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Genetic polymorphism of Indian tobacco types as revealed by amplified fragment length polymorphism

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During the past five decades, a large number of tobacco varieties have been developed for different end uses in India through pure line selection from local land-races, mutation breeding and hybridization involving local selections and exotic introductions followed by pedigree selection. No systematic effort has been made to understand the existing diversity pattern in these varieties, which is crucial to define future breeding strategy in this important commercial crop. Amplified fragment length polymorphism (AFLP) analysis was used to determine genetic variation in 54 varieties of cultivated tobacco (*Nicotiana tabacum* and *N. rustica*) and three accessions of exotic germplasm. Nine oligonucleotide primer-pair combinations resolved a total of 967 AFLP fragments, of which 785 (81.2%) were polymorphic. The mean genetic distance among the 49 cultivars and three exotic accessions of *N. tabacum* was 15.35%; 22% among the five cultivars of *rustica*. Genetic polymorphism present among the cultivars of tobacco was low, as evidenced by the high degree of similarity in the AFLP profiles of different tobacco types. All the five cultivars of *N. rustica* can be readily identified using the primer pairs E-ACT/M-CAG and E-AAC/M-CTG. Two major clusters were formed on the basis of species and seven sub-clusters were formed on the basis of manufacturing quality traits in the cultivars of *N. tabacum*. Cultivated flue-cured varieties were clustered separately from other air cured types. Species-specific markers identified in this study would be useful in identification of the true hybrids and monitoring introgression of useful genes from the wild relatives. The markers found specific to the varieties can be used in correct identification of the carrier genotypes in trade and commerce.

Keywords: AFLP markers, flue and air-cured varieties, genetic diversity, Indian tobacco types.

THE genus *Nicotiana* is a member of the family Solanaceae. Out of 64 recognized species[1,2] in the genus *Nicotiana*, two species, namely *tabacum* and *rustica*, which are natural amphidiploids ($2n = 48$), are grown commercially in the world. India is the only country where different

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types of tobacco, viz. Flue-Cured Virginia (FCV), burley, natu, cigar filler, cigar wrapper, cheroot, hookah, bidi and chewing are grown under different agro-climatic conditions. These types are defined to a large extent by the method of curing, intended use in manufacturing, and by some distinct morphological and chemical characteristics. The types of tobacco grown at different locations differ considerably depending on the climatic conditions and nutrient supply. Hence, breeding and selection of tobacco varieties is specific to the intended use and location, right from the choice of parents to the final stages of evaluation and selection.

Prior to 1970, tobacco cultivation in India was confined to a few introductions such as Harrison Special, Chatam, Delcrest and Virginia Gold. During 1970, the emphasis was on pure line selection from local landraces, which led to the development of popular varieties such as CTRI Special (FCV), Hema (FCV) and DP-401 (chewing). During this period, cross-hybridization involving the introductions and local selections was also carried out leading to the release of many high-yielding varieties, including Kanakaprabha, Gauthami and VT1158 (all FCV). After 1980, emphasis shifted towards development of varieties for biotic and abiotic stress tolerance through recombination breeding and induced mutations. This approach resulted in the development of many stress-tolerant varieties, including Jayasri MR (tobacco mosaic virus-resistant), Bhavya (black shank and root-knot nematode resistant) and Lanka Special (powdery mildew-resistant). Varieties suited for different end uses and locations such as Viswanath (natu), Sendarapatty Special (cheroot), GT-6 (bidi) and Meenakshi (chewing) were also developed[3]. In addition, Indian tobacco is considered to be of superior quality since most of the varieties of FCV and burley type contain lower levels of carcinogenic substances like tobacco-specific nitrosoamines (TSNA; 0.42–1.44 ppm) compared to tobacco from other countries[4]. Though a large number of varieties belonging to different commercial types have been developed in India, no information is available on their genetic base.

Molecular genetic markers have become useful tools to provide a relatively unbiased estimation of genetic diversity in plants[5]. PCR-based markers like AFLP have been used as a powerful molecular marker technique for the construction of linkage maps, marker-assisted breeding and molecular typing. AFLP is being used in the analysis of genetic diversity in crop plants because of maximum coverage of the genome with single reaction in a short time. Generally, AFLP produced more polymorphic loci in diversity studies per primer than RFLP, SSR or RAPD[6]. In addition, for AFLP analysis prior knowledge about the genome is not a pre-requisite, which also makes this a favourable method for diversity analysis of different crops like tobacco, where the genome is not much explored. In tobacco, RAPD has been used mainly to identify markers linked to genes for resistance to pathogens[6–8]. Del Piano *et al.*[9] carried out a preliminary analysis of genetic diversity in 12 varieties of *N. tabacum* using three random primers in RAPD. AFLP markers were also employed with a few sets of varieties and species to study inter and intra-specific variations in the genus *Nicotiana*[7]. This communication reports the genetic diversity based on AFLP analysis among 54 Indian tobacco varieties belonging to two cultivated species of *Nicotiana* which are currently being cultivated in different agro-climatic zones of India, and three exotic germplasm accessions which have special characters.

Forty-nine genotypes of *N. tabacum* and five genotypes of *N. rustica* were used in the study. This collection included different types of tobacco defined by their intended manufacturing (viz. cigar filler, cigar wrapper and bidi), method of curing (viz. air-cured and flue-cured) and morphological and biochemical characteristics (natu and chewing) and were used to determine the genetic relationship, inter- and intra-specific variation (Table 1). Three accessions of exotic origin from USA were also included to compare their relationship with the Indian cultivars.

Total genomic DNA was isolated[10] from 30-day-old seedlings, and quantity and concentration of the extracted DNA were estimated on 0.8% agarose gel using diluted uncut lambda DNA as standard. AFLP fingerprints were generated based on the protocol by Vos *et al.*[11] with slight modifications. Genomic DNA (250 ng) was restricted with *Eco*RI and *Mse*I (1.5 U/μl each) in a restriction buffer (50 mM Tris-HCl, pH 7.5, 50 mM magnesium acetate, 250 mM potassium acetate) in a total volume of 25 μl. *Mse*I and *Eco*RI adapters were subsequently ligated to a digested DNA fragment. The adapter-ligated DNA was pre-amplified using the following cyclic parameters: 20 cycles of 30 s at 94°C and 60 s at 72°C. The pre-amplified DNA was diluted in the ratio of 1 : 50 and was used as a template for selective amplification using +3 primers (*Eco*RI and *Mse*I). The *Eco*RI primers were labelled with γ-^{33}P ATP. The cycling parameters were: 1 cycle of 30 s at 94°C, 30 s at 65°C and 60 s at 72°C. The annealing temperature was lowered by 0.7°C per cycle during the first 12 cycles followed by 23 cycles at 94°C for 30 s, 56°C for 30 s and 72°C for 60 s. The samples were resolved on 5% polyacrylamide gel containing 7 M urea, dried and autoradiographed[12]. The size of the fragments was estimated using 20 bp DNA ladder (MBI Fermantas, Lithuania).

Clearly resolved AFLP fragments in the size range 100–700 bp were scored for presence (1) or absence (0). Data on 967 fragments generated by the nine pair-wise combinations of primers were used for analysis of genetic diversity. The NTSYS-pc software[13], version 2.02 was used to calculate Jaccard's[14] similarity coefficients among 57 accessions. Based on UPGMA and SAHN clustering, a dendrogram depicting genetic relationship among the varieties was prepared. Mean similarity of individual varieties with the rest and among varieties within a particular clus-

Table 1. Tobacco cultivars investigated in the study using AFLP

Species	Variety	Parentage	Type	Use	Area of cultivation
Nicotiana tabacum					
1	Kanakaprabha	Delcrest × DB101	FCV	Cigarette	AP
2	Dhanadayi	Delcrest × Bissette	FCV	Cigarette	AP
3	CTRI Spl	Local selection	FCV	Cigarette	AP
4	CTRI Spl MR	TMVRR-2 × CTRI Special	FCV	Cigarette	AP
5	Jayasri MR	TMV44-3 × Jayasri	FCV	Cigarette	AP
6	VT 1158	L617 × CTRI special	FCV	Cigarette	AP
7	Hema	Local selection	FCV	Cigarette	AP
8	CY79	Jayasri × L617	FCV	Cigarette	AP
9	Bhayva	FCV Special × Virgina 145	FCV	Cigarette	KA
10	Ratna	FCV Special mutant	FCV	Cigarette	KA
11	FCV Spl	(H.Spl × V. bright) × (V. Spl × Chatham)	FCV	Cigarette	KA
12	CM 12	Chemical Mutant of McNair 12	FCV	Cigarette	AP
13	Mc Nair 12	Exotic introduction (USA)	FCV	Cigarette	AP
14	Gauthami	L 617 × Delcrest	FCV	Cigarette	AP
15	Godavari Spl	TMVRR1 × Special Cross purified	FCV	Cigarette	AP
16	Swarna	Kuofan × Kanakaprabha	FCV	Cigarette	AP
17	Lanka Spl	DR1 × kuofan	Lanka	Cigar	AP
18	Burley 21	Exotic introduction	Burley	Cigarette	AP
19	BSRB 2	Delcrest (4 ×) *N. Plumbaginifolia* × La Burley 21 × Burley 58	Burley	Cigarette	AP
20	Natu spl	KVT × Toka AKU	Natu	Cigarette	AP
21	Viswanath	I 452 × Karuvazhai	Natu	Cigarette	AP
22	Pyruvithanam	Local selection	Natu	Cigarette	AP
23	Jati chama	Jati local type	Hookah	Hookah	WB
24	Jati podali	Jati local type	Hookah	Hookah	WB
25	Dixie shade	Exotic introduction (USA)	Cigar wrapper	Cigar wrapper	WB
26	S-5	Dixie sh × ade × Rangpur Sumatra	Cigar wrapper	Cigar wrapper	WB
27	GT-4	70-6-6 × Anand 119	Bidi	Bidi	GJ
28	GT-5	GT-4 × 108-9-101	Bidi	Bidi	GJ
29	GT-6	783-51-11-13-61 × Smyrna	Bidi	Bidi	GJ
30	GT-7	930-47 × Anand 2	Bidi	Bidi	GJ
31	NPN-190	(169-119 × Olor) × 121-1	Bidi	Bidi	GJ
32	Anand 2	Sokhadu × 88-47	Bidi	Bidi	GJ
33	Bhavyasree	NPN-190 × PL-5	Bidi	Bidi	KA
34	CGH 1	CMS bidi line	Bidi	Bidi	GJ
35	Sendarapatti special	OK-1 × VV2	Cheroot	Cheroot	TN
36	Olor	Exotic introduction (Malavi)	Cigar filler	Cigar filler	TN
37	Vairam	I 64 × VTK-I	Chewing	Chewing	TN
38	Maragadam	PV-7 × I 115	Chewing	Chewing	TN
39	Thangam	I 64 × PV-7	Chewing	Chewing	TN
40	Bhagyalakshmi	I 64 × I-735	Chewing	Chewing	TN
41	Meenakshi	PV-7 × HV67-9	Chewing	Chewing	TN
42	Abirami	I-64 chemical Mutant	Chewing	Chewing	TN
43	Pusatobacco 76	HP 60-1 × Bori	Chewing	Chewing	BR
44	Prabha	Desila madhata × Hingla kiratpur	Chewing	Chewing	BR
45	Gandakbahar	Local selection	Chewing	Chewing	BR
46	Sona	Borimalingar tohra × DP-401	Chewing	Chewing	BR
47	Vaishali Spl	Bandi × Sona	Chewing	Chewing	BR
48	DP 401	Local selection	Chewing	Chewing	BR
49	Rajkhand	Local selection	Chewing	Chewing	BR
50	Shamboon	Germplasm collection (exotic)	FCV type		USA
51	Sybilla	Germplasm collection (exotic)	FCV type		USA
52	Banana leaf	Germplasm collection (exotic)	FCV type		USA
Nicotiana rustica					
53	GC 1	Local selection	Chewing	Chewing	GJ
54	GC 2	GC 1 × Coker 1	Chewing	Chewing	GJ
55	HD65-40	BVH-5 × Sel 47	Hookah	Hookah	WB
56	DD-437	Selection from Hemti bulk crop	Hookah	Hookah	WB
57	Dharla	C304 × DD437	Hookah	Hookah	WB

AP, Andhra Pradesh; BR, Bihar; KA, Karnataka; GJ, Gujarat; TN, Tamil Nadu; WB, West Bengal.

ter was computed from the similarity matrix table. Polymorphism information content (PIC) was used to identify primers that would distinguish cultivars most efficiently using the formula PIC = $2 \times P_i Q_J$, where P_i is the frequency of presence of band and Q_j is the frequency of absence of band[15]. PIC values for all the polymorphic fragments for a primer or primer pair were averaged to provide PIC value for a primer-pair.

AFLP analysis provided a large number of distinct scorable fragments per primer (Figure 1). All bands that could be reliably read within the size range 100–700 bp were treated as individual dominant loci. A total of 967 bands were obtained from genus *Nicotiana* with nine primers, of which 785 (81.2%) were polymorphic and 182 (18.8%) monomorphic (Table 2). The presence of 18.8% monomorphic bands indicated that these markers have remained conserved in evolution in these species. Sequence analysis of multiple nuclear fragments from such potentially conserved regions of the genome would be useful in establishing phenetic trees and characterizing patterns of divergence. The number of fragments amplified by each primer ranged from 79 to 149, with an average of 107.4 fragments/primer combinations. The earlier reported AFLP analysis of cultivated tobacco accessions detected only 92 bands per primer pair[16], which is less than that obtained in the present study. Similarly, in another study 13 RAPD primers amplified 106 fragments in 12 tobacco lines[9], which accounted for only 8.15 fragments per primer. This is also much less than that obtained in the present study, and clearly indicates the power of the AFLP assay. The higher level of AFLP loci amplified in the present study with Indian tobacco cultivars was due to inclusion of local selections, exotic introductions and improved varieties developed using a wide range of introductions and local landraces over a period of three decades.

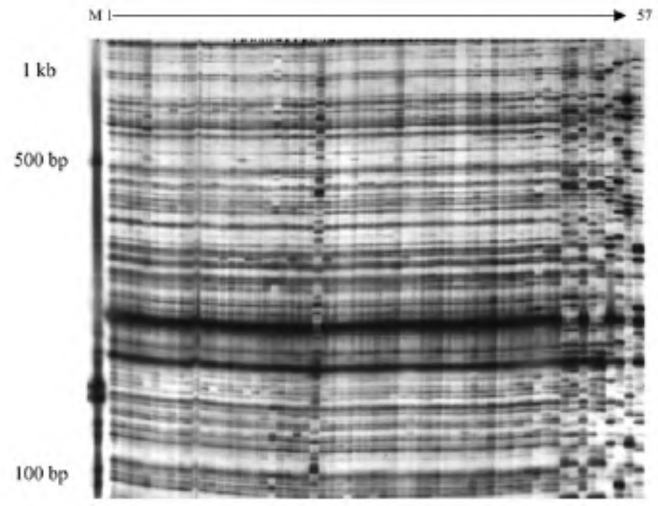

Figure 1. AFLP profile showing genetic polymorphism detected among cultivated accession lines of *Nicotiana tabacum* and *N. rustica* using selective primers combination E-AAG/M-CAA. Numbers 1–57 above each lane correspond to accession lines listed in Table 1.

The maximum number of fragments was amplified by the primer pairs E-AAG/M- CAA (149), E-AAC/M-CTG (130) and E-ACA-M-CAT (104), whereas minimum fragments were amplified by the pair E-ACA/M-CAC (79). In *N. tabacum* cultivars, a total of 758 fragments were amplified, of which 446 (58.8%) were polymorphic and 312 (41.2%) monomorphic. In cultivars of *N. rustica*, 675 bands were amplified, of which 320 (47.4%) were polymorphic and 355 (52.6%) monomorphic. Maximum fragments were amplified by the primer combination E-AAG/M-CAA (119) while minimum fragments were amplified by E-ACA/M-CAC (62) in *N. tabacum*, whereas in *N. rustica*, maximum (95) and minimum (54) fragments were amplified by the primer combinations E-AAG/M-CAA and E-ACT/M-CAG respectively. All the primer pairs showed high PIC values (0.72–0.87; Table 2), which is a measure to know the usefulness of the primer for diversity analysis. The primer combination E-AAC/M-CAC gave the highest PIC across all cultivars, whereas E-AAC/M-CTT and E-ACG/M-CAG gave the lowest score. Primer pairs with high PIC values identified in this study would be of use in rapid evaluation of diversity in the germplasm of tobacco.

In cultivars of *N. tabacum*, the average of pair-wise similarities was 0.73–0.97, whereas it was 0.63–0.94 in cultivars of *N. rustica*, suggesting that the use of introductions and local selections has not been effective in significant broadening of the base of the Indian commercial cultivars, although diversity level is higher than that in *N. rustica* and *N. tabacum* cultivars studied earlier[9]. This narrow genetic variation may also be attributed to the preferential use of some varieties as parents in the breeding programme, because of prepotency of these varieties to produce desirable qualitative traits and their adaptability to different agro-climatic conditions. Compared with the measured levels of polymorphism among the varieties in other plant species[17,18], the degree of polymorphism recognized among the varieties of cultivated tobacco was relatively low, suggesting that the large differences observed in 'manufacturing quality traits' may result from a relatively small number of genetic differences among the cultivars. All cultivars of both the species shared 123 common bands, showing evolutionary conservation in the species of *Nicotiana*.

Cluster analysis performed with Jaccards' similarity matrix corresponded well with the traditional classification. Though minor changes were evident in the sub-clusters of *N. tabacum* and *N. rustica*, the relative positions of the major clusters were preserved. All the cultivars of *Nicotiana* were separated into two main clusters (A and B) corresponding to two cultivated species (Figure 2). All varieties and three exotic accessions of *N. tabacum* were grouped into a major cluster A with seven sub-clusters.

Table 2. Analysis of polymorphism, polymorphism information content (PIC) values, and identification of unique markers in tobacco cultivars as revealed by AFLP

	N. tabacum + *N. rustica*		*N. tabacum*		*N. rustica*				
Primer pair	Amplified bands	Polymorphic bands	Amplified bands	Polymorphic bands	Amplified bands	Polymorphic bands	Common bands in both species	Unique bands per primer	PIC
E-ACT/M-CCT	83	63 (75.9)	68	38 (55.8)	59	27 (45.7)	15	7	0.84
E-ACA/M-CAC	79	60 (75.9)	62	49 (79)	60	20 (33.3)	15	4	0.87
E-ACT/M-CAG	82	65 (79.2)	70	38 (54.2)	54	25 46.2)	10	6	0.82
E-AAC/M-CTT	95	80 (84.2)	77	52 (67.5)	89	64 (71.9)	7	23	0.72
E-ACG/M-CAG	96	83 (86.4)	80	61 (76.25)	68	44 (64.7)	7	22	0.72
E-AAC/M-CTG	130	114 (87.69)	92	43 (46.7)	94	41 (43.6)	12	16	0.79
E-AAG/M-CAA	149	101 (67.7)	119	47 (39)	95	31 (32.6)	39	6	0.73
E-ACA/M-CAT	104	92 (88.4)	81	49 (60.4)	70	32 (45.7)	7	11	0.80
E-AAG/M-CAC	149	127 (85.2)	109	69 (63.3)	86	36 (41.8)	11	5	0.77

Values in parenthesis represent % polymorphism.

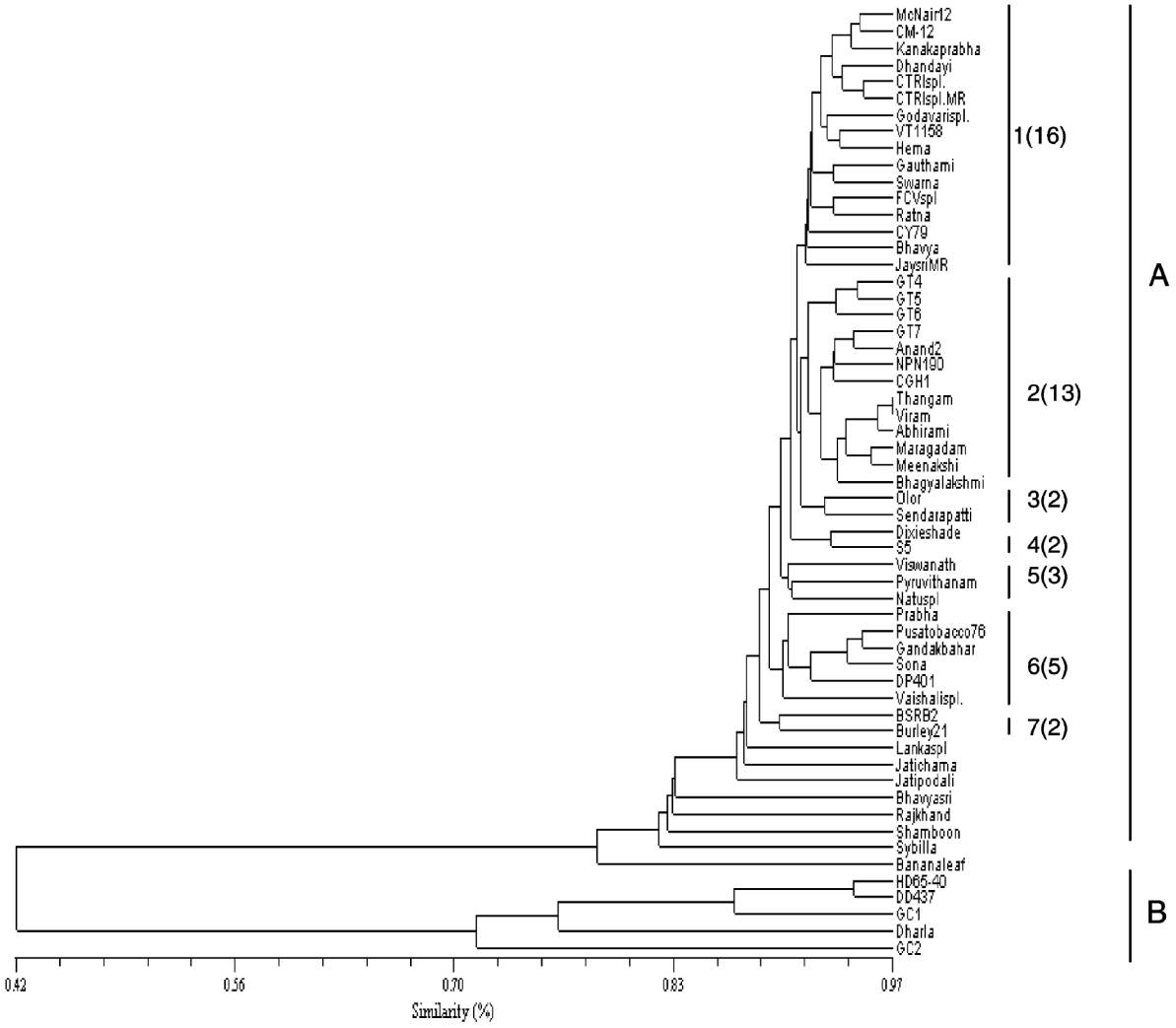

Figure 2. Dendrogram representing genetic relationship among cultivars of tobacco by AFLP.

Sub-grouping of the varieties within the main cluster was formed mostly based on manufacturing trait and parentage. Sixteen varieties of FCV, which were used in cigarette manufacture, remained together in sub-cluster 1. Almost all the varieties of bidi type (except the variety Bhavyasree) grouped together in sub-cluster 2. The non-grouping of Bhavyasree with other bidi varieties may be due to its different parentage and cultivation in a different

agro-climatic zone in Karnataka. All the varieties of chewing tobacco grown in Tamil Nadu were grouped together in sub-cluster 2, and maximum varieties of chewing tobacco (five out of six) varieties grown in Bihar were grouped together in sub-cluster 6. The only variety of cigar filler (Olar) and cheroot (Sendarapatti Special) type of tobacco were grouped together in the third sub-cluster, whereas varieties of cigar wrapper grown in West Bengal were grouped in sub-cluster 4. All the varieties of natu and burley type of tobacco were grouped into the fifth and seventh sub-cluster respectively. The variety Jatichama (hookah) and chewing varieties (Jatipodali) were not included in any of the clusters. The three exotic germplasm lines did not form one cluster and they merged at higher fusion level, independently. The low-nicotine germplasm lines were positioned side by side and the accession Banana leaf was at the end of the main cluster of *N. tabacum*.

The parentage of the varieties also contributed to the grouping pattern in both species. For instance, in sub-cluster 1, the variety CTRI Special MR, which was a derivative of CTRI Special; Mc Nair 12 and its chemical mutant CM12 and variety FCV Special and its derivatives Ratna and Bhavya were grouped together. Varieties Gauthami and VT1158 had a similar parental line, i.e. L617 as one of the parents and were grouped together. Varieties CY 79, Jayasri MR, CTRI Special, CTRI Special MR, Godavari Special and VT 1158 have an ancestral background of the variety FCV Special. Another set of varieties, viz. Gauthami, Kanakaprabha, Dhanadayi and Jayasri MR were developed in the background of the variety Delcrest. For incorporation of TMV resistance, the line TMVRR1 was used in the development of the variety Godavari Special, TMVRR2 used for development of CTRI Special MR and TMVV44-3 (derivative of TMVRR1) was used in the development of Jayasri MR. Thus among many of the FCV tobacco varieties, a genetic relationship existed and these were clustered together. Clustering of varieties based on closer parentage was also observed in the bidi varieties (GT-5 and its parent GT-4; GT-7 and its parent Anand-2), chewing varieties of Tamil Nadu (Thangam, Viram, Bhagyalakshmi and Abirami, which have a common parent, line I-64), chewing varieties of Bihar (DP-401 was one of the parents of Sona, and Sona was one of the parents of Vaishali Special), cigar-wrapper varieties (S-5 and its parent, Dixie shade) and burley varieties (Burley 21 and its derivative, BSRB-2).

Cluster B was included in all the cultivars of *N. rustica*. The genetic distance among them was 0.28. In this cluster, varieties of different manufacturing traits GC1, GC2 (chewing types), HD65-40, DD437 and Dharla (hookah types) cultivated in different agro-climatic regions were grouped together based on species specificity. The parentage of the cultivars has also contributed towards the clustering pattern. Variety DD437 was one of the parents of Dharla, and GC1 was one of the parents of GC2, and these were grouped in the same cluster.

The three exotic germplasm accessions, Shamboon (low-nicotine type), Sybilla and Banana leaf (broadleaf-type of tobacco) were included in the present study to find out the relationship between these accessions and the cultivated tobacco varieties. However, AFLP profiles showed that they were distinctly related to the cultivated varieties.

Unambiguous identification of inter-specific/generic hybrids is an important prerequisite for the success of introgression breeding in tobacco. It would therefore be essential to monitor introgression of target genes/chromosome segments from the parental species/genus. Use of species and genus-specific markers could greatly facilitate an objective genetic variation of derived progeny and thus help in the monitoring of introgression programmes.

Interestingly, this study revealed a number of markers that are specific to species of *N. tabacum* and *N. rustica*. A total of 100 species-specific markers were identified, of which 34 were specific to *N. tabacum* and 66 specific to *N. rustica*. The highest number of species-specific markers was revealed by the primer combination E-AAC/M-CTT (23), followed by the combination E-AGC/M-CAG (22). The combination E-AAC/M-CTT generated eight fragments specific for *N. tabacum* and 15 for *N. rustica* which would have a large influence on their separation in the evolutionary tree. The highest number of species-specific markers was identified in *N. rustica*, reflecting the presence of unique regions in the genomic structure of this species. The species-specific AFLP markers identified in this study would be useful for monitoring introgression programmes, identifying unknown accessions and classifying germplasm collections.

In the previous reports, varieties with different manufacturing quality traits have been analysed and the measured extent of polymorphism was relatively small with RAPD[9]. Also, the clustering of cultivars was not based on the manufacturing traits[16]. Moreover, tobacco being a genetically large amphidiploid genome ($2n = 48$) requires the use of AFLP for wider genome coverage, which provides a more realistic measure of genetic diversity.

Evolutionary relationships in the genus *Nicotiana* have been analysed and a significant amount of morphological and cytological information is available. However, little information is available on the extent of genetic variation within commercially cultivated tobacco of *N. tabacum* and *N. rustica*. In this study, we used AFLP analysis to gain insight into the degree of intra- and inter-specific variation in the cultivars of *N. tabacum* and *N. rustica*. The results indicated that with appropriately defined specific combination of selective primers, it is possible to yield a specific number of polymorphic fragments to allow meaningful comparison among cultivated tobacco accessions.

In conclusion, AFLP analysis using nine primer-pair combinations showed sufficient resolution to distinguish among the closely related cultivars and allowed distinct sub-groups with similar manufacturing-quality traits to be recognized. AFLP was also found efficient in determin-

ing the extent of intra- and inter-specific genetic diversity existing in *N. tabacum* and *N. rustica*. The species and genus-specific AFLP markers identified in this study would be useful in introgression breeding programmes of tobacco.

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Differential accumulation of manganese in three mature tree species (Holoptelia, Cassia, Neem) growing on a mine dump

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Three trees, including *Cassia siamea* (Cassia), *Azadirachta indica* (Neem), *Holoptelia integrifolia* (Holoptelia) belonging to three different families were identified from a manganese mine tailing dump. Manganese content in dump soil and in the stem, green leaves and dry, fallen leaves of the plants was determined. Values were compared with similar samples collected from normal vegetation. Under control condition, manganese content was highest in Cassia. Distribution of metal in samples collected from the dump site revealed that Holoptelia has a special ability to accumulate high amounts of manganese under stress condition followed by Cassia and Neem. There is no literature on metal accumulation in Holoptelia. Mechanism of manganese sequestration in Holoptelia is different from the other two trees growing in the same soil.

Keywords: *Azadirachta indica*, *Cassia siamea*, *Holoptelia integrifolia*, hyperaccumulator, manganese, mine dump.

MANGANESE (Mn) is a trace element found in varying amounts in all tissues and is among the mostly used elements in the industry. It is an essential micronutrient and activator for enzymes involved in tricarboxylic acid cycle. However, Mn is toxic when in excess and consequently it represents an important factor in environment contamination and causes various phytotoxic effects[1].

Phytoremediation is an environmental clean-up strategy in which selected green plants are employed to remove, contain or render environmentally toxic contaminants harmless. This is an emerging biotechnological application and operates on the principles of biogeochemical cycling[2]. This remediation approach is attracting attention from various governments as a cost-effective and environment-friendly green technique to clean-up heavy metal polluted soil using hyperaccumulators[3]. The generation of scientific information on heavy metal-accumulating plants is so extensive that in the last decade a commercial industry has been developed for the application of phytoextraction to restore heavy metal-contaminated sites[4].

Most experimental studies of heavy metal tolerance confirm that populations growing in metal-contaminated

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