ranges from 8.00 cm to 13.50 cm and for parents, the value cob length was 12.00 cm in DI-103 whilst, in teosinte, the ear length was 4.00 cm (19). The yield contributing traits, like several cobs, ranged from 2 to 5. The most significant variation in the parents among all morphological characteristics was found in the number of cobs per plant, i.e., DI-103 (2.00), whereas teosinte had 417 cobs. Likewise, our findings observed 2 cobs in popcorn and 16 cobs per plant in teosinte. In RILs, 17 RILs had 12 or more than 12 cobs identified. The study showed that the BC_1F_5 lines derived from a cross of teosinte and maize and the kernel rows per ear ranged from 2.67-16. Similarly, the present study observed it from 3 to 8 (20). The parent teosinte possess kernel rows per ear were fewer, i.e., 2, whereas maize (DI-103) had 12.66 an average basis. Maize typically flowers earlier than Mexicana (9). Thus, popcorn matures earlier than teosinte. In the present analysis, the popcorn matures earlier (95 days), while the wild progenitor teosinte matures in 142 days. Overall, the results suggest that popcorn and teosinte differ in their maturity time and other important traits and further research is needed to understand the genetic basis of these differences better.

SSR marker-based genotyping of popcorn and teosinte

250 SSR markers covering all ten chromosomes of the maize genome were used for SSR-based genotyping of popcorn and teosinte. The SSR markers were chosen for all the chromosomes covering different bin regions. Out of 250, 70 SSR markers were polymorphic between parental lines. These polymorphic markers were rechecked to determine their reproducibility and it was found that 35 were reproducible, showing an overall 14 percent polymorphism (Table 3). The maximum per cent polymorphism was observed on chromosomes 1, 4 and 6, whereas the minimum was on chromosomes 3, 7 and 10.

Table 3. List of polymorphic SSR markers and their chromosomal location

Sr. No.	Marker name	Chromosome number	Sr. No.	Marker name	Chromosome number
1	bnlg176	1	19	bnlg2305	5
2	bnlg1953	1	20	phi126	6
3	umc1035	1	21	bnlg238	6
4	bnlg1025	1	22	bnlg1371	6
5	bnlg1720	1	23	umc1006	6
6	bnlg1017	2	24	umc1805	6
7	bnlg1297	2	25	bnlg2132	7
8	bnlg1036	2	26	umc1695	7
9	bnlg1447	3	27	umc1034	8
10	umc1641	3	28	umc1984	8
11	umc2278	4	29	bnlg1823	8
12	umc1017	4	30	bnlg1131	8
13	bnlg490	4	31	umc1492	9
14	umc1031	4	32	umc1519	9
15	umc1869	4	33	umc1231	9
16	umc1679	5	34	umc1053	10
17	bnlg2323	5	35	bnlg1360	10
18	phi087	5			

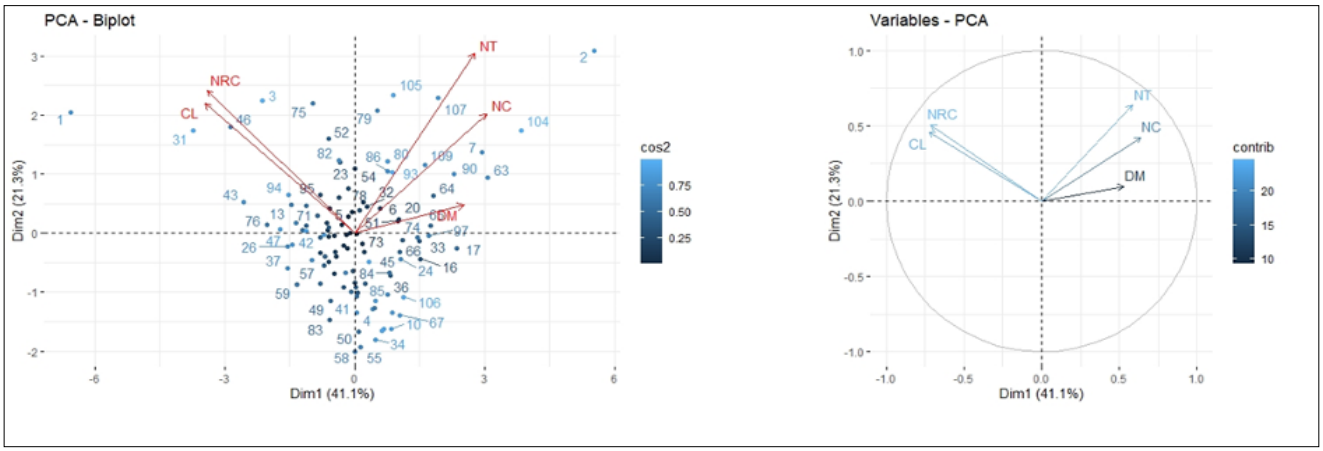

Fig. 2. Biplot analysis based on A. PCA1, B. PCA2. DM: Days to maturity, NT: No. of tillers per plant, NC:No. of cobs per plant, NRC:No. of rows per cob, CL: Cob length.

GGT analysis

The percentage of Introgression for Teosinte was maximum (66.8%) in RIL421 and minimum (8.8%) in RIL339 and for Popcorn was highest (48.6%) in RIL443 and lowest (8%) in RIL352. The introgression data of all the 105 RILs was compared with the morphological data. The Inbred line with more cobs, tillers, seed rows per cob and cob length with few days of maturity were selected compared to parental lines. Afterwards, the genotypic profiling of these selected lines was analyzed and markers that might be linked to traits on each chromosome were found. After analysis of introgression profile, some common markers in RILs were identified for each trait. For days to maturity, marker bnlg1131 on chromosome 8 was found to be common in maximum number of RILs with less maturity (Table 4). Marker bnlg1297 is present on chromosome 2 and is found in most RILs with more cobs and tillers (Table 5-6). For seed rows per cob, marker umc1984 present on chromosome 8 was common in the maximum number of RILs having more seed rows per cob (Table 7). Marker bnlg1131 on chromosome 8 was common in the maximum number of RILs with more cob length (Table 8). So, these genomic regions might be associated with the trait of interest.

In genetic improvements of cultivated crops, wild crop relatives play a significant role (21). Molecular markers are now a potent tool for finding the genomic regions associated with target traits and following the possible introgressions of genomic regions (22, 23). There is a need for systematic efforts to introduce a wide range of wild relative diversity into crop plants, aiming to produce a genetic toolbox from which natural adaptations for traits like disease resistance, tolerance to climatic changes with more productivity and good agronomic characteristics (24). This can be feasible through systematic introgressions to rapidly recover both wild relative stress tolerance from wild progenitor and cultivated agronomic traits of interest from desirable maize lines by generation backcrosses (25).

In our investigation, an SSR marker analysis of Popcorn and Teosinte was carried out using 250 SSR markers. The GGT analysis showed an association between the marker and introgressed genomic region on all chromosomes. After comparing the introgression profile with morphological traits, we identified a common allele, bnlg1297, on chromosome 2 from the introgression profile between several tillers and the number of cobs. In one finding, they genotyped maize and teosinte using 76 SSR markers and found 377 alleles with an average of 5 alleles per marker loci (26). However, using molecular markers to identify associations between specific genomic regions and traits is a common approach used in genetic studies and the current studys' findings are consistent with the broader literature in this field. Graphical genotyping allows for visualizing introgressed regions of the parental

Table 4. Introgression lines (lesser maturity days) showing markers associated to the parent (Popcorn, Teosinte)

Sr. No.	Population	Days to maturity	CHR 1	CHR 2	CHR 3	CHR 4	CHR 5	CHR 6	CHR 7	CHR 8	CHR 9	CHR 10
1	RIL302	124	bnlg1953, bnlg1025	bnlg1036	umc1641	umc1017, umc1869	bnlg2305			bnlg1823	umc1519	
2	RIL304	125	bnlg176, bnlg1720			umc1017, umc1031	umc1679, bnlg2305	umc1006		bnlg1823, bnlg1131		
3	RIL306	120			bnlg1447	bnlg490, umc1031, umc1869	umc1679, bnlg2323, phi087	umc1006		umc1034, umc1984, bnlg1131	umc1519	bnlg1360
4	RIL309	124	bnlg176, bnlg1720			umc1031	bnlg2305	umc1006		bnlg1131		umc1053
5	RIL311	119	bnlg176, bnlg1720		umc1641	umc1869	umc1679, bnlg2305	umc1006		bnlg1823	umc1519	
6	RIL315	118	bnlg176, bnlg1720		bnlg1447	umc1017, umc1031	umc1679, bnlg2306	bnlg1371	bnlg2132, umc1695	umc1034, umc1984, bnlg1131		
7	RIL330	123	umc1035, bnlg1720		bnlg1447	umc1017, umc1031, umc1869	phi087					bnlg1360
8	RIL344	118	umc1035, bnlg1720			umc1869				umc1034, umc1984, bnlg1131	umc1519	umc1053
9	RIL352	121	bnlg1720					bnlg1371		bnlg1131		bnlg1360
10	RIL355	118	bnlg1720	bnlg1036			bnlg2305		umc1695	bnlg1131		umc1053
11	RIL365	120		bnlg1036	bnlg1447, umc1641	umc1017			bnlg2132	bnlg1823	umc1519	

Sr. No.	Population	Days to maturity	CHR 1	CHR 2	CHR 3	CHR 4	CHR 5	CHR 6	CHR 7	CHR 8	CHR 9	CHR 10
12	RIL366	124	bnlg176, umc1035, bnlg1025		bnlg1447, umc1641		phi087	umc1006		bnlg1131		
13	RIL370	124	bnlg176, bnlg1025						bnlg2132	umc1034, umc1984, bnlg1823, bnlg1131		
14	RIL372	118	bnlg1720			umc1017, umc1869	phi087	phi126, bnlg238, bnlg1371, umc1006		umc1034, bnlg1823, bnlg1131	umc1492, umc1231	umc1053
15	RIL375	121	bnlg1720	bnlg1036	umc1641	umc1017	phi087			umc1984, bnlg1823, bnlg1131	umc1492	
16	RIL378	122	bnlg176		umc1641	umc1017	bnlg2305		umc1695	bnlg1131	umc1231	
17	RIL379	123	bnlg176, bnlg1025	bnlg1036	umc1641		bnlg2305		umc1695	umc1034, umc1984, bnlg1823, bnlg1131		
18	RIL383	120	bnlg1720		umc1641	umc1031	bnlg2305	bnlg1371	bnlg2132	umc1984, bnlg1131	umc1492, umc1519, umc1231	
19	RIL388	118	bnlg1953, umc1035, bnlg1720			umc1869	umc1679, phi087, bnlg2305			umc1984, bnlg1823, bnlg1131	umc1519	umc1053
20	RIL391	118	bnlg176, umc1035, bnlg1720			umc2278, umc1869	bnlg2305	bnlg1371		bnlg1131	umc1492, umc1519	bnlg1360
21	RIL392	121	bnlg176, bnlg1720			umc1017	umc1679, bnlg2305	bnlg1371	bnlg2132	bnlg1131	umc1519	
22	RIL416	123	bnlg1720			umc1869	umc1679, bnlg2305		bnlg2132	bnlg1131	umc1492	
23	RIL418	119	bnlg1953, umc1035, bnlg1720		umc1641	umc1031, umc2278	phi087			umc1984	umc1519	umc1053
24	RIL421	118	bnlg176, umc1035				umc1679, bnlg2305					
25	RIL422	118	bnlg176, bnlg1953, umc1035	bnlg1036		umc1017	umc1679, bnlg2323, bnlg2305	umc1805	bnlg2132	bnlg1823, bnlg1131		
26	RIL423	117	bnlg176, bnlg1720	bnlg1036		umc1017	umc1679	bnlg1371		bnlg1984, bnlg1131		
27	RIL426	118	bnlg1720			umc1869	bnlg2323, phi087, bnlg2305			bnlg1131	umc1231	umc1053
28	RIL444	118	bnlg1720	bnlg1036	umc1641	umc1031, umc1869			umc1695	umc1034, bnlg1823, bnlg1131		bnlg1360
29	RIL445	124	bnlg176, bnlg1720			umc2278, umc1031	bnlg2305	bnlg1371, umc1805	bnlg2132	umc1984	umc1519	umc1053
30	RIL448	120	bnlg176, bnlg1953	bnlg1036			umc1679, bnlg2305	umc1805	bnlg2132	bnlg1823	umc1492, umc1519	
31	RIL450	118	bnlg1953			umc1017, umc1031	umc1679	bnlg1371		bnlg1131	umc1231	umc1053

Table 5. Introgression lines (more number of cobs) showing markers associated to the parent (Popcorn, Teosinte)

Sr No.	Population	No. of cobs	CHR 1	CHR 2	CHR 3	CHR 4	CHR 5	CHR 6	CHR 7	CHR 8	CHR 9	CHR 10
1	RIL306	13	bnlg176, bnlg1953, umc1035, bnlg1025	bnlg1297	umc1641	umc1017		phi126, bnlg238, umc1805	bnlg2132, umc1695	bnlg1823	umc1231	
2	RIL330	18	bnlg1953	bnlg1017, bnlg1297, bnlg1036	umc1641	bnlg490	bnlg2323, bnlg2305	umc1006, umc1805	bnlg2132	bnlg1131	umc1492, umc1519, umc1231	
3	RIL370	12	bnlg1953, bnlg1720	bnlg1017, bnlg1297, bnlg1036	bnlg1447	umc2278, bnlg490, umc1031		phi126, bnlg238, umc1006, umc1805			umc1492, umc1231	
4	RIL381	12	bnlg176, bnlg1953, bnlg1025, bnlg1720	bnlg1297	bnlg1447	umc1017, bnlg490	bnlg2323	phi126, bnlg238, umc1006, umc1805	bnlg1131	umc1519, umc1231		
5	RIL391	18	bnlg1953	bnlg1017, bnlg1297	bnlg1447, umc1641	umc1017, bnlg490, umc1031	umc1679, bnlg2323, phi087	umc1006	bnlg2132	bnlg1823	umc1231	
6	RIL428	22	bnlg1025	bnlg1017, bnlg1297			bnlg2323, phi087, bnlg2305	umc1805		umc1034, umc1984, bnlg1131		
7	RIL429	19	bnlg176, bnlg1953, bnlg1720	bnlg1297	bnlg1447		phi087	umc1006, umc1805	umc1695		umc1492, umc1519, umc1231	
8	RIL430	17		bnlg1017, bnlg1297, bnlg1036			umc1679, bnlg2323, phi087, bnlg2305	phi126, bnlg238, umc1006, umc1805	umc1695		umc1492, umc1519, umc1231	
9	RIL431	14	bnlg176	bnlg1297		umc1017, umc1031	umc1679, bnlg2323, bnlg2305	bnlg1371, umc1805		bnlg1823, bnlg1131		
10	RIL444	15	bnlg1953	bnlg1017, bnlg1297		umc1017	bnlg2323, phi087, bnlg2305	phi126, bnlg238, bnlg1371		umc1984	umc1492	
11	RIL445	13		bnlg1017, bnlg1297		bnlg490	bnlg2323	phi126, bnlg238, umc1006	umc1695	bnlg1823, bnlg1131		
12	RIL459	13	bnlg1720	bnlg1017, bnlg1297, bnlg1036		umc1017	umc1679, bnlg2323	umc1805		bnlg1131		umc1053
13	RIL469	14	bnlg176, umc1035, bnlg1720	bnlg1017, bnlg1297	bnlg1447, umc1641	umc1017, umc1869	bnlg2323	umc1006, umc1805		bnlg1131	umc1492, umc1519, umc1231	
14	RIL479	18	bnlg176, bnlg1953, bnlg1025, bnlg1720	bnlg1017, bnlg1297		umc2278, umc1031	bnlg2323, phi087, bnlg2305		umc1695			
15	RIL482	21	bnlg1025, bnlg1720	bnlg1017, bnlg1297	bnlg1447	umc1017	umc1679, bnlg2323, phi087	phi126, bnlg238, umc1805	umc1695	umc1034, bnlg1131		umc1053
16	RIL484	12	bnlg1953	bnlg1297		umc2278	bnlg2323	phi126, bnlg238, umc1805				
17	RIL485	13	bnlg176, bnlg1953	bnlg1297		umc2278, umc1017	bnlg2323	phi126, bnlg238, umc1805				

Table 6. Introgression lines (more tiller number) showing markers associated with the parent (Popcorn, Teosinte)

Sr. No.	Population	No. of tillers	CHR 1	CHR 2	CHR 3	CHR 4	CHR 5	CHR 6	CHR 7	CHR 8	CHR 9	CHR 10
1	RIL308	4	bnlg176, umc1035	bnlg1297	umc1641	umc2278, umc1017, bnlg490	bnlg2323	phi126, bnlg238, umc1805	bnlg2132	bnlg1823		
2	RIL329	3	bnlg1953, bnlg1025, bnlg1720	bnlg1297	bnlg1447, umc1641	umc1017, bnlg490	bnlg2323, phi087	phi126, bnlg238, bnlg1371, umc1805	umc1695		umc1231	umc1053
3	RIL337	3	bnlg1953	bnlg1017, bnlg1297, bnlg1036		umc2278, umc1017, umc1869	bnlg2323	phi126, bnlg238, umc1805			umc1492, umc1519, umc1231	
4	RIL341	3		bnlg1297		umc2278, bnlg490	bnlg2323	phi126, bnlg238, umc1805		umc1034, bnlg1131	umc1519	bnlg1360
5	RIL392	3	bnlg1953, umc1035, bnlg1025	bnlg1297, bnlg1036	bnlg1447, umc1641	bnlg490, umc1031	bnlg2323, phi087	phi126, bnlg238		umc1984	umc1231	
6	RIL417	3	bnlg1953	bnlg1297	umc1641			phi126, bnlg238, bnlg1371, umc1805	bnlg2132, umc1695	umc1984, bnlg1131	umc1231	
7	RIL446	3		bnlg1017, bnlg1297			bnlg2323, phi087	phi126, bnlg238, umc1006	bnlg2132, umc1695	umc1034, bnlg1823, bnlg1131		
8	RIL459	3	bnlg1720	bnlg1017, bnlg1297, bnlg1036		umc1017	umc1679, bnlg2323	umc1805		bnlg1131		umc1053
9	RIL479	4	bnlg176, bnlg1953, bnlg1025, bnlg1720	bnlg1017, bnlg1297		umc2278, umc1031	bnlg2323, phi087, bnlg2305		umc1695			
10	RIL480	3	bnlg176, umc1035, bnlg1720	bnlg1297	bnlg1447, umc1641	umc2278	bnlg2323, bnlg2305	bnlg1371, umc1006	umc1695			
11	RIL482	3	bnlg1025, bnlg1720	bnlg1017, bnlg1297	bnlg1447	umc1017	umc1679, bnlg2323, phi087	phi126, bnlg238, umc1805	umc1695	umc1034, bnlg1131		umc1053
12	RIL485	3	bnlg176, bnlg1953	bnlg1297		umc2278, umc1017	bnlg2323	phi126, bnlg238, umc1805				

Table 7. Introgression lines (more seed rows per cob) showing markers associated with the parent (Popcorn, Teosinte)

Sr No.	Population	Seed rows per cob	CHR 1	CHR 2	CHR 3	CHR 4	CHR 5	CHR 6	CHR 7	CHR 8	CHR 9	CHR 10
1	RIL302	8	bnlg1953, bnlg1025, bnlg1720	bnlg1036	umc1641	umc1017, umc1031, umc1869	bnlg2305			bnlg1823	umc1519	
2	RIL307	6	bnlg176	bnlg1017		umc1017, umc1031, umc1869	umc1679, bnlg2305	umc1006		umc1984, bnlg1131	umc1519	umc1053
3	RIL313	6	bnlg176, bnlg1025, bnlg1720	bnlg1017, bnlg1297, bnlg1036	umc1641	umc2278, umc1869			bnlg2132, bnlg1695	umc1984, bnlg1131		
4	RIL318	6	bnlg1953	bnlg1036	umc1641	umc1017, umc1031	umc1679, bnlg2305	bnlg1371	umc1695	umc1984	umc1492, umc1519	umc1053
5	RIL339	6	bnlg1720	bnlg1036		umc1031	phi087, bnlg2305	umc1805	bnlg2132	bnlg1823	umc1231	umc1053
6	RIL358	8	umc1035, bnlg1025	bnlg1036				phi126, bnlg238	bnlg2132	bnlg1823, bnlg1131		
7	RIL379	8	bnlg176, bnlg1025	bnlg1017, bnlg1036	umc1641		bnlg2305		umc1695	umc1034, umc1984, bnlg1823, bnlg1131		
8	RIL382	7	bnlg176, bnlg1025, bnlg1720		umc1641	umc1017, umc1031			bnlg2132	umc1034, umc1984	umc1519	
9	RIL383	6	bnlg1720		umc1641	umc1031	bnlg2305	bnlg1371	bnlg2132	umc1984, bnlg1131	umc1492, umc1519, umc1231	
10	RIL441	8	bnlg1953, bnlg1035, bnlg1720		bnlg1447	umc1031	umc1679, bnlg2305	umc1006			umc1519	umc1053
11	RIL446	6	bnlg176, umc1035, bnlg1720			umc1017, umc1031	bnlg2305	bnlg1371, umc1805		umc1984	umc1519	umc1053, bnlg1360
12	RIL465	6	bnlg176, umc1035, bnlg1025, bnlg1720	bnlg1297		umc1017, umc1869	umc1679		umc1695	umc1984, bnlg1823		
13	RIL480	6	bnlg1953, bnlg1025	bnlg1017		umc1017, bnlg490, umc1869	umc1679, phi087	umc1805		umc1984, bnlg1823, bnlg1131	umc1492, umc1519	umc1053

Table 8. Introgression lines (more cob length) showing markers associated with the parent (Popcorn, Teosinte)

Sr. No.	Population	Cob length	CHR 1	CHR 2	CHR 3	CHR 4	CHR 5	CHR 6	CHR 7	CHR 8	CHR 9	CHR 10
1	RIL302	10	bnlg1953, bnlg1025,	bnlg1036	umc1641	umc1017, umc1031,	bnlg2305			bnlg1823	umc1519	
2	RIL311	9	bnlg176, bnlg1720		umc1641	umc1031, umc1869	umc1679, bnlg2305	umc1006		bnlg1823	umc1519	
3	RIL315	10	bnlg176, bnlg1720	bnlg1017	bnlg1447	umc1017, umc1031	umc1679, bnlg2305	bnlg1371	bnlg2132, umc1695	umc1034, umc1984, bnlg1131		
4	RIL318	9	bnlg1953	bnlg1036	umc1641	umc1017, umc1031	umc1679, bnlg2305	bnlg1371	umc1695	umc1984	umc1519	umc1053
5	RIL338	10	umc1035			umc1031	bnlg2305		bnlg2132	bnlg1823, bnlg1131		bnlg1053
6	RIL341	9	bnlg176, bnlg1953		umc1641	umc1017	bnlg2305		bnlg2132, umc1695	bnlg1823		
7	RIL343	9	bnlg176, bnlg1953, bnlg1025		umc1641	umc2278, umc1031			bnlg2132, umc1695	bnlg1131		umc1053
8	RIL344	9	umc1035, bnlg1720	bnlg1017		umc2278, umc1869				umc1034, umc1984, bnlg1131	umc1519	umc1053
9	RIL358	12	umc1035, bnlg1025	bnlg1036				phi126, bnlg238	bnlg2132	bnlg1823, bnlg1131		
10	RIL369	9			umc1641	umc1017, umc1031	phi087			bnlg1131		umc1053
11	RIL373	9	bnlg176, bnlg1720	bnlg1036		umc1017, umc1869	phi087, bnlg2305			umc1034	umc1492	umc1053
12	RIL378	9	bnlg176		umc1641	umc1017	bnlg2305		umc1695	bnlg1131	umc1231	
13	RIL380	9	bnlg176, umc1035		umc1641	umc1031	bnlg2305	phi126, bnlg238	bnlg2132	bnlg1131	umc1519, umc1231	umc1053
14	RIL382	13	bnlg176, bnlg1025, bnlg1720		umc1641	umc1017, umc1031			bnlg2132	umc1034, umc1984	umc1519	
15	RIL383	9	bnlg1720		umc1641	umc1031	bnlg2305	bnlg1371	bnlg2132	umc1984, bnlg1131	umc1492, umc1519, umc1231	
16	RIL392	9	bnlg176, bnlg1720	bnlg1017		umc1017	umc1679, bnlg2305	bnlg1371	bnlg2132	bnlg1131	umc1519	
17	RIL416	9	bnlg1720	bnlg1017		umc1869	umc1679, bnlg2305		bnlg2132	bnlg1131	umc1492	
18	RIL425	10	bnlg176, umc1035, bnlg1025, bnlg1720	bnlg1036	umc1641	umc1031	bnlg2305			umc1984, bnlg1131	umc1519	umc1053, bnlg1360
19	RIL435	9	bnlg176	bnlg1017	umc1641	umc1017, umc1031, umc1869	umc1679, phi087	umc1805	umc1695	umc1984, bnlg1131	umc1492, umc1519	umc1053
20	RIL437	10	umc1035	bnlg1297			bnlg2305			bnlg1131, bnlg1823		
21	RIL438	9	bnlg1953, bnlg1025, bnlg1720	bnlg1036		umc1017						
22	RIL443	11	bnlg1953, bnlg1025, bnlg1720, umc1035	bnlg1036	bnlg1447	umc1017, umc1031, umc1869		umc1006				
23	RIL449	10	bnlg176, bnlg1953, bnlg1025		umc1641	umc2278, umc1031, umc1869	umc1679, bnlg2305	bnlg1371		bnlg1823	umc1519	umc1053
24	RIL455	9	bnlg176, bnlg1720	bnlg1017, bnlg1297		umc1017, umc1869	umc1679, bnlg2305		bnlg2132	umc1984, bnlg1131	umc1231	umc1053
25	RIL465	9	bnlg176, umc1035, bnlg1025, bnlg1720	bnlg1297		umc1017, umc1869	umc1679		umc1695	umc1984, bnlg1823		
26	RIL467	11	umc1035, bnlg1720	bnlg1017, bnlg1297, bnlg1036			umc1679, bnlg2323			umc1984, bnlg1131	umc1519	umc1053
27	RIL470	10	bnlg176, bnlg1953	bnlg1017, bnlg1297, bnlg1036	umc1641	umc1031	umc1679		umc1695	umc1034, bnlg1131	umc1519	bnlg1360
28	RIL478	9	bnlg1953, bnlg1720			umc1017, umc1031	umc1679	bnlg1371		umc1984, bnlg1823, bnlg1131		umc1053
29	RIL484	9	umc1035, bnlg1720	bnlg1036		umc1869	umc1679		umc1695	umc1984, bnlg1823, bnlg1131	umc1519, umc1231	bnlg1360, umc1053

genome in a population (27).

In this study, graphical genotyping was used to observe the intrinsic profile of both parents and derived inbred lines, followed by an analysis of the introgression profile. The percentage of Introgression for Teosinte was maximum (66.8%) in RIL421 and minimum (8.8%) in RIL339 and for Popcorn was highest (48.6%) in RIL443 and lowest (8%) in RIL352. Similarly, the graphical genotyping analysis carried out to know parental allelic introgression in five teosinte-derived maize BC_1F_4lines and have reported 34.1% to 53.4% teosinte and 34.1% to 54.5% maize allelic introgression (19). From the introgression profile, common markers in RILs were identified for each trait were identified. Marker bnlg1131 on chromosome 8 was found to be shared in the maximum number of RILs with less maturity days (Table 4). Marker bnlg1131 on chromosome 8 was common in the maximum number of RILs with more cob length (Table 8). From these observations, it can be concluded that these genomic regions might be associated with traits of interest and can be further dissected.

Based on the phenotypic data, RILs exhibiting good maturity, along with high numbers of cobs, seeds per cob and tiller count, or those with a combination of these traits, could be promising candidates for further introgression studies. These lines show potential for improving yield and agronomic performance, making them valuable for future breeding efforts. RIL 302 and RIL 383 demonstrated good maturity, with the highest seed no. per cob measuring 10 cm and 9 cm in length, respectively. RIL 306 produced 13 cobs and matured in 120 days. RIL 311 and RIL 315 matured in 118 to 119 days, with cob lengths ranging from 9 to 10 cm. RIL 318 had a cob length of 9 cm and yielded 6 seeds per cob. RIL 330 had the highest production with 18 cobs, which was maturing in 123 days. RIL 341 had 3 tillers and a cob length of 9 cm. RIL 358 produced the maximum seeds per cob at 8, with a cob length of 12 cm. RIL 370, RIL 378, RIL 391, RIL 444 and RIL 445 each yielded between 8 to 13 cobs, maturing within 118 to 124 days. RIL 379 had 6 seeds per cob and matured in 120 days. RIL 392 matured in 121 days with a cob length of 9 cm and produced 3 tillers. RIL 416 matured in 123 days with a cob length of 9 cm. RIL 446 and RIL 480 each yielded 6 seeds per cob and had 3 tillers. RIL 459 produced 13 cobs and also had 3 tillers. RIL 479 and RIL 482 exhibited the highest cob production, with 18 to 21 cobs and 3 to 4 tillers. RIL 484 produced 12 cobs with a cob length of 9 cm, while RIL 485 yielded 13 cobs and had 3 tillers.

It is stated that the teosinte introgressed maize lines are superior to maize in several aspects such as flowering time and ear numbers, test weight and yield (26). Hence, these lines could be an excellent material for researchers as it offers researchers new opportunities to undertake complementary multi-location, multi-year trials for yield and agronomic performance, response to abiotic and biotic stresses and quality traits. Graphical genotype showed a greater extent of teosinte allelic introgression in maize lines, which also leads to a wide range of variation in terms of morphological traits. Such variation in the maize germplasm provides a better opportunity for breeders to improve traits of interest through parent selection, hybridization and recombination of desirable genotypes (26).

Conclusion

In our study, after comparing the introgression profile with morphological traits, we found that 31 lines had less maturity days, 17 lines had more cobs, 12 lines had more tillers, 13 lines had more seed rows per cob and 29 lines had more cob length. A standard marker, bnlg1297 on chromosome 2, was identified from the introgression profile between several tillers and cobs. The genomic regions can be further dissected with the help of more markers to identify the genes linked to the traits using mapping techniques. After identification of the genes, gene cloning studies can be carried out to clone these genes in various popcorn inbred lines. Thus, improving popcorn varieties can increase popcorns' industrial value and productivity.

Acknowledgements

The financial assistance provided by the School of Agricultural Biotechnology, PAU, Ludhiana, through different grants, is gratefully acknowledged.

Authors' contributions

PS, YV and VT worked on conceptualizing the experiment. VT and PS developed plant material. VT and ND conducted phenotypic evaluation. RK and AS retrieved genotypic data. RK and AS wrote the draft of the manuscript. PS and VT critically revised and edited the manuscript.

Compliance with ethical standards

Conflict of Interest: The authors declare that they have no conflict of interest

Ethical issues: None

Competing Interest: The authors have declared that no competing interest exists.

References

1. Karn A, Gillman, JD, Flint-Garcia SA. Genetic analysis of teosinte alleles for kernel composition traits in maize. G3: Genes, Genomes, Genetics. 2017;7(4):1157-64. https://doi.org/10.1534/g3.117.039529
2. Doebley J, Bacigalupo A, Stec A. Inheritance of kernel weight in two maize-teosinte hybrid populations: implications for crop evolution. J Hered. 1995;85(3):191-95. https://doi.org/10.1093/oxfordjournals.jhered.a111434
3. Flint-GarciaSA. Genetics and consequences of crop domestication. J Agric Food Chem. 2013;61(35):8267-76. https://doi.org/10.1021/jf305511d
4. FAOSTAT. Food and Agriculture Organisation of United Nations [internet]. FAOSTAT statistical database; 2020 [cited 2024 Oct 18]. Available from: https://www.fao.org
5. Yang N, Wang Y, Liu X, Jin M, Vallebueno-Estrada M, Calfee E, Chen L, Dilkes BP, Gui S, Fan X, Harper TK. Two teosintes made modern maize. Science. 2023;382(6674):eadg8940. https://doi.org/10.1126/science. adg8940
6. Mangelsdorf PC. Introgression in maize. Euphytica. 1961;10(2):157-68. https://doi.org/10.1007/bf00022207
7. Anderson E, Hubrich L. Hybridization in Tradescantia. III. The

evidence for introgressive hybridization. Am J Bot. 1938;396-402. https://doi.org/10.2307/2436413

8. Leonova IN. Molecular markers: Implementation in crop plant breeding for identification, introgression and gene pyramiding. Rus J Gen Appl Res. 2013;3(6):464-73. https://doi.org/10.1134/s2079059713060051
9. Hufford MB, Lubinksy P, Pyhäjärvi T, Devengenzo MT, Ellstrand NC, Ross-Ibarra J. The genomic signature of crop-wild introgression in maize. PLoS Gen. 2013;9(5):e1003477. https://doi.org/10.1371/journal.pgen.1003477
10. Yang L, Yang CJ, Cheng Q, Xue W, Doebley JF. Mapping Prolificacy QTL in Maize and Teosinte. J Hered.2016;107(7):674-78. https://doi.org/10.1093/jhered/esw064
11. Watson SA. Description, development, structure and composition of the corn kernel. In: White PJ, Johnson LA, editor. Corn: chemistry and technology. vol 2. St. Paul: American Association of Cereal Chemists; 2003. p. 69-106.
12. Flint-Garcia, Sherry A, Bodnar AL, Scott MP. "Wide variability in kernel composition, seed characteristics and zein profiles among diverse maize inbreds, landraces and teosinte. Theor App Genet. 2009; 119:1129-42. https://doi.org/10.1007/s00122-009-1115-1
13. Yang LiYan YL, Yang ChinJian YC, Cheng Qi CQ, Xue Wei XW, Doebley JF. Mapping prolificacy QTL in maize and teosinte. J Hered. 2016;107(7):674-78. https://doi.org/10.1093/jhered/esw064
14. Karen SP, Lopes de Souza Junior C, Pereira de Souza A, Augusto Franco Garcia A. QTL mapping for yield components in a tropical maize population using microsatellite markers. Hereditas. 2008;145 (4):194-203. https://doi.org/10.1111/j.0018-0661.2008.02065.x
15. Lin Z, Zhou L, Zhong S, Fang X, Liu H, Li Y, Lin Z. A gene regulatory network for tiller development mediated by Tin8 in maize. J Exp Bot. 2022;73(1):110-22. https://doi.org/10.1093/jxb/erab399
16. Wei T, Simko V, Levy M, Xie Y, Jin Y, Zemla J. Package corrplot. Statistician. 2017;56:316-24.
17. Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA, Nucl Acid Res. 1980;8:4321-25. https://doi.org/10.1093/nar/8.19.4321
18. Doebley JF. Molecular systematics of Zea (Gramineae). Maydica. 1990;35(2):143-50.
19. Kumar A, Singh NK, Adhikari S, Joshi A. Morphological and molecular characterization of teosinte derived maize population. Indian J Genet Plant Breed. 2019;79(04):670-77. https://doi.org/10.31742/ijgpb.79.4.4
20. Adhikari S, Joshi A, Singh NK. Phenotypic characterization and microsatellite marker analysis of elite maize inbred and teosinte (*Zea mays* ssp. parviglumis) accession. Pantnagar J Res. 2019;17 (2):123-28.
21. Canci H, Toker C. Evaluation of annual wild Cicer species for drought and heat resistance under field conditions. Genet Resour Crop Evol. 2019;56:1-6. https://doi.org/10.1007/s10722-008-9335-9
22. Timonova EM, Leonova IN, Röder MS, Salina EA. Marker-assisted development and characterization of a set of Triticum aestivum lines carrying different introgressions from the *T. timopheevii* genome. Mol Breed. 2013; 31:123-36. https://doi.org/10.1007/s11032-012-9776-x
23. Mallick N, Vinod Sharma JB, Tomar RS, Sivasamy M, Prabhu KV. Marker‐assisted backcross breeding to combine multiple rust resistance in wheat. Plant breed. 2015;134(2):1727. https://doi.org/10.1111/pbr.12242/full
24. Warschefsky E, Penmetsa RV, Cook DR, Von Wettberg EJ. Back to the wilds: tapping evolutionary adaptations for resilient crops through systematic hybridization with crop wild relatives. Am J Bot. 2014;101(10):1791-1800. https://doi.org/10.3732/ajb.1400116
25. Tanksley SD, McCouch SR. Seed banks and molecular maps: unlocking genetic potential from the wild. Science. 1997;277 (5329):1063-66. https://doi.org/10.1126/science.277.5329.106
26. Adhikari S, Joshi A, Kumar A, Singh NK, Jaiswal JP, Jeena AS. Revealing the genetic diversity of teosinte introgressed maize population by morphometric traits and microsatellite markers. J Plant Biochem. 2022;31.4:720-73. https://doi.org/10.1007/s13562-021-00710-z
27. Young ND, Tanksley SD. Restriction fragment length polymorphism maps and the concept of graphical genotypes. Theor Appl Genet. 1989;77(1):95-101. https://doi.org/10.1007/BF00292322