

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.

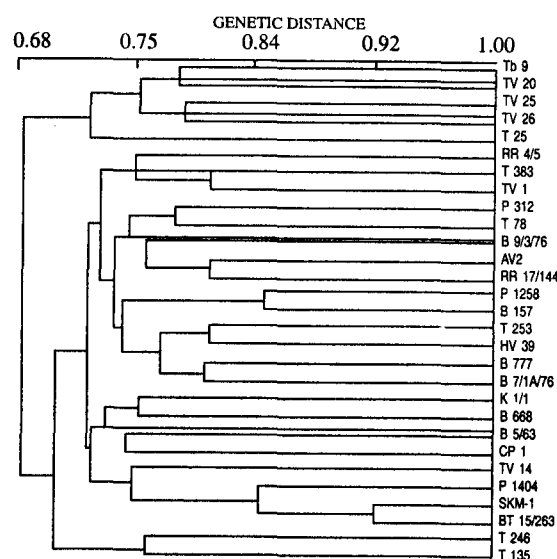
Leaves of tea plants from Ging Tea Estate, Darjeeling were used. Genomic DNA from young tea leaves were extracted using modified CTAB method of Gawel and Jarret (1991). AFLP analysis was carried out by using GIBCO-BRL AFLP analysis system-1 kit. Data scored from AFLPs generated with 8 primer pairs were 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

**Table 1. AFLP generated among 29 tea clones from Darjeeling region**

Primer Pair	Total no. Bands	No. of Polymorphic Bands
E <sub>at</sub> M <sub>ctt</sub>	121	82
E <sub>ta</sub> M <sub>cat</sub>	37	19
E <sub>tg</sub> M <sub>cta</sub>	99	76
E <sub>tc</sub> M <sub>cta</sub>	111	79
E <sub>ag</sub> M <sub>cta</sub>	96	66
E <sub>ac</sub> M <sub>cta</sub>	48	36
E <sub>aa</sub> M <sub>caa</sub>	78	45
E <sub>tg</sub> M <sub>ctg</sub>	87	66
Total	677	469

of polymorphic fragments per primer pair is 58.6. Using the polymorphism data generated from AFLP of 29 tea clones, a similarity matrix was constructed which was used for preparing a dendrogram using UPGMA method. The dendrogram obtained, clearly divided the tea clones into three groups - each group containing plants that are conventionally assigned to China, Assam and Cambod types on the basis of their morphological characters (Fig. 1). It was interesting to note that the clones T246 and T135 conventionally assigned to the Assam group, appeared, from our DNA fingerprinting studies, to be separated from the three conventionally recognized clusters. This suggests that these two clones have arisen through extensive cross breeding between species within the genus *Camellia*. Among all the genotypes studied the similarity level was found to be above 68% Paul *et al.* (1997) reported a similarity level above 70% among tea clones of Indian and Kenyan origin. From studies on lilac, Marsolais *et al.* (1993) have shown that similarity coefficient about 50% is indicative of origin through inter-specific hybridization. Our finding that similarity level among tea genotypes, regardless of morphological grouping, ranged from 69.7% to 92% suggest that the clones represent intra-as well as inter-specific hybridization.

**Fig.1 Dendrogram illustrating genetic relatedness among 29 Tea plants**

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