male spikelets in wild populations of teosinte occurs at a low frequency. Its significance can be questioned because, rather than being part of the natural variation in teosinte, it could just be a result of introgression from corn."

Recently the author has found these paired female spikelets in populations of teosinte race Chalco (Figure 1). Two types of "double kernel fruitcases", as the author has called them, were found: a) fruitcases with the two spikelets in parallel within the single cupule with an indurated outer glume covering each spikelet, as shown in the middle row of Figure 1; b) two spikelets grown in a different manner, one within the cupule covered by an indurated outer glume in the same way as the normal teosinte fruitcase with a single spikelet, and the second spikelet developing outside the cupule, due to elongation of the pedicel or rachilla, and covered by the floral bracts or glumes with a variously indurated outer glume. Frequently, the outer kernel becomes naked or almost so; the elongated rachilla is grown parallel to the rachis axis, therefore, this spikelet usually is positioned at the top of the fruitcase; and either one of the two spikelets can grow outward from the cupule. No case with the two kernels growing outside the cupule has been observed so far (see bottom row in Figure 1).

Figure 1. Normal and double kernel fruit cases of teosinte from the region of Chalco-Amecameca, State of Mexico-

The parallel orientation of the elongated pedicel of the external kernel in double kernel fruitcases in relation to the rachis axis, seems to indicate that they are not a consequence of introgression from maize into teosinte because the rachilla elongation in maize is perpendicular to the rachis axis. Besides, as Galinat (Univ. Massachusetts, Agric. Expt. Sta. Amherst, Bull. No. 585, 1970) states, "In a hybrid between modern maize and teosinte, the rachilla is shortened and the paired spikelets inclined and partially embedded within the cupule." However, because the present report is based on preliminary observations, the maize introgression hypothesis cannot be discarded completely yet until more detailed studies are made on these paired spikelet female fruitcases from teosinte race Chalco populations. In any event, the evolutionary significance of these findings is that the teosinte populations of 8,000 to 10,000 years ago probably produced the natural variation, which included the paired spikelet female fruitcases with naked kernels among other variants, before man of that time, upon observing this variation was motivated to start domestication of maize from teosinte. This evolutionary process intermediate between teosinte and maize is what Galinat (1988) called the "primary step" toward the origin of maize by means of "a reactivation of the second female spikelet".

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Molecular characterization of selected maize landraces in India using Simple Sequence Repeat (SSR) markers

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Intensive efforts have been initiated in the last few years on phenotypic as well as molecular characterization of the maize landraces in India. Significant variability in plant, ear, and tassel characteristics of maize landraces has been observed in Northeastern and Northwestern highlands of India with relatively less varietal diversity for those collected from the plains (Prasanna and Sharma, Indian J. Plant Genet Resources 18:155-168, 2005).

In the present study, a set of 48 Indian maize landraces was selected for SSR genotyping. These landraces are all important for breeding purposes, since they are mostly early maturing, with excellent yield characters and adaptability, along with biotic and abiotic stress tolerance. The selected germplasm represents diverse agro-ecological zones of India, spanning both NEH (29 landraces) and other regions (19 landraces). Thirty were obtained from the National Gene Bank at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, and 18 were collected by the Maize Genetics Unit, IARI, from Sikkim state in the NEH region in November 2005 (Table 1). SSR analysis employed fluorescent-dye labeled SSR primers (CIMMYT Applied Biotechnology Center's Manual of Laboratory Procedures (http://www.cimyt.org/ABC/Protocols/manual ABC.html) (Fig. 1).

Table 1. List of accessions selected for molecular characterization.

S.No.	Accession	State (India)*	S.No.	Accession	State (India)*
1	IML112	HP	25	IML429	Rajasthan
2	IML115	J&K	26	IML436	Rajasthan
3	IML132	Uttarakhand	27	IML452	MP
4	IML181	HP	28	IML454	MP
5	IML196	Manipur	29	IML479	MP
6	IML203	Nagaland	30	IML550	Sikkim
7	IML210	AP	31	IML558	Sikkim
8	IML215	Ar.P	32	IML560	Sikkim
9	IML232	Ar.P	33	IML565	Sikkim
10	IML235	Ar.P.	34	IML567	Sikkim
11	IML255	Meghalaya	35	IML587	Sikkim
12	IML267	Sikkim	36	IML588	Sikkim
13	IML282	Ar.P.	37	IML589	Sikkim
14	IML290	Jharkhand	38	IML590	Sikkim
15	IML293	Bihar	39	IML592	Sikkim
16	IML295	Jharkhand	40	IML594	Sikkim
17	IML297	WB	41	IML595	Sikkim
18	IML298	Meghalaya	42	IML602	Sikkim
19	IML321	Bihar	43	IML608	Sikkim
20	IML415	MP	44	IML610	Sikkim
21	IML420	Rajasthan	45	IML615	Sikkim
22	IML423	Bihar	46	IML616	Sikkim
23	IML427	Rajasthan	47	IML618	Sikkim
24	IML428	Rajasthan	48	IML637	Mizoram

*HP: Himachal Pradesh; J&K: Jammu and Kashmir; AP: Andhra Pradesh; Ar.P.: Arunachal Pradesh; WB: West Bengal; MP: Madhya Pradesh.

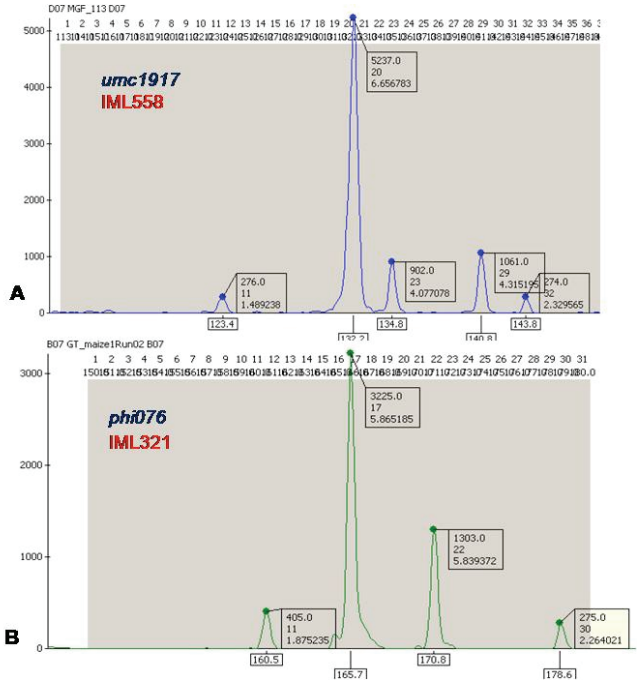

Figure 1. Images of some SSR alleles (seen as peaks) resolved using a MegaBACE DNA Sequencer: (A) *umc1917*; (B) *phi076*.

A total of 547 alleles were detected for 42 SSR markers. We found, on average, a higher number of alleles per marker (13.0) (Table 2) than reported in a study on maize populations from Europe and America using 24 SSR markers (Dubriel et al., Maydica 51:281-291, 2006) where the average number of alleles observed was 7.8. Similarly, Warburton et al. (Crop Sci 42:1832-1840, 2002), in an analysis of seven CIMMYT populations and 57 CML lines, reported mean numbers of SSR alleles of 6.3 and 4.9, respectively. The higher number of alleles per locus observed in this study indicates considerable diversity among the selected landraces in India. The PIC values of the 42 SSR loci in the present study ranged from 0.18 (*phi062*) to 0.85 (*phi083* and *phi331888*), with a high mean PIC value of 0.60, reinforcing the importance and utility of SSR markers in the study of population genetics. The frequency of the major allele at each locus ranged from 0.21 (*phi96100*) to 0.89 (*phi062*). Rare alleles (frequency less than 0.005) were identified at all loci, with an average of 9 alleles/locus.

Genetic relationships among the selected Indian maize landrace accessions are depicted in Figure 2. The distinct feature of the dendrogram is the grouping of 'Sikkim Primitives' into one cluster. 'Sikkim Primitives', first described by N. L. Dhawan in 1964 (MNL 38:69-70), have some unique features, including a complete lack of apical dominance; prolificacy (5-9 ears) with uniformity in ear size; erect leaves; top bearing habit and drooping tassel (Sachan and Sarkar, MNL 56:122-124, 1982). This landrace stays green after maturity, and thus serves well for fodder purpose for the local farmers. The 'Sikkim Primitive' landrace is still grown in remote and isolated villages of Sikkim state in the NEH region. Accessions from other regions in India were found to form different clusters, based on geographical region.

Estimation of Wright's *F*-statistics revealed F_{IS} values ranging from -0.11 (IML602) to 0.13 (IML592), indicating low levels of in-

Table 2. Marker-wise summary statistics for the selected landraces.

S.No.	SSR locus	Observed size range (bp)	No. of alleles	Major Allele		PIC*
				Size (bp)	Frequency	
1	*nc130*	133-148	6	141	0.65	0.46
2	*nc133*	99-120	4	116	0.54	0.51
3	*phi014*	140-172	7	158	0.80	0.31
4	*phi029*	148-162	10	149	0.54	0.57
5	*phi031*	170-230	17	186	0.38	0.79
6	*phi034*	120-150	10	123	0.33	0.79
7	*phi041*	190-220	9	200	0.58	0.59
8	*phi046*	55-70	7	63	0.49	0.58
9	*phi059*	140-180	10	153	0.53	0.60
10	*phi062*	156-162	4	162	0.89	0.18
11	*phi063*	145-191	32	172	0.49	0.67
12	*phi065*	124-152	12	131	0.29	0.77
13	*phi075*	199-245	15	228	0.53	0.57
14	*phi076*	150-180	10	171	0.50	0.55
15	*phi079*	160-210	11	187	0.24	0.78
16	*phi083*	110-140	21	125	0.23	0.85
17	*phi084*	150-162	10	158	0.53	0.65
18	*phi090*	110-150	7	141	0.83	0.24
19	*phi093*	282-291	11	288	0.28	0.82
20	*phi102228*	118-135	9	124	0.66	0.54
21	*phi108411*	112-128	11	123	0.42	0.68
22	*phi109188*	140-181	14	165	0.64	0.56
23	*phi112*	129-170	19	152	0.68	0.52
24	*phi115*	275-320	18	293	0.52	0.52
25	*phi123*	115-185	15	145	0.58	0.62
26	*phi127*	96-135	19	112	0.53	0.67
27	*phi227562*	302-324	8	308	0.38	0.71
28	*phi299852*	99-165	19	122	0.37	0.80
29	*phi308707*	110-160	18	134	0.38	0.76
30	*phi331888*	115-139	16	119	0.25	0.85
31	*phi96100*	245-305	22	297	0.21	0.84
32	*umc1161*	125-160	17	145	0.56	0.64
33	*umc1196*	129-185	14	148	0.63	0.56
34	*umc1266*	130-149	10	134	0.86	0.25
35	*umc1304*	97-141	21	131	0.54	0.62
36	*umc1332*	108-150	14	145	0.40	0.69
37	*umc1367*	130-165	7	159	0.87	0.22
38	*umc1447*	110-127	8	124	0.46	0.56
39	*umc1545*	60-85	16	80	0.36	0.76
40	*umc1917*	121-147	11	132	0.53	0.64
41	*umc2047*	119-140	10	135	0.59	0.59
42	*umc2250*	135-163	18	152	0.75	0.41

*PIC: Polymorphism Information Content.
bp and PIC determined using FreqsR and Fto software (Dubreuil et al., Maydica 51:281-291, 2006) developed under the Generation Challenge Program (GCP) Project titled "Characterization of global maize populations: tracking the maize migration route from the center of origin", coordinated by CIMMYT, Mexico.

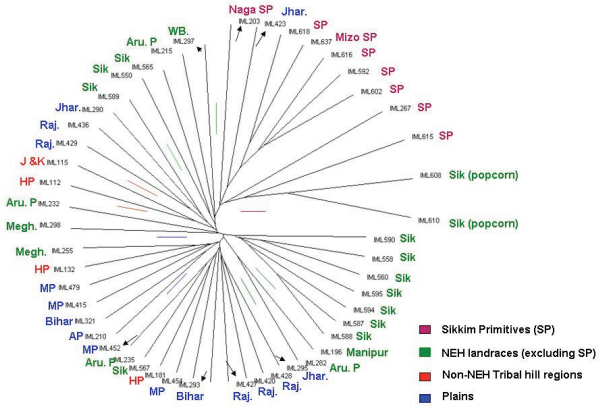

Figure 2. Genetic relationships among selected Indian maize landrace accessions. Pair-wise genetic distances were based on SSR allele frequencies determined using Rogers (1972) distance, followed by cluster analysis using UPGMA; the resulting dendrogram was prepared using TREEVIEW (Page, 1996). Note the occurrence of 'Sikkim Primitive' accessions in a distinct cluster.

breeding and negligible non-random mating within each population. Significant variation in the F_{ST} values was found among accessions, with a mean of 0.38, indicating high genetic differentiation between accessions. AMOVA using Arlequin v2.0 revealed that 60% of the genetic variation in these accessions was within the individuals, while 37% of the variation was among populations within a group.

More extensive efforts are in progress at the Maize Genetics Unit, IARI, New Delhi, with regard to phenotypic and molecular characterization of landraces in India. The goals are to identify 'core collections' with potential utility in breeding programmes, and in mining favourable alleles towards influencing productivity and other target traits.

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Evolutionary divergence of the genes *dek1* and *Agpsem* (*agp1*) of *Tripsacum* and *Zea mays*

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Eastern gamagrass (*Tripsacum dactyloides* L.), a perennial cereal, is a distant relative of maize and is found widely throughout South and Central America, and southern and eastern areas of North America. Several cytotypes are encountered in nature, diploid *(2n=36)*, triploid *(2n=3x=54)*, tetraploid *(2n=4x=72)* and hexaploid *(2n=6x=108)* forms. Diploids follow a strictly sexual mode of reproduction, whereas polyploids are apomictic. Hybridisation among these species may occur spontaneously. Thus, *Tripsacum andersonii* J.R. Gray, a *Zea* (maize) x *Tripsacum* hybrid with 54 chromosomes which clonally reproduces through budding, has been found in nature and is considered to be a spontaneous hybrid of the two species *Tripsacum dactyloides* and *Zea mays*.

Modern maize x *Tripsacum* hybridisations are also used in plant breeding, primarily for introgression of various agronomic traits. These hybridisations have made it possible to improve maize resistance to high temperatures, to obtain valuable forms of polyunsaturated fatty acids, and to produce apomictic maize x *Tripsacum* hybrids. The use of both molecular mapping and molecular-cytological methods (FISH) has demonstrated that there is a high degree of homology between maize and *Tripsacum* genomes. Molecular probes derived from maize genes can be used for marking, or tagging, *Tripsacum* chromosomes. However, the precise level of genome homology has yet to be determined.

The goal of this research was to determine the primary sequence structure of some *Tripsacum dactyloides* L. genes and to compare them to candidate orthologous maize genes. A *Tripsacum dactyloides* L. specimen, 4N=72, was obtained from the Tashkent Botanic Garden and used as a representative of the species. This particular specimen is of historic significance in that it originated from the materials of N.I. Vavilov's expedition. In addition, this plant accession has also been used to produce apomictic maize x *Tripsacum* hybrids.

Total DNA extraction was carried out from young leaves using cetyltrimethylammonium bromide (CTAB). The DNA was then quantitated and used in polymerase chain reaction (PCR) amplification of specific fragments. The primer pair used for fragment amplification of gene *dek1* was dek1-F (5'-GGGTGCTTTAACTTCAGTTGCA -3') and dek1-R (5'-GCCANGTTCAAATCCAATAGCTG - 3'). For fragment amplification of gene *Agpsem,* the primers Agp-F (5'- GA-TATCCCNGTCAGCAACTG T - 3') and Agp-R (5' - TTTTGGTANTCCATACGGTAC - 3') were used. PCR was carried out in 20 μl of reactions using BIOTAQ™ Red DNA Polymerase by Bioline Enterprise and 10 ng of the total DNA. Amplification cycle reactions were as follows: initial denaturation — 95^{0}C, 3 min.; amplification for 35 cycles: 94^{0}C, 30 sec., annealing — 56^{0}C, 30 sec., extension — 72^{0}C, 60 sec.; final extension — 5 min. Amplification products were visualized in a 1% agarose gel. Total maize DNA extracted from accession C435 (VIR Collection, St.-Petersburg) was used as control. Determination of initial PCR product sequences was carried out in the Inter-Institutional Centre for DNA Sequencing, SB RAS, Novosibirsk using a ABI PRISM® BigDye™ Terminator v3.1 Ready Reaction Cycle Sequencing Kit. The same primers were used for the sequencing and amplification reactions. Only part of the initial PCR product sequences were used in the comparative analysis as determined using direct and reverse primers. The maize gene sequences used for comparison were obtained from GenBank (http://www.ncbi.nlm.nih.gov).

Using the primers dek1-F and dek1-R, the expected PCR product size was 886 nucleotides. The fragment of the putative *Tripsacum dek1* gene was determined to be 797 bases long. Direct comparison of initial sequences of the PCR-generated putative *Tripsacum dek1* gene fragment and that of maize demonstrated very high homology, i.e., 99%, and only 1% of differences, for 6 one-nt replacements and 1 three-nt deletion/insertion (Fig. 1). Three one-nt replacements were localised in an exon region, the other three were found in the intron region of maize gene *dek1*. The *Tripsacum* three-nt deletion was found in the gene exon relative to maize.

			48				264			612		
		27			161			468			739	
T. dactyloides	(1) ..	C ..	T ..	..	A ..	..	G ..	..	A ..	T ..	---	..
Zea mays	(5066) ..	A ..	C ..	..	G ..	..	C ..	..	C ..	C ..	ACT	..

Figure 1. Comparison of nucleotide sequences for the *Zea mays dek1* gene (AY061804) and the putative PCR-generated *dek1* gene of *Tripsacum*. Exons of the *Zea mays dek1* gene are shown in grey.

The maize gene *dek1* (*defective kernel1*) is approximately 24k nucleotides long and plays a very important role through its participation in the structural maintenance and functioning of Ca^{2+} ion trans-membrane transport channels. For the maize *dek1* gene, not only is the whole primary structure known, but also intron and exon positions, as well as the structure of its protein product. Therefore, open coding frames may be determined on the primary sequence of the putative *Tripsacum dek1* gene fragment in the areas corresponding to comparative exons of the maize *dek1* gene, and thus it is possible to determine if revealed comparative sequence differences lead to changes at the level of the amino acid sequence. As seen in Fig. 2, the difference in position 161 between maize and *Tripsacum* leads to amino acid replacement. Replacements at nt positions 468 and 612 do not lead to differences in amino acids, i.e., they are synonymous. Absence of an