in *R. indica* protoplast culture. Maintenance of high osmotic condition (0.6 M glucose) up to the second week of culture is necessary for the stability of the protoplasts. Gradual lowering of the osmoticum by lowering of glucose, increasing sucrose, and a gradual lowering of auxin (both 2,4-D and NAA in the RA_2 medium and finally elimination of 2,4-D in RA_3, RA_4 and LR1 media) are important for high plating efficiency of protoplasts and high-frequency of plant regeneration from calli of protoplast origin in *R. indica*.

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Molecular diversity in *Phyllanthus amarus* assessed through RAPD analysis

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A collection of *Phyllanthus amarus* was made from various parts of India to determine the extent of genetic variability using analysis at DNA level. RAPD profiling of 33 collections from different locations, covering states of Tamil Nadu, Karnataka, Maharashtra, Gujarat, Assam, West Bengal, Tripura, Uttar Pradesh, Punjab and Haryana was generated. Analysis through UPGMA revealed up to 65% variation among these accessions. However, intra-population variation was found to be much larger in the accession from the southern part of the country. Nevertheless, inter-population variation also overlaps in the phylogenetic clustering, which is understandable from the natural dissemination of this plant species as a weed that has spread across the geographical boundaries. The study indicates the random hybridization across the populations falling within the range of possible cross-pollination in terms of physical distance. A study of these accessions at a single location, allowing free mating, would throw light on the extent of cross-pollination and genetic flow.

THE genus *Phyllanthus* L. of the family Euphorbiaceae consists of about 800 species, of which 200 are American, 100 African, 70 from Madagascar and the remaining Asian and Australasian[1]. *Phyllanthus amarus* is an important medicinal plant species due to its antiviral properties, useful against hepatitis infection. The species is also used in stomach ailments like dyspepsia, colic, diarrhoea, dysentery, dropsy, urinogenital problems and also as external application for oedematous swelling and inflammation. This is an important ingredient in many ayurvedic preparations, especially for the treatment of jaundice. The taxonomic revision on this genus by Webster included closely-related genera *P. amarus,* under the sub-section *Swartiziani* of the section *Phyllanthus*. The nomenclature, taxonomic distinctness and close relatives of *P. amarus* were addressed in detail based on morphology and geographical distribution[2–4]. It is said to be related to *P. abnormis*, which is endemic to sandy areas in Texas and Florida of southern USA[2]. It is therefore most likely that *P. amarus* originated in the Caribbean area as a vicarious species of *P. abnormis* of the southern United

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States and has spread around the tropics by trading vessels[2].

This species is distributed all over India and is considered as the most widely occurring species of *Phyllanthus* in India[4]. The presence of dioceous cymules[3] at the end of the branches is considered to be a unique character, though it resembles in many respects its close relatives, *P. debilis* and *P. fraternus* of the same sub-section Swartziani[1]. This is the only sub-section in the section *Phyllanthus*, which consists of most widespread herbaceous species throughout the tropics. Considering widespread distribution and marked morphological diversity, including growth habit of plants within species, it is important to analyse the genetic diversity using molecular tools.

PCR-based markers have been used extensively for assessing genetic variation within the species to measure the genetic diversity[5]. So far, genetic diversity analysis in this genus has been carried out regionally for the species *P. emblica*[6]. In the present analysis RAPD markers were used to assess the genetic diversity within *P. amarus* species collected from different geographical locations of India.

Table 1. *P. amarus* collection from different parts of the country

Accession no.	Location	State
CIMAP/PAC	Lucknow	Uttar Pradesh
CIMAP/PA2	Tinisular, Chennai	Tamil Nadu
CIMAP/PA5	Mundiyanakau, Arcot	Tamil Nadu
CIMAP/PA6	Ullundurpet, Arcot	Tamil Nadu
CIMAP/PA11	Adivaran, Salem	Tamil Nadu
CIMAP/PA21	Thirumangalam, Madurai	Tamil Nadu
CIMAP/PA22	Kayathar, Tirunelveli	Tamil Nadu
CIMAP/PA23	Vallioor, Tirunelveli	Tamil Nadu
CIMAP/PA25	Kanyakumari	Tamil Nadu
CIMAP/PA26	Palayamkottai	Tamil Nadu
CIMAP/PA28	Attyapuram, Tuticorin	Tamil Nadu
CIMAP/PA30	Paramakudi	Tamil Nadu
CIMAP/PA37	Pudukkottai	Tamil Nadu
CIMAP/PA39	Kumbakonam	Tamil Nadu
CIMAP/PA53	Sepahijala Wildlife Sanctuary	Tripura
CIMAP/PA54	Barjalu, Jirania	Assam
CIMAP/PA74	Momealli	Assam
CIMAP/PA76	GKVK, Bangalore	Karnataka
CIMAP/PA77	Sanakana	Karnataka
CIMAP/PA78	CFTRI Campus, Mysore	Karnataka
CIMAP/PA80	Kusal Nagar	Karnataka
CIMAP/PA84	Harnahatti	Karnataka
CIMAP/PA98	Dhaiadam	Karnataka
CIMAP/PA111	Gurgaon	Haryana
CIMAP/PA112	Ferozpur Jhinka, Gurgaon	Haryana
CIMAP/A117	Karnal	Haryana
CIMAP/PA120	Panipat	Punjab
CIMAP/PA134	Kalmegha	West Bengal
CIMAP/PA135	Rishra	West Bengal
CIMAP/PA136	Bithur	Uttar Pradesh
CIMAP/PA140	Shakti Nagar	Karnataka
CIMAP/PA144	Agra	Uttar Pradesh
CIMAP/PA145	Aghar	Gujarat

Extensive field trips were carried out to collect *P. amarus* from different geographical locations in India. Each accession consisted of 20 plants within a radius of 5 m, and seeds from same locality. The pooled seeds were stored and maintained in the seed gene bank of CIMAP, Lucknow. Out of the total germplasm collections of *P. amarus*, 33 random accessions were taken for analysis (Table 1). DNA was isolated from the leaf tissue, essentially according to the protocol described earlier[7], from 20 plants per accession and pooled. PCRs were carried out in 25 µl volume. A reaction tube contained 25 ng DNA, 0.2 unit Taq DNA polymerase, 100 mM of each dNTP, 1.5 mM $MgCl_2$ and 5 pmol decanucleotide primers. The amplifications were carried out using the DNA Engine thermal cycler (MJ Research, USA) using 94, 35 and 72°C temperatures for 40 cycles[8]. The amplified products were separated on 1.2% agarose gel containing 0.5 µg ml^{-1} of ethidium bromide and photographed with Image master VDS (Pharmacia). The bands were analysed using Image master 1D elite software, and the graphic phenogram of the genetic relatedness among the accessions was produced by means of UPGMA (unweighted pair group method with arithmetic average) cluster analysis. Custom-made decanucleotide primers were synthesized in the laboratory on Applied Biosystems 392 DNA–RNA Synthesizer and were designated as MAP01 to MAP20. The sequences of the primers MAP01 to MAP20 were AAATCGGAGC, GTCCTACTCG, GTCCTTAGCG, TGCGCGATCG, AACGTACGCG, GCACGCCGGA, CACCCTGCGC, CTATCGCCGC, CGGGATCCGC, GCGAATTCCG, CCCTGCAGGC, CCAAGCTTGC, GTGCAATGAG, AGGATACGTG, AAGATAGCGG, GGATCTGAAC, TTGTCTCAGG, CATCCCGAAC, GGACTCCACG, AGCCTGACGC, respectively.

Pairwise comparisons for the presence and absence of bands in the RAPD profiles (Figure 1) were made to calculate similarity indices using the method of Nei and Li[9]. The average of similarity matrices was used to generate a tree by UPGMA.

The similarity indices as given in Table 2, clearly show that *P. amarus* accessions from the country show a variation up to 65%. Also, there are collections as close as 98% in terms of similarity index, even though they may be belonging to geographically distinct locations. This situation can arise in natural populations when there is a possibility of free/random pollen flow and fertilization, as is the case in most cross-pollinated species. On the other hand, further amplification of such cross-hybridized seeds through dissemination by natural modes like wind is possible. This is probably the reason that accessions like PA21 and PA117 appeared closely related at the genetic level, although geographically they are from distinct zones of highly distinct locations of the country (Tamil Nadu and Haryana).

The subclusters in the dendrogram (Figure 2) could be to some extent correlated to the geographical distribution

Table 2. Similarity index of *Phyllanthus amarus* accessions using RAPD

	5	25	26	28	37	39	53	80	120	140	145	C	2	6	11	21	22	23	30	54	74	76	77	78	84	98	111	112	117	134	135	136	144
PA5	1.00																																
PA25	0.63	1.00																															
PA26	0.75	0.72	1.00																														
PA28	0.80	0.65	0.91	1.00																													
PA37	0.66	0.59	0.83	0.76	1.00																												
PA39	0.84	0.57	0.80	0.90	0.73	1.00																											
PA53	0.65	0.66	0.57	0.67	0.43	0.69	1.00																										
PA80	0.76	0.86	0.79	0.77	0.69	0.66	0.64	1.00																									
PA120	0.72	0.71	0.86	0.88	0.75	0.77	0.55	0.85	1.00																								
PA140	0.76	0.67	0.90	0.92	0.79	0.81	0.58	0.81	0.96	1.00																							
PA145	0.58	0.70	0.61	0.55	0.43	0.48	0.72	0.59	0.46	0.51	1.00																						
PAC	0.59	0.51	0.76	0.69	0.93	0.66	0.43	0.61	0.68	0.73	0.50	1.00																					
PA2	0.80	0.83	0.73	0.76	0.56	0.70	0.77	0.79	0.70	0.74	0.78	0.49	1.00																				
PA6	0.40	0.43	0.76	0.68	0.80	0.65	0.46	0.54	0.62	0.66	0.47	0.74	0.48	1.00																			
PA11	0.67	0.44	0.75	0.77	0.73	0.73	0.55	0.61	0.72	0.76	0.46	0.68	0.57	0.91	1.00																		
PA21	0.65	0.68	0.58	0.61	0.41	0.55	0.73	0.64	0.55	0.59	0.75	0.36	0.85	0.54	0.63	1.00																	
PA22	0.67	0.68	0.60	0.63	0.43	0.57	0.75	0.66	0.57	0.61	0.76	0.38	0.87	0.56	0.65	0.97	1.00																
PA23	0.72	0.52	0.78	0.80	0.63	0.72	0.60	0.64	0.74	0.79	0.59	0.58	0.70	0.81	0.90	0.76	0.78	1.00															
PA30	0.67	0.51	0.75	0.78	0.61	0.76	0.63	0.61	0.72	0.76	0.52	0.59	0.62	0.79	0.88	0.76	0.70	0.91	1.00														
PA54	0.65	0.72	0.58	0.61	0.41	0.55	0.81	0.67	0.55	0.59	0.83	0.41	0.88	0.44	0.53	0.89	0.86	0.66	0.59	1.00													
PA74	0.67	0.54	0.82	0.83	0.67	0.72	0.60	0.68	0.79	0.83	0.39	0.69	0.65	0.84	0.93	0.71	0.73	0.96	0.93	0.61	1.00												
PA76	0.64	0.44	0.69	0.72	0.55	0.64	0.52	0.56	0.71	0.75	0.52	0.45	0.62	0.72	0.82	0.73	0.75	0.92	0.83	0.59	0.88	1.00											
PA77	0.73	0.64	0.81	0.83	0.59	0.73	0.63	0.64	0.78	0.82	0.58	0.53	0.70	0.61	0.70	0.65	0.67	0.80	0.78	0.65	0.83	0.72	1.00										
PA78	0.67	0.51	0.75	0.84	0.61	0.76	0.63	0.61	0.72	0.76	0.52	0.56	0.62	0.79	0.88	0.69	0.70	0.71	0.95	0.59	0.93	0.77	0.78	1.00									
PA84	0.62	0.62	0.80	0.72	0.57	0.62	0.56	0.59	0.66	0.70	0.61	0.51	0.62	0.76	0.75	0.68	0.70	0.65	0.83	0.58	0.88	0.76	0.85	0.83	1.00								
PA98	0.66	0.51	0.72	0.75	0.62	0.66	0.54	0.62	0.73	0.77	0.53	0.57	0.65	0.70	0.79	0.75	0.77	0.89	0.80	0.61	0.85	0.87	0.75	0.55	0.73	1.00							
PA111	0.73	0.72	0.57	0.63	0.53	0.68	0.66	0.68	0.59	0.63	0.64	0.48	0.86	0.40	0.51	0.77	0.79	0.57	0.51	0.75	0.51	0.55	0.53	0.51	0.45	0.64	1.00						
PA112	0.64	0.59	0.50	0.55	0.42	0.59	0.67	0.56	0.47	0.49	0.66	0.37	0.76	0.55	0.63	0.87	0.89	0.68	0.63	0.76	0.63	0.74	0.55	0.63	0.58	0.66	0.85	1.00					
PA117	0.63	0.67	0.56	0.59	0.40	0.53	0.71	0.62	0.53	0.58	0.73	0.35	0.83	0.53	0.61	0.98	0.95	0.74	0.67	0.87	0.69	0.72	0.63	0.67	0.66	0.77	0.79	0.89	1.00				
PA134	0.63	0.66	0.48	0.54	0.45	0.59	0.70	0.63	0.51	0.54	0.67	0.39	0.79	0.41	0.52	0.80	0.82	0.58	0.52	0.75	0.52	0.55	0.54	0.52	0.46	0.61	0.88	0.89	0.78	1.00			
PA135	0.63	0.69	0.55	0.60	0.45	0.59	0.71	0.67	0.57	0.61	0.69	0.39	0.82	0.51	0.62	0.92	0.94	0.74	0.67	0.88	0.69	0.71	0.62	0.67	0.64	0.77	0.84	0.91	0.90	0.88	1.00		
PA136	0.66	0.69	0.51	0.57	0.48	0.62	0.72	0.66	0.53	0.57	0.69	0.41	0.82	0.43	0.54	0.77	0.79	0.60	0.54	0.84	0.54	0.52	0.57	0.54	0.48	0.58	0.85	0.86	0.75	0.97	0.85	1.00	
PA144	0.62	0.68	0.53	0.58	0.43	0.57	0.69	0.43	0.55	0.59	0.68	0.38	0.80	0.50	0.61	0.90	0.93	0.72	0.66	0.79	0.67	0.60	0.61	0.66	0.63	0.79	0.87	0.93	0.93	0.86	0.97	0.83	1.00

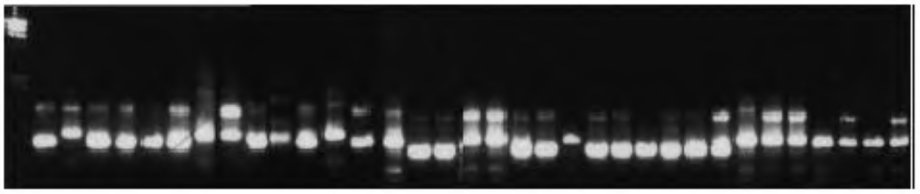

Figure 1. Representative RAPD profile of *Phyllanthus amarus* accessions (legend of the lane according to Table 1; marker λDNA *Hin*dIII digest) with one of the primers (5′ AACGTACGCG 3′).

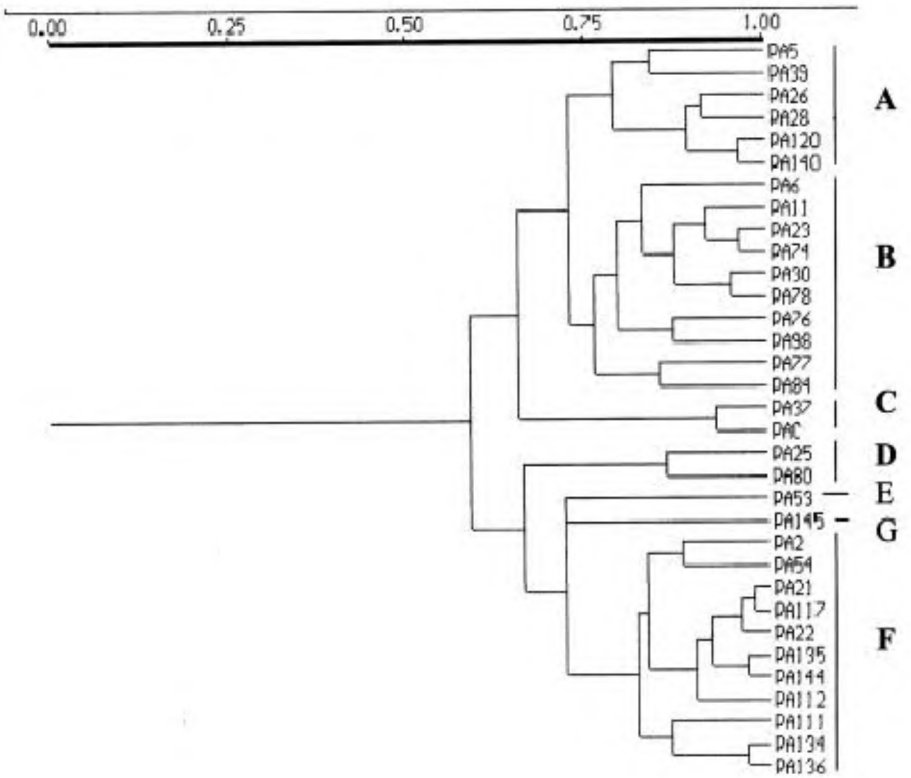

Figure 2. Dendrogram showing diversity of *Phyllanthus amarus* accessions.

(Table 3). Group A of the first major cluster represents all the accessions from southern part of the country (Tamil Nadu and Karnataka), except one accession which is a collection from Punjab. In this subcluster, the southern representation is mostly from the coastal belt. In the other subcluster (B), all the accessions are from the southern part of the country, representing the Deccan Plateau. Interestingly, the accessions collected from Tamil Nadu are distributed in all the subclusters, indicating the differences in genotypes with wide habitat diversity. However, the accessions of *P. amarus* also show different habit forms such as procumbent or woody herbs, branches with unisexual cymules or bisexual cymules, which created confusion among some researchers about the taxonomy of *P. amarus*[10] and its close relatives. Similarly, varied habit forms with high degree of genetic similarity (92%) were observed in this study, as in the case of CIMAP/PA140 (procumbent) and CIMAP/PA28 (woody). High genetic similarity is expected among *P. amarus* accessions in the southern part of country, due to the same geographical location. But, in contrast, they showed broad genetic base indicating earlier introduction of this species to coastal plain, and subsequently leading to accumulation of variation.

The association between the accessions CIMAP/PAC (CIMAP farm Lucknow) and CIMAP/PA37 (Pudukottai, Tamil Nadu) in subcluster C, sharing 92% RAPD markers, is indicative of escapes from selected earlier collections at CIMAP, similar to that of CIMAP/PA37. The other major cluster (F) represents a total of 11 accessions from mixed geographical locations. *P. amarus* is considered the most widespread species, with high recruitment ratio and dispersal. Moreover, this species as a major weed in the crop fields, has also the possibility of spreading and respreading to different geographical regions as contaminant in the seeds. The accessions from Gujarat

Table 3. Representation of accessions in different clusters

Cluster	Accession	Location	State
A	CIMAP/PA5	Mundiyanakau, Arcot	Tamil Nadu
	CIMAP/PA39	Kumbakonam	Tamil Nadu
	CIMAP/PA26	Palayamkottai	Tamil Nadu
	CIMAP/PA28	Attyapuram, Tuticorin	Tamil Nadu
	CIMAP/PA120	Panipat	Punjab
	CIMAP/PA140	Shakti Nagar	Karnataka
B	CIMAP/PA6	Ullundurpet, Arcot	Tamil Nadu
	CIMAP/PA11	Adivaran, Salem	Tamil Nadu
	CIMAP/PA23	Vallioor, Tirunelveli	Tamil Nadu
	CIMAP/PA74	Momealli	Assam
	CIMAP/PA30	Paramakudi	Tamil Nadu
	CIMAP/PA78	CFTRI Campus, Mysore	Karnataka
	CIMAP/PA76	GKVK, Bangalore	Karnataka
	CIMAP/PA98	Dhaiadam	Karnataka
	CIMAP/PA77	Sanakana	Karnataka
	CIMAP/PA84	Harnahatti	Karnataka
C	CIMAP/PA37	Pudukkottai	Tamil Nadu
	CIMAP/PAC	Lucknow	Uttar Pradesh
D	CIMAP/PA25	Kanyakumari	Tamil Nadu
	CIMAP/PA80	Kusal Nagar	Karnataka
E	CIMAP/PA53	Sepahijala Wildlife Sanctuary	Tripura
F	CIMAP/PA2	Tinisular, Chennai	Tamil Nadu
	CIMAP/PA54	Barjalu, Jirania	Assam
	CIMAP/PA21	Thirumangalam, Madurai	Tamil Nadu
	CIMAP/PA117	Karnal	Haryana
	CIMAP/PA 22	Kayathar, Tirunelveli	Tamil Nadu
	CIMAP/PA135	Rishra	West Bengal
	CIMAP/PA144	Agra	Uttar Pradesh
	CIMAP/PA112	Ferozpur Jhinka, Gurgaon	Maharashtra
	CIMAP/PA111	Gurgaon	Maharashtra
	CIMAP/PA134	Kalmegha	West Bengal
	CIMAP/PA136	Bithur	Uttar Pradesh
G	CIMAP/PA145	Aghar	Gujarat

(west India) and Tripura (northeast India) outgrouped from the rest, forming distinct clusters (E and G) according to expectation, as they are from two entirely different geographical locations. Though *P. amarus* has distinct characters to substantiate the species status and constant chromosome number[11], the RAPD profiles display vast genetic variation indicative of the evolving nature of the taxa.

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Pattern of species succession of soft-bottom macrofauna in the estuaries of Goa, west coast of India

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Multivariate techniques, chord normalized expected species shared (CNESS) and principal component analyses of hypergeometric probability of species matrices (PCA-H) were applied to soft-bottom macrofauna data of Goa estuaries, west coast of India, to assess the pattern of species succession at different sites. These analyses revealed three groups of species that produced three-stages or triangular species succession pattern, corresponding to the three seasons, namely post-, pre- and southwest monsoon. Each site exhibited a different pattern of species succession and compostion. A total of 58 species were recorded among which 18 were new to the local fauna. Dominant species that controlled the orientation of this succession were Polychaetes (*Prioniospio pinnata*, *Clymene annandalei*, *Nereis capensis*), Bivalves (*Meretrix casta*, *Cardium flavum*), Amphipoda (*Urothoe platydactyla*), Echiurida (*Thalassema* sp.) and Nema-

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