

## Origin, Evolution and Diffusion of Maize (*Zea mays* L.)

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### **Pattern of evolution in maize and its wild relatives:**

To study the (1) phylogeny of maize (2) origin of maize and (3) antiquity of maize in India, multidisciplinary approach *e.g.* chromosome differentiation, isoenzyme polymorphism, knob-heterochromatin distribution, crossability relationships, exine sculpture, ethnobotany *etc.* have been adopted. A large number of collections of North-Eastern Himalayan (NEH) maize along with established Mesoamerican races and the wild relatives of maize such as teosinte (*Zea diploperennis*, *Z. luxurians*, *Z. parviglumis*, *Z. mexicana*), *Coix* spp., *Chionachne* spp. and *Trilobachne cookei* have been subjected to above studies.

*Cytological characterization of North-Eastern Himalayan (NEH) maize:* Because of the distance from the centre of origin, the antiquity and diversity of NEH maize is of considerable evolutionary significance. The NEH maize diversity has been characterized by the distribution pattern of constitutive heterochromatin, discernible by knobs, C- and Q-bands. Specific geographic pattern of C-heterochromatin has been observed. Frequency and combination of various knobs is the characteristic of different complexes.

Altogether 26 knob forming positions have been observed in NEH maize. Four new knob forming positions *i.e.* 1Lb, 2Lb, 2Lt and 9Lb have been identified in NEH maize. The knob constellation 2La, 4L, 6S, 6Lb, 8La and 9St is the characteristic feature of NEH maize. The occurrence of knob at 1La, 3L, 5L, 6Lc, 7St, 7L and 8Lb positions is less frequent. The NEH maize has been further characterized and grouped into two complexes (a) high knob number, strains with frequent positions at 2La, 4L, 6S, 8L and 9St, and (b) low knob number strains having frequent knobs at 3L, 6LB and 7L.

The Sikkim Primitive (SP) maize has been treated separately because of its unique characteristics. The knob constellation of SP maize (T-2 and Murlia) and the Nal-Tel, a Mexican primitive race share the following common knob positions. 2La, 4L, 5L, 6Lb, 6Lc, 7L, 8La, 8Lb, 9S and 9L. The lineage of SP maize has been assigned

to Nal-Tel Chapalote complex of Mexico. This suggests that Sikkim Primitive maize is the prototype of pre-historic wild corn.

*Karyotypic studies in maize and its wild relatives:* The study of the karyotype has been realized as an aid to evolutionary significance. When 28 collections of *Coix* from different geographical regions were used for their karyotypic studies, it was found that different populations of Indian origin largely fell into 2 major groups, *i.e.* (i) those with chromosome complement of  $2n = 10$  (*Coix aquatica*) and (ii) *C. lacryma-jobi* with  $2n = 20$ . The genomic length of *C. aquatica* was smallest (33.25  $\mu\text{m}$ ) with mean chromosome length 6.64  $\mu\text{m}$ . The genomic length of *C. lacryma-jobi* and *Zea mays* were 80.64  $\mu\text{m}$  and 87.43  $\mu\text{m}$ , respectively. The relative length, arm ratio and centromeric index widely varied in these three taxa. The karyotype of maize is symmetrical and entirely different from *Coix*.

*Trilobachne cookei* showed bimodal karyotype ( $2n=20$ ) having six telocentric, two metacentric and two submetacentric pairs of chromosomes. The arm ratio greatly varied from 1.09 to 7.5. *Chionachne koenigii* also showed  $2n = 20$  karyotype with nine pairs of submetacentric and one pair of metacentric chromosomes. Maize and teosinte showed similar karyotype. The karyotypes of *Coix*, *Chionachne* and *Trilobachne* did not show any relationship with *Zea*.

A new cytotype of *Coix-28* with chromosome number  $2n=18$  without any meiotic abnormality has been identified. This suggests that basic chromosome number in *Coix* spp. is 5.

*Distribution of constitutive heterochromatin in maize and its wild relatives:* The distribution of C- and Q-bands in maize and teosinte were terminal and sub-terminal which were comparable to each other. Chromosome banding patterns of maize and teosinte showed reciprocal introgression of C-heterochromatin. Striking differences in the distribution of C-heterochromatin have been observed between American and Asiatic genera of Maydeae. Among the *Coix* spp. only cultivated *Coix*

showed 22 small terminal bands while wild *Coix* did not reveal any band, however, chromosomes were differentiated by enhanced and reduced fluorescence. *Trilobachne* manifested minute centromeric C-heterochromatin in all the chromosomes. *Chionachne* did not reveal any C- or Q-band.

*Pachytene analysis of Coix and maize*: For the first time detailed study of pachytene chromosomes of *C. aquatic* along with maize and teosinte have been made. The relative length of chromosomes in 26 collections of *Coix* ranged from 13.55-28.28. The total genomic length in maize, teosinte and *Coix* was 565  $\mu\text{m}$ , 547  $\mu\text{m}$  and 838  $\mu\text{m}$ . The genome of *Coix* is quite dissimilar to teosinte (*Zea diploperennis*, *Z. luxurians*, *Z. parviglumis*) and maize.

The wild *Coix* chromosomes are devoid of knobs. However, cultivated coix possess small terminal knobs.

*Coix*-21 and *Coix*-63 have shown structural heterozygosity. The hexavalents have been consistently observed. The pollen fertility was 75% and the normal seed set has been observed.

*Genetic distance studies based on isoenzyme polymorphism*: Elaborate studies on electrophoretic movement of different isoenzymes e.g. esterase, glutamate dehydrogenase, peroxidase, superoxide dimutase, catalase, malate dehydrogenase, acid phosphatase have been undertaken. Among the tribe Maydeae genetic distance was the least (0.30) between maize and teosinte. While *Chionachne* and *Coix* (GD=0.83) were apart from maize and teosinte. *Coix* and *Chionachne* were distantly related with (GD=0.65) each other. Based on GD the heirarchical order of Maydeae is as follows:

Maize: Teosinte > *Coix* > *Trilobachne* > *Chionachne*

Analysis of isozyme polymorphism in Maydeae indicated the prevalence of wider diversity not only at inter-generic level but also at inter- and intra-specific levels. Based on phylogenetic relationships genetic distances between teosinte and maize, three groups have been formed. Teosinte(s) (with large amount of C-heterochromatin) e.g. *Zea diploperennis*, *Z. luxurians* and *Z. mexicana* are distinct from a group and lie at one end. The maize races with low C-heterochromatin e.g. Confite Morocho and Palomero Taluqueno lie at another extreme end. While the races like Pira and Sikkim Primitive with intermediate C-heterochromatin lie in the middle indicating that these races are product of teosinte introgression into maize.

*Scanning electron microscopic studies of pollen grains in Maydeae*: Detailed study of pollen exine surface in various collections of maize, *Coix*, *Chionachne*, *Trilobachne*, teosinte and sorghum have been made through SEM. In these genera pollen grain size was found to vary between 31.25  $\mu\text{m}$  (*Chionachne*) to 124.70  $\mu\text{m}$  (SP maize). The mean pollen grain size in maize was 106.14  $\mu\text{m}$  followed by teosinte (85.11  $\mu\text{m}$ ), *Coix* (55.99  $\mu\text{m}$ ) and *Chionachne* (34.70  $\mu\text{m}$ ).

Enormous variation in distribution pattern of spinules on the pollen surface has been observed in different genera of Maydeae. Pollen surface of the three species of teosinte and maize was similar. The spinules were dense and uniformly distributed. In *Coix* spinules were thinly distributed and followed curved path. In *Chionachne* spinules were sparse and in sorghum they tend to form groups of 8-12. These studies suggest that maize and teosinte are closely related and other genera of Asiatic Maydeae are not related.

*Ethnobotanical studies and the antiquity of maize in India*: Beaded ornamentation of the objects in the hands of deities in the temples of Somnathpur, Halebid and Belur of Karnataka state has aroused considerable interest regarding presence of maize in peninsular India in medieval period. However, through our meticulous studies based on religious, linguistic, sculptural, archaeological, agricultural and botanical records, it is concluded that the beaded objects in the hands of sculptural friezes do not represent maize. The pre-Columbian antiquity of Sikkim Primitive maize of the NEH region has no relevance with the maize like objects in Karnataka temples. There is no evidence of diffusion of NEH maize to peninsular India.

#### *Studies on Distant Hybridization*

*Crossability relationship in maize and its wild relatives*: To overcome the pre- and post-fertilization barriers in crosses between maize and its wild relatives pollen tube growth in vivo has been studied through fluorescence microscopy. It was observed that the pollens of *Chionachne*, *Coix*, *Trilobachne* and *Tripsacum* do germinate on the silks of maize but pollen tubes do not travel up to the ovule. By cutting the silks and pollinating the maize ears at the base on the silk, the seed development up to 15 days has been observed. Crosses between maize x *Chionachne* have shown seed development up to 100% and in maize x *Coix* spp. up to 25%. Therefore, wide crosses are within reach through embryo rescue.

Fluorescence study of pollen tube growth of SP maize on *Coix* has shown that maize pollen tube fertilizes the *Coix* embryo.

**Chiasma frequency and chromosome association in wide crosses:** Observation of chiasma frequency and chromosome configuration in maize x teosinte (*Zea diploperennis*, *Z. luxurians*, *Z. parviglumis*) have shown reciprocal differences in chiasma frequency. In general there was decrease in chiasma frequency in all reciprocal crosses as compared to direct crosses e.g. CM 105 x *Z. diploperennis* chiasma frequency per cell was 19.55 as compared to its reciprocal cross (18.24). The chiasma frequency was reduced significantly per cell in reciprocal crosses (deviation range 1.3 to 2.68)/cell.

Reduction in chiasma frequency (per cell) was observed in all the maize x teosinte crosses. The deviation observed to be maximum in maize x *Zea diploperennis* (-5.28) and minimum in maize x *Zea parviglumis* (-1.49) over the mean parental values. This suggests that *Z. diploperennis* is more primitive than *Z. parviglumis*. Based on the degree of chiasma frequency following relationships have been deduced: *Z. diploperennis* > *Z. luxurians* > *Z. parviglumis* > *Z. mays*.

**Amphiploid theory of maize origin:** The secondary association of bivalents in various maize-teosinte (*Zea diploperennis*, *Z. luxurians*, *Z. parviglumis*) have suggested the amphiploid ( $2n=4x = 2.0$ ) nature of maize origin. The secondary association of 5:5, 6:4 and 3:2:2:2 of bivalents at metaphase-I has been consistently observed.

When stock-6 derived haploid plants were meiotically analyzed, chromosome grouping was very much pronounced at diakinesis and metaphase I. About 75% of the cells showed grouping of 5.5. Five groups of two bivalents were noticed in 15% of the cells. These observations suggest that basic chromosome number in maize is 5.

**Biphyletic origin of maize:** In wide hybridization studies a series of maize-teosinte crosses were made and advanced progenies were generated. Primitive and advanced races of maize were crossed to diploid perennial teosinte (*Zea diploperennis*,  $2n = 20$ ), *Z. luxurians*, *Z. parviglumis* and *Z. mexicana*. Strikingly, segregating population of primitive maize and the most primitive teosinte (*Z. diploperennis*) consistently exhibited the phenomenon of catastrophic sexual transmutation (CST). This CST theory envisages that maize ear is the transformed, feminized central spike of tassel terminating the primary

branches of teosinte. The teosinte alone never transforms into maize. Only the primitive maize x *Z. diploperennis* combination transforms into maize. This suggest the biphyletic origin of maize. One ancestor being *Z. diploperennis* and other being primitive wild corn.

**Reduction in knob number in maize x teosinte hybrids:** Interesting phenomenon of disappearance of knob in maize x teosinte crosses have been noticed. This has adaptive significance of an organism under genomic stress environment.

Material	Terminal knobs	Inter-calary knobs
CM111	1	7
Pira	1	9
<i>Zea diploperennis</i>	6	6
<i>Z. luxurians</i>	10	5
CMIII x <i>Z. dip.</i>	1 (Reduction 83%)	4 (Reduction 43%)
CMIII x <i>Z. lux.</i>	2 (Reduction 80%)	5 (Reduction 29%)
Pira x <i>Z. lux.</i>	3 (Reduction 70%)	8 (Reduction 11%)

The reduction of terminal knob was more than the inter-calary knobs.

**Clonal and Micropropagation in Maize:** Homeotic transformation of male florets into plantlets *in vivo* in maize x teosinte hybrids have been studied. Lemma and palea are transformed into first and second leaf of the plantlet. These plantlets grow *in situ* and develop into complete miniature plants of 20-80 cm in height and produce aerial roots, tassel and seeds. In certain cross combinations direct emergence of plantlets have been observed from almost all the glumes e.g. CMIII x *Zea diploperennis* (98%) and in Palomero toluqueno x *Zea parviglumis* cross 88% of the glumes transformed into plantlets.

When these plantlets are removed from the mother plant and directly transplanted into the field, these plantlets grow like normal maize teosinte  $F_1$  plants. These plants ( $R_1$ ) again show the same phenomenon of *in vivo* regeneration. These observations suggest that explants of maize x teosinte hybrids and their derivatives will be totipotent with high regeneration ability. *In vitro* response of glume explant of these crosses is amazingly very high. The glume cultured at tassel initiation stage developed into whole plant without rooting or shooting media. Glume culture of CMIII x *Zea diploperennis*

in callusing medium (MS + 0.2 mg/l 2-4-D) produced somatic embryos in almost all the cases and regeneration of maize plant in hormone free medium. These studies open up new frontiers for genetic engineering, fixation of heterosis and chromosome manipulation in maize.

*Wide crosses for fodder, baby corn and other useful traits:* Maize x teosinte crosses particularly Sikkim Primitive maize x *Zea diploperennis* and Pira x *Z. diploperennis* have shown great promise for developing fodder maize hybrids (750 q/ha). In coordinated trials, teo-maize has ranked first in Central and North-Eastern zones. Similarly Teo-6 has first rank in North-West and

Central zones. These hybrids are tillering, luxuriant high yielding and free from diseases and have shown antibiosis to shoot borer. Novel trait which doubles the pollen shedding periods has been transferred from *Z. diploperennis* to maize.

Cross derivatives of Sikkim Primitives from Tripura (T-2) and Sikkim (S-2) have proved to be an excellent material for development of baby corn varieties. The baby corn population developed is prolific, early and vigorous. Brace root stocks suitable for waterlogging conditions has been developed through distant hybridization.

## DNA Fingerprinting and Genetic Relationship Study of Tea Plants Using Amplified Fragment Length Polymorphism (AFLP) Technique

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Cultivated tea consists of three species within the genus *Camellia* viz. -*Camellia sinensis* China type *C. assamica* Assam type, *C. assamica lasiocalyx* - Cambod type (Wight, 1962); of these *Camellia sinensis* was the earliest known and used in tea cultivation. Intermediate plant types are available due to extensive cross-breeding between the species.

Till date, selection of tea plants from the wide generation available, for tea making industry is based on phenotypic characters which is time consuming. Heterogeneity in genome composition compounded with environmentally influenced variations in the field makes it difficult for precise characterization of tea plant/bushes on the basis of morphological characters.

In recent years characterization based on DNA sequence information have been used as the ultimate means of plant individualization. An array of methods, based on polymerase chain reaction using a DNA primer and a thermostable DNA polymerase are now available for DNA fingerprinting based documentation of plants (Song *et al.* 2000; William *et al.* 1990; Joshi *et al.* 1999 and Parani *et al.* 1997).

A relatively recent approach in the PCR mediated

amplification of specific DNA fragments for genome analysis is the AFLP study (Vos *et al.* 1995; Maughan *et al.* 1996; Barrett and Kidwell, 1998). This technique permits inspection of polymorphism at a large number of loci within a very short period; reproducibility of the technique is ensured by the use of restriction sites-specific adaptors and adaptor-specific primers with a variable number of selective nucleotides.

We present DNA fingerprinting pattern of some tea clones using AFLP technique. The genetic relationship between the clones is also presented through a cluster analysis study.

Leaves of tea plants from Ging Tea Estate, Darjeeling were used. Genomic DNA from young tea leaves were extracted using modified CTAB method of Gawel and Jarret (1991). AFLP analysis was carried out by using GIBCO-BRL AFLP analysis system-1 kit. Data scored from AFLPs generated with 8 primer pairs were used to compile a binary matrix for cluster analysis using the NTSYS-pc version 1.8 package.

Using 8 primer pair combinations in 29 tea clones, 677 PCR products were observed of which 469 were polymorphic (Table 1), the calculated average number