Phenotypic characterization and microsatellite marker analysis of elite maize inbred and teosinte (*Zea mays ssp. parviglumis*) accession

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ABSTRACT : Teosinte (*Zea mays ssp. parviglumis*), a weedy morphologically diverse sub-species is now considered as the most probable progenitor of modern maize (Zea mays ssp. mays). To determine differentiation between maize and its progenitor toesinte, an investigation on phenotypic and genomic assessment was carried out. Photoperiod sensitivity is one of the traits where maize differs from teosinte. Teosinte grows for a longer time, bears tillers, more than one tassel and takes more duration in flowering. Ear morphology and kernels were entirely different in maize from teosinte. Genomic analysis using 91 microsatellites loci identified 164 alleles with a mean of 1.8 alleles per locus. Of the 91 loci, 55 were polymorphic, 31 were monomorphic, whereas five loci showed null allele between maize and teosinte. Polymorphism information content (PIC) value of polymorphic markers was 1.0. Maize and teosinte were found only 25% similar as indicated by Jaccard's similarity coefficient and dendrogram analysis of SSR data. The results therefore indicates that both at phenotypic and genome level, maize and teosinte are quite diverse probably because of the mutations in some major and regulatory genes followed by selection during evolutionary domestication. Large genetic diversity in teosinte from maize may help in domestication of wild alleles as well diversification and maize germplasm enhancement which are essentially and urgently required in maize improvement programme.

Key words: Maize, Teosinte, diversity, SSR markers

Maize (*Zea mays* L.) is one of the most important cereal grains. It is important because of the highest productivity and production worldwide, adapted to diverse climatic conditions, used as model plant for genetics and plant breeding theories and principles, and utilized from staple food to animal feed and largest number of processed and industrial products (Haarhoff and Swanepoel, 2018; Tigchelaar *et al.* 2018). Maize plant has well-structured and defined characteristics having male inflorescence in the form of tassel on the top and female inflorescence in the form of ear in the mid-ways constituting a typical monoecious, protoandrous and highly cross pollinated crops. Maize has been leader in transforming low productive land races and open pollinated varieties in to highest genetic potential of in the form of hybrids. Origin of such a wonderful crop still unclear and has many theories and hypotheses. There have been three general theories regarding the origin of maize: (1) that it originated from pod-corn, which differs from normal maize primarily by a single dominant gene governing the development of a brittle, disarticulating rachis and the production of prominent glumes enclosing the seeds; (2) that maize originated from teosinte, a wild grass native to Guatemala and Mexico, by direct selection, by large-scale mutations or by the hybridization of teosinte with a grass now unknown; (3) that Zea, teosinte and Tripsacum, the three American Maydeae, have descended along divergent and independent lines from a remote common ancestor (Mangelsdorf, 1940).

Most historians believe maize was domesticated in the Tehuacán Valley of Mexico. Recent research in the early 21st century has modified this view somewhat; scholars now indicate the adjacent Balsas River Valley of south-central Mexico as the center of domestication of maize from a weedy species teosinte (*Zea mays* ssp. *parviglumis*). However, there are wide morphological differences and low co-linearity between modern maize and its most probable progenitor teosinte-*parviglumis*. Scientist believed that maize evolved from teosinte by small number of macro and micro mutation events. George Beadle (1980) was one of the first scientists to fully appreciate the close relationship between teosinte and maize. He calculated that about 5 genes were responsible for the notable differences between teosinte and primitive strain maize by applying basic laws of genetic inheritance which was further supported by Doebley and Stec (1993). Over and above variations and differentiation between teosinte and maize are assumed to be resulted from aim and objectives of artificial selection

in the process of domestication (Wang *et al.,* 1999; Matsuka*et al.* 2002). Surprisingly, both maize and teosinte are seems to be quite similar at genome level. In fact, both have the same number of chromosomes (2n=20) and a remarkably similar arrangement of genes. Teosinte can also cross-breed with modern maize varieties to form fertile maize-teosinte hybrids that can reproduce naturally Singh *et al.* (2017). Intra- or inter-species diversity has always been the basis for productivity enhancement to support continuous increasing demand. Being fully crossable in nature, teosinte (*Zea mays* ssp. *parviglumis*) may constitute a potential candidate source for domestication of wild alleles and diversification of maize germplasm. Being one of the species with greater genetic diversity, molecular analysis of the maize genome suggests that a single domestication event has lowered diversity when compared with wild relative (Warburton *et al*., 2008). Further, most maize commercial varieties in the world has limited genetic diversity, whereas, today the germplasm base in maize breeding programs is relatively narrow (Liu *et al*., 2016). Domestication and breeding bottlenecks have resulted in genomewide reductions in genetic variation in maize relative to teosinte (Tenaillon *et al*., 2004). Additional studies indicated that approximately 2-4% of genes were targets for artificial selection during domestication and breeding (Hufford *et al*., 2012).

Morphological differences between teosinte and modern maize are evidently visible. However, the information on differences between modern maize and its progenitor teosinte at molecular level is scanty. Many molecular marker systems are available to analyse the genomic differences, however simple sequence repeats (SSRs) markers are seems to be better choice because of high polymorphisms, co-dominant nature, distribution across the genome and ease in assay. The present investigation was therefore planned with objectives to determine level of phenotypic and genomic differentiation from modern maize and its probable progenitor teosinte (*Zeamays* ssp. *parviglumis*).

MATERIALS AND METHODS

The present investigation was undertaken at N. E. Borlaug Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand by taking wild progenitor (population) of maize i.e. teosinte (*Z. mays* ssp. *parviglumis*) collected locally and a maize inbred line DI 103. The inbred line DI 103 is a promising inbred line developed at Pantnagar and has been used in crossing programme. These two sub-species were grown in 10 rows of 4.o meter length in 2018 *kharif* season and characterised for 17 morphological characters namely days to anthesis, days to silking, anthesis–silking interval (ASI), number of ears per plant, anthocynin colouration of leaf sheath, male and female flowering behavior, angle between leaf and stem, anthocynin colouration of tassel (base), anthocynin colouration of tassel (glumes), anthocynin colouration of anther, tassel density of spikelets, tassel length (cm), branching, cob length, cob diameter, kernel rows per cob and kernel per row in order to determine morphological differentiation from probable progenitor to modern maize. Data were recorded on 15 randomly selected plants for all the characters.

Genomic Diversity

Genomic DNA extraction

The genomic DNA from each genotype was isolated from pooled young healthy leaves from 10 plants of 30 days old. DNA was extracted using CTAB (Cetyltrimethyl ammonium bromide) method. The quality of DNA was assessed by gel electrophoresis (0.8 per cent agarose) and quantity was estimated by using spectrophotometer. RNAse treated DNA samples were diluted to a working concentration of 100 ng /µl and stored for further PCR amplification.

PCR amplification

Ninety one SSR markers covering whole genome were synthesized from Eurofins Genomics India Pvt. Ltd .The original source, repeat motifs, primer sequences and chromosomal position of these markers can be found in the http://maize.gdb. Ninety one SSR markers were used to screen the diversity between maize inbred DI-103 and teosinte. 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 3 units/µl Taq DNA polymerase. The PCR reaction was performed in an Agilent and Prima 96 plus PCR machines. The PCR cycle conditions for SSR markers consisted of initial denaturation of DNA at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 40 seconds, annealing at 55°C for 40 seconds and elongation at 72°C for 1 minute and final extension at 72°C for 10 min. The amplicons generated were resolved on 2.5 per

cent agarose gel using horizontal gel electrophoresis assembly. After 75 per cent of the gel run, the amplicons were visualized and photographed under UV light (Alpha Innotech Corporation, USA).

Data analysis

Marker data were recorded in binary format as '1' refers to presence of specific allele at the locus while '0' refers to absence of the same allele. Marker data were analyzed using NTSYS-pc version 1.8 statistical software and dendogram was constructed (Rohlf, 1992). The polymorphism information content (PIC), also named expected heterozygosity (Nei, 1987) for each SSR marker was determined as described by Smith *et al*. (1997). PIC is a measure of allele diversity at a locus and is equal to 1 - where *fi* is the frequency of the i^{th}
allele. The PIC calculation was performed using Microsoft Excel.

RESULTS AND DISCUSSION

Phenotypic characterization of maize and teosinte

Data recorded on different morphological characters on maize and teosinte were analysed simultaneously to determine differences between them (Table 1). In maize inbred line anthesis and silk emergence took place in 55 and 57 days where as in teosinte it took 81 and 78 days after sowing. In fact *parviglumis* teosinte is photo-sensitive and flowering duration varies according month of sowing. If teosinte planted late in *kharif,* it takes less duration in flowering than the teosinte planted earlier. In general *parviglumis* takes longer duration to flower than even the late genotype of maize. Maize derived from *parviglumis* takes relatively less duration in flowering probably because of the selection practiced for earliness during domestication process to fit into different cropping system. In case of maize, ASI was 2-3 days whereas -3.0 days in case of teosinte because of the early blooming of female inflorescence than the anthesis. In fact, some of the female inflorescence in teosinte blooms earlier than the anthesis. In both the cases, tassel born on the top of the stem. Side branches in case of teosinte also bears tassel on the top and ears on the mid way. Thus, teosinte bears many tassels whereas maize bears only one tassel. Tassel on the main shoot of teosinte is larger and prolific pollen producer than the maize. Differences in tassel size are again justified on the basis of continuous selection for small tassel during the development of maize inbred lines. In fact, bulky tassel produces pollen grains many hundred times of the required number to affect the complete pollination. To avoid the energy required in development large tassel and more number of pollen grains, concept of small/lax tassel has been adopted and selection has been practiced regularly to develop small tassel in maize. Prolificacy condition is one of the major distinguished characteristics of teosinte and therefore it bears more number of cobs (245). However, in general, maize inbred had single or double cobs per plant. Reduction in cob numbers in maize may be compensated by increased kernel rows per cob and kernels per row. In maize, ears are longer with wide diameter and bear more number of grains (more than 300)

Table 1: Morphological differences between maize and teosinte (*Zea mays ssp pariglumis*)

S. No.	Characters	Maize	Teosinte
1	Days to anthesis	55	81*
2	Days to silking	57	78 *
3	ASI	2	-3
4	Number of cobs per plant	1.4	245
5	Anthocynin colouration of leaf sheath	Absent	Present
6	Male and female flowering behavior	protoandrous	protogynous
7	Angle between leaf and stem	>450	<450
8	Anthocynin colouration of tassel (base)	Absent	Present
9	Anthocynin colouration of tassel (glumes)	Absent	Present
10	Anthocynin colouration of anther	Absent	Present
11	Tassel density of spikelets	Sparse	Dense
12	Tassel length (cm)	>30	<30
13	Branching	Absent	Present
14	Cob length (cm)	12	4
15	Cob diameter (cm)	3.3	0.75
16	Kernel rows per cob	12	2
17	Kernel per ear	310	8

*Anthesis and silking durations depends on the time/month of sowing.

on the central axis of cob. Kernels of maize are naked without any protection from predation. In case of teosinte, ears are small about 4-5 cm and bears 8 kernels. Teosinte kernels are not naked and covered with stony casing, collectively kernels and stony casing is known as fruit case. Anthocynin colour was absent in maize (DI-103) leaf sheath, glume base and anther whereas presence was reported in teosinte. Anthocyanins often appear transiently at specific developmental stages and may be induced by a number of environmental factors including visible and UVB radiation, low temperatures and water stress. The subsequent production and localization of anthocyanins in root, stem and especially leaf tissues may allow the plant to develop resistance to a number of environmental stresses (Scott, 1999). As teosinte was growing under natural condition this feature make it capable to survive under adverse climatic conditions. Tassel with sparse spikelets was observed in case of maize inbred, whereas teosinte possessed dense spikelets. Tassel length in case of maize was found >30 cm, whereas in case of teosinte was <30 cm. In fact, tassel branches are much more in teosinte than the maize.

Comparison of maize and teosinte alleles for the level of teosinte branched 1 (*tb1*) transcript accumulation indicates that the maize allele is expressed at about twice the level of the teosinte allele in immature axillary branches and the inflorescence primordial **(**Dorweiler and Doebley, 1997; Lukens and Doebley, 1999**)**. Further, *in situ* hybridization in teosinte showed no sign of *tb1* expression in axillary buds, where maize shows strong expression **(**Hubbard *et al.,* 2002). Therefore, maize *tb1* mutant showed reduction in apical dominance with proliferation of basal tiller. In teosinte *tb1* gene expression is off resulted in branching behavior whereas maize has strong expression leading to strong apical dominance and absent of tillering in maize. The phenyotypic assessment indicated significant differentiation of modern maize from its wild relative teosinte. The observation therefore indicates great diversity between maize and teosinte. Further, such diverse, desirable and adaptive alleles, probably lost during the evolution and artificial selection, can be domesticated from teosinte through pre-breeding approaches.

Genomic characterization

Genomes of maize and teosinte were analysed using 91 SSR markers and amplified alleles were separated on agarose gel (Fig. 1). A total of 164 alleles were observed at 91 microsatellites loci, on an average being 1.8 alleles per marker (Table 2). Wietholter (2008) also observed an average of 2.7 alleles per locus at 23 SSR loci. The allele size varied from 70 to 300 bp, which is in agreement with the results of Matsouka *et al.*(2002) and Senior *et al*. (1998).

In the present investigation, alleles per locus were low in comparison to other marker studies. The reason for low number of alleles is because of inclusion of one line of teosinte and one line of maize. Because of only two samples, maximum possible alleles per locus is 2 whereas in other studies where alleles per locus is more consisted of more than two samples (Tarter *et al*., 2004; Wietholter, 2008). Of the 91 markers, 55 markers were polymorphic, 31 were monomorphic and 5 markers had null allele either in maize or in teosinte. The polymorphism information content (PIC) indicates the discriminatory power of a marker. More the PIC value,

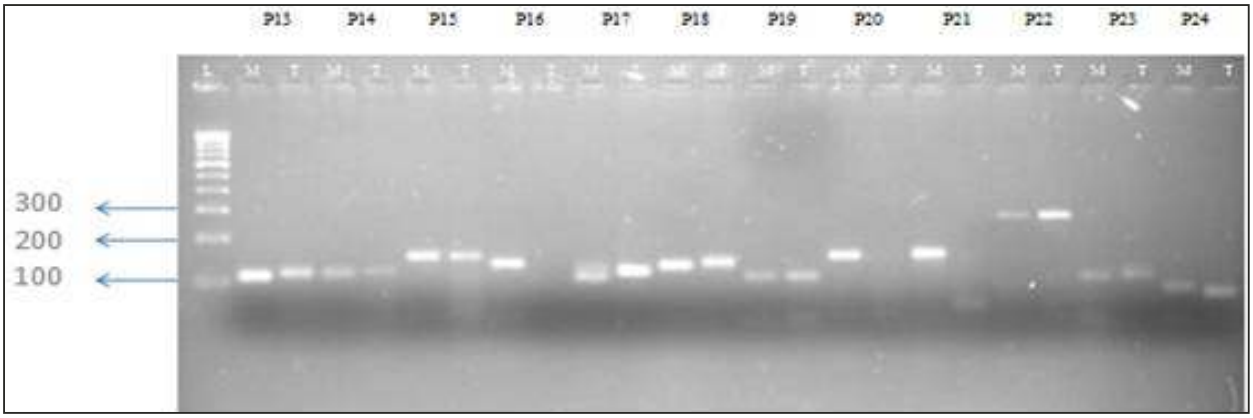

Fig.1: SSR profile of Maize and Teosinte with primer umc1156, umc1303, umc2182, phi035, phi035, umc1500, umc2255, umc1444, umc2099, umc1294, nc013 and phi089

Table 2: Summary of SSR markers analysis of maize and teosinte genomes

Particulars	Number/value
Number of SSR markers used	91
No. of polymorphic markers	55
No. of monomorphic markers	31
No. markers with null allele	5
Total alleles	164
Average alleles per locus	1.8
Size of alleles	70-300 bp
PIC value of polymorphic markers	1.0

more is the usefulness of markers in differentiation of genotypes. The 55 polymorphic markers had PIC value of 1.0 (Table 2). Five markers exhibited null alleles i.e. absence/presence type of variation rather than usual size variations. Such variations are generally rare and make complications in analysis with SSR markers, therefore they need re-validation.

Upon repeated PCR analysis, five markers exhibited null alleles were found to show null allele again and again and we consider the situation is not because of the experimental errors but probably because of the loss of either of the primer binding sites due to addition or deletion of nucleotide sequence. Thirty one markers exhibited uniform allelic pattern throughout the maize and teosinte genomes were considered as monomorphic. Based on the marker data, 31 SSR loci exhibiting monomorphism, can be considered to be conserved between maize and teosinte. Matsuoka *et al.* (2002) found an average PIC of 0.62 (varying from 0.18 to 0.89) and 0.73 (varying from 0.22 to 0.91) in microsatellite analysis in maize lines and joint analysis of teosinte and maize, respectively. Jaccard similarity coefficients and dendrogram (Fig. 2) generated using markers data indicate 25% genetic similarity in maize and teosinte at genome level. Thus, based on the present observations, maize and teosinte are diverse to each other by 75%.

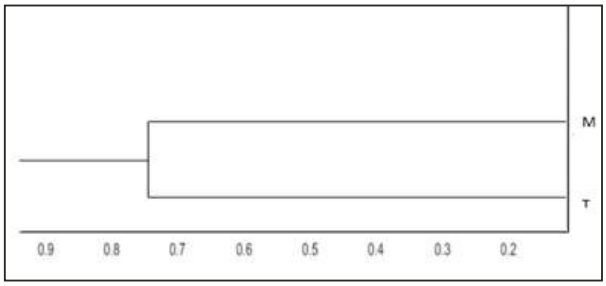

Fig. 2: Dendrogram showing the genetic relationship between maize and teosinte based on SSR profile

Terra *et al*., (2011) also observed 78% genetic distance between teosinte (*Zea mays mexicana*) and maize. *Zea mays* ssp. *parviglumis* is now believed as progenitor of maize, however higher level of morphological and genome sequence variations are evident in different investigations. In addition to mutations at many loci followed by artificial selection in the process of domestication might have made significant changes in maize from its progenitor teosinte (Wang *et al.,* 1999; Matsuka *et al.* 2002).

CONCLUSION

In the investigation, maize and teosinte-parviglumis were investigation for phenotypic and genomic differences. Phenotypically, maize was observed entirely different in many characteristics, prominent being plant and ear morphology and kernel characteristics. Such a higher level of differentiation from teosinte to maize is assumed to be because of mutations at many loci followed by selection during the domestication. Of the 91 SSR markers, 55 were polymorphic, 31 were monomorphic and 5 SSR markers exhibited null allele. Dendrogram based on marker data indicate that 25% of the genomes are similar while 75% are dissimilar between maize and teosinte-*parviglumis*. Teosinte-*parviglumis* are easily crossable with maize producing fertile progenies. Unique and adapted allelic variation of teosinte can be a potential source of maize improvement through pre-breeding approaches. Thus, domestication of wild adaptive alleles, probably those lost during artificial selection, can be introgressed in to maize for improving biotic and abiotic stresses and quality traits and also for the diversification and enhancement of maize germplasm.

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Received: May 29, 2019
Accepted: June 11, 2019