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Biochemical variation in turmeric (*Curcuma longa* Linn.) accessions based on isozyme polymorphism

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SUMMARY
Fifteen accessions of *Curcuma longa* L. collected from different geographical areas in India, along with a few seedling progenies, were studied for variation based on isozyme polymorphism. A high degree of variability (63.8–96% similarity) was seen in the population studied. Phenetic analyses revealed several groups with interesting features. Two seedling progenies, which showed maximum similarity, stood distinctly from the clonally propagated material. Other pairs of accessions showing high similarity (above 90%), were from the same geographical area, indicating that accessions collected based on vernacular names and those collected from adjoining areas, need not be genetically distinct.

Turmeric (*Curcuma longa* syn. *C. domestica*, Zingiberaceae) is an ancient and sacred rhizomatous spice of India which is considered to have originated in South-East Asia. At present India is the major producer and exporter of turmeric in the world. Although more than 50 cultivars of turmeric are known in the country, it is difficult to discriminate these varieties solely on rhizome morphology. The Indian Institute of Spices Research (IISR), Calicut, has a mandate for collection and conservation of turmeric germplasm. At present, the National Repository of Turmeric Germplasm at IISR maintains more than 700 accessions of turmeric, including land races, improved varieties, open pollinated progenies (OP), related species and taxa. Collection of these accessions, especially the land races and cultivars are done mainly based on the vernacular names. There is every possibility that a particular cultivar or race is known by different vernacular names in different places. This fact coupled with a lack of clear-cut distinguishable morphological features, could have resulted in the accumulation of duplicates in the collection. The aim of the present study is to determine variability in isozymes for 15 accessions (Accs) of turmeric, with a view to using this technique to eliminate duplicate entries.

Isozyme profiles are useful in identifying variation existing in plant populations. This technique of isozyme electrophoresis, developed some 30 years ago, has since been widely used to study genotypic variation in living organisms, and still remains a popular method as evidenced by its extensive use. The use of isozyme markers in germplasm characterization has been demonstrated for several crops, such as barley (Zhang *et al.*, 1994); *Brassica* spp. (Simonsen and Heneen, 1995); common bean (Acquaah *et al.*, 1994; Paredes and Gepts, 1995); cotton (Wendel *et al.*, 1994); eucalyptus (Prober and Brown, 1994); maize (Ordas *et al.*, 1994); pearl millet (Tostain, 1994); pineapple (Aradhya *et al.*, 1994); rubber (Paiva *et al.*, 1994); silver fir (Giannini *et al.*, 1994); soybean (Griffin and Palmer, 1995) and sweet potato (Zheng and Cheng, 1993). Isozymes, being multiple forms of enzyme proteins are primary gene products; variation in their structure should give reliable information about the variability in the genome and be less susceptible to environmental influence than the so-called secondary metabolites. Isozymes are theoretically well suited to identify closely related individuals or clones, simply by comparing phenotypic banding patterns. The main restriction is the degree of polymorphism in the available enzyme systems in the population of interest, in relation to the number of genotypes which have to be differentiated and the highly biased sampling (Forrest, 1994).

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MATERIALS AND METHODS

Plant material

Fifteen accessions of turmeric, obtained from the germplasm collection of IISR, were planted in pots (30 cm diameter), and grown in uniform conditions. Details of the accessions are given in Table I. These accessions were chosen (as part of a project to screen the entire germplasm) to represent a reasonable spectrum of morphological and geographical variability.

Electrophoresis assay

From a preliminary study using rhizomes, pseudostems and leaves of different maturity, the first fully unfurled leaf was found to be most suitable for

TABLE I
Details of turmeric accessions used for screening

Acc. No.	Popular names	Location*
32	Mananthody	Kerala
37	Sugandham	Andhra Pradesh
38	Sugandham	Orissa
42	Wynad Local	Kerala
64	Edapalayam	Kerala
88	Kaziranga	Assam
98	Maran	Assam
109	Palappally	Kerala
119	Singhihat	Manipur
143	CLS No. 5A	Andhra Pradesh
210	Daboka	Assam
358	Seedling progeny 5C	Unknown
363	Seedling progeny	Moovattupuzha, Kerala
364	Seedling progeny	Shillong, Meghalaya
366	Seedling progeny	Amalapuram, Andhra Pradesh

*Refer to Figure 2.

the study. Enzyme extracts were prepared by thoroughly homogenizing 3–3.5 g of the tissue in 5 ml of prechilled extraction buffer, using the method of Bhat *et al.* (1992) – 0.05 M Tris-HCl, pH 7.4, containing 0.1% cysteine. HCl, 0.1% ascorbic acid and 17% sucrose. The homogenate after filtering through a muslin cloth, was centrifuged at 15,930 **g** for 20 min at 4°C. The supernatant was kept frozen until use.

Polyacrylamide gel electrophoresis (PAGE) was performed using the mini-dual model of the Genei vertical slab gel electrophoresis system (Genei, India). The dimensions of the gel were 8.0 × 7.0 × 1 cm. The separations were performed on a 2.5% stacking gel consisting of 0.5 M Tris-HCl, pH 6.8 buffer and 7.5% resolving gel consisting of 3 M Tris-HCl, pH 8.8 buffer. The reservoir buffer contained 0.025 M Tris and 0.19 M Gly, pH 8.3, according to the method of Hames (1994). Twenty-five to fifty µl of the enzyme extract was loaded. The electrophoresis was carried out at a constant 70 V until proper stacking was achieved and then at 150 V until the tracking dye reached the end of the gel.

The staining procedures were those of Harris and Hopkinson (1976) for catalase (EC 1.11.1.6) and esterase (EC 3.1.1.2), of Ravindranath and Fridovich (1975) for superoxide dismutase (SOD) (EC 1.15.1.1), of Shimoni and Reuveni (1988) for peroxidase (PRX) (EC 1.11.1.7), of Mahadevan and Sridhar (1986) for polyphenol oxidase (PPO) (EC 1.14.18.1) and of Sadasivam and Manikam (1992) for acid phosphatase (EC 3.1.3.2). After staining, the gels were fixed in 7% CH_3COOH and bands were marked, the Em (electrophoresis mobility) values were calculated for each band and zymograms constructed. Two replications of each accessions were done.

From comparisons of the isozyme profiles, the paired affinity indices (PAI) for the 15 accessions were calculated from the ratio between the number of shared bands between two accessions and the total number of bands (Payan and Dickson, 1990). The per cent similarity between the accessions based on all the six isozymes, was calculated from the per cent of the sum of individual PAI values. Using the per cent similarity values, cluster analysis was done using the MVSP package (Ver 1.3, Warren L. Kovach, Department of Biology, Indiana University, USA). The clusters were depicted in the form of dendrograms.

RESULTS AND DISCUSSION

Of the six isozymes studied, acid phosphatase demonstrated the maximum number of bands (3–10), followed by SOD (2–7), esterase (3–6),

TABLE II
Paired affinity indices (per cent similarity) between 15 accessions of turmeric based on six isozyme profiles

Acc. No.	32	37	38	42	64	88	98	109	119	143	210	358	363	364	366
32	—	17/47 36%	18/51 35%	17/43 40%	17/46 37%	16/40 40%	14/41 34%	16/44 36%	12/39 31%	13/42 31%	17/47 36%	11/42 26%	12/43 28%	13/37 35%	17/46 37%
37		—	29/62 47%	20/54 37%	22/57 39%	19/52 37%	21/53 40%	21/55 38%	15/50 30%	19/53 36%	21/58 36%	16/53 30%	18/54 33%	16/50 32%	21/57 37%
38			—	21/58 36%	24/61 39%	21/55 38%	24/57 42%	25/59 42%	19/54 35%	22/57 39%	21/62 34%	18/57 32%	19/58 33%	18/54 33%	24/61 39%
42				—	24/53 45%	21/47 45%	20/49 41%	19/51 37%	16/46 35%	18/49 37%	19/54 35%	16/49 33%	16/50 32%	15/46 33%	20/53 38%
64					—	22/50 44%	23/52 44%	22/54 41%	16/49 33%	18/52 35%	19/57 33%	17/52 33%	18/53 34%	19/49 39%	22/56 39%
88						—	21/46 46%	20/48 42%	17/43 40%	18/46 39%	16/51 31%	14/46 30%	14/47 30%	17/43 40%	19/50 38%
98							—	22/50 44%	17/45 38%	19/48 40%	17/53 32%	14/48 29%	15/49 31%	17/45 38%	22/52 42%
109								—	19/47 40%	22/50 44%	19/55 35%	18/50 36%	16/51 31%	13/47 28%	21/54 39%
119									—	20/45 44%	14/50 28%	14/45 31%	15/46 33%	15/42 36%	20/49 41%
143										—	18/53 34%	16/48 33%	17/49 35%	17/45 38%	19/53 37%
210											—	18/53 34%	19/54 35%	15/50 30%	19/51 33%
358												—	24/50 48%	15/45 33%	16/51 31%
363													—	15/46 33%	17/51 32%
364														—	18/51 37%
366															—

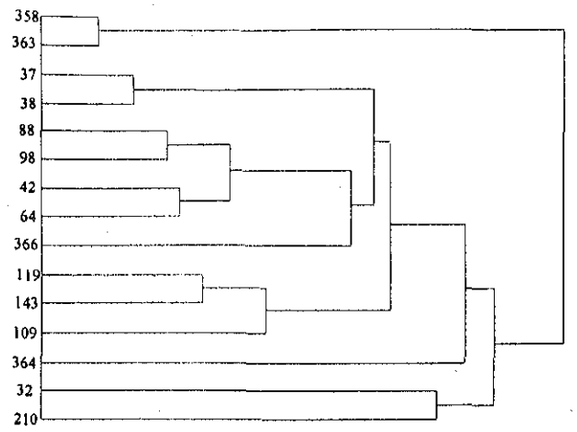

FIG. 1
Cluster analysis dendrogram of turmeric accessions based on six isozyme profiles.

PPO (3–6), PRX (2–5) and catalase (1–2). PPO and PRX isozymes were the most consistent and reproducible within replicates.

The PAI and per cent similarity values for the accessions are presented in Table II. The per cent similarity ranged between a maximum of 96% between Accs 358 and 363, to a minimum of 52.4%, between Accs 32 and 358.

The groups derived from cluster analysis and their average similarity are presented in Table III. The pairs of Accs 358 and 363, 37 and 38, 88 and 98, and 42 and 64 had a high per cent of average similarity, above 90%. Nodes 6 and 7 had an average similarity between 84 and 87%. The remaining nodes were less similar, 64–79%. Figure 1 represents the dendrogram constructed from the above data.

In general, there was a high degree of variability among the turmeric accessions screened, as shown in Figure 1. Accs 358 and 363, both seedling progenies, were closest in similarity, but the other two seedling progenies, Accs 364 and 366 showed a similarity of only 74%. This behaviour of seedling progenies could not be resolved in the absence of clear information about their parentage. However, seedling progenies are expected to have more variability than the clonally propagated material on account of sexual reproduction and sexual recombination. Accs 37 and 38, 'Sugandham' from Andhra Pradesh (AP) and 'Sugandham' from Orissa were the next most similar pairs of accessions. Accs 88 and 98, 'Kaziranga' and 'Maran', both from Assam, were the third most similar group, followed by Accs 42 and 64, 'Wynad Local' and 'Edapalayam' respectively, both from Kerala. The last two pairs of Accs (Nodes 3 and 4) were found to have an average similarity of 86.8%. Accs 119 and 143, 'Singhihat' from Manipur and CLS No. 5A from AP, respectively, were the next most similar group with an average similarity of 88.8%. Accs 119 and 143, released as 'Suguna' and 'Sudarsana', respectively, have fallen under the same cluster, separate from accession 98, released as 'Suvarna' (NRCS Annual Report 1989–90, 1991–92). Cultivars Suguna and Sudarsana resemble each other very closely not only in the morphology of the rhizome (plump and long) but also in agro-morphological characters (plant stature, high fresh yield per bed, low dry matter recovery (12%) and low curcumin content (5%)). It is quite possible that the cvs Suguna and Sudarsana were released as two separate varieties inadvertently in the absence of clear information about their origin or pedigree. However, 'Suvarna' is distinct from these two varieties in morphological and agronomical features. These differences are reflected in the isozyme profile as well. The remaining groups of Accs were much more dissimilar as can be seen from Table III.

The pattern of clustering of the accession thus reinforce the belief that Accs collected from the same geographical area (refer to Figure 2 for an impression of the relative geographical distances), although in some cases having different local names, may not be genetically distinct, e.g., Accs 88 and 98, as well as Accs 42 and 64. Accs 88 and 98 were collected as two separate Accs, 'Kaziranga' and 'Maran', respectively, from Assam, whereas Accs 42 and 64 were collected under the names 'Wynad Local' and 'Edapalayam' from Kerala. Similarly, the Accs collected from distant areas, for example Accs No. 210 from Assam and Accs No. 32 from Kerala showed only 72.4% similarity, indicating that they are genetically distant. Further, based on their isozyme profiles, these two Accs stood out separately

TABLE III
Cluster analysis of the 15 accessions of turmeric based on six isozome profiles

Node	Group I	Group II	Average similarity (%)	No. of objects in fused group
1	Acc. No. 358	Acc. No. 363	96.0	2
2	Acc. No. 37	Acc. No. 38	92.0	2
3	Acc. No. 88	Acc. No. 98	91.4	2
4	Acc. No. 42	Acc. No. 64	90.6	2
5	Acc. No. 119	Acc. No. 143	88.8	2
6	Node 4	Node 3	86.8	4
7	Acc. No. 109	Node 5	84.4	3
8	Node 6	Acc. No. 366	78.6	5
9	Node 2	Node 8	76.7	7
10	Node 9	Node 7	75.8	10
11	Acc. No. 32	Acc. No. 210	72.4	2
12	Node 10	Acc. No. 364	70.4	11
13	Node 11	Node 12	68.6	13
14	Node 13	Node 1	63.8	15

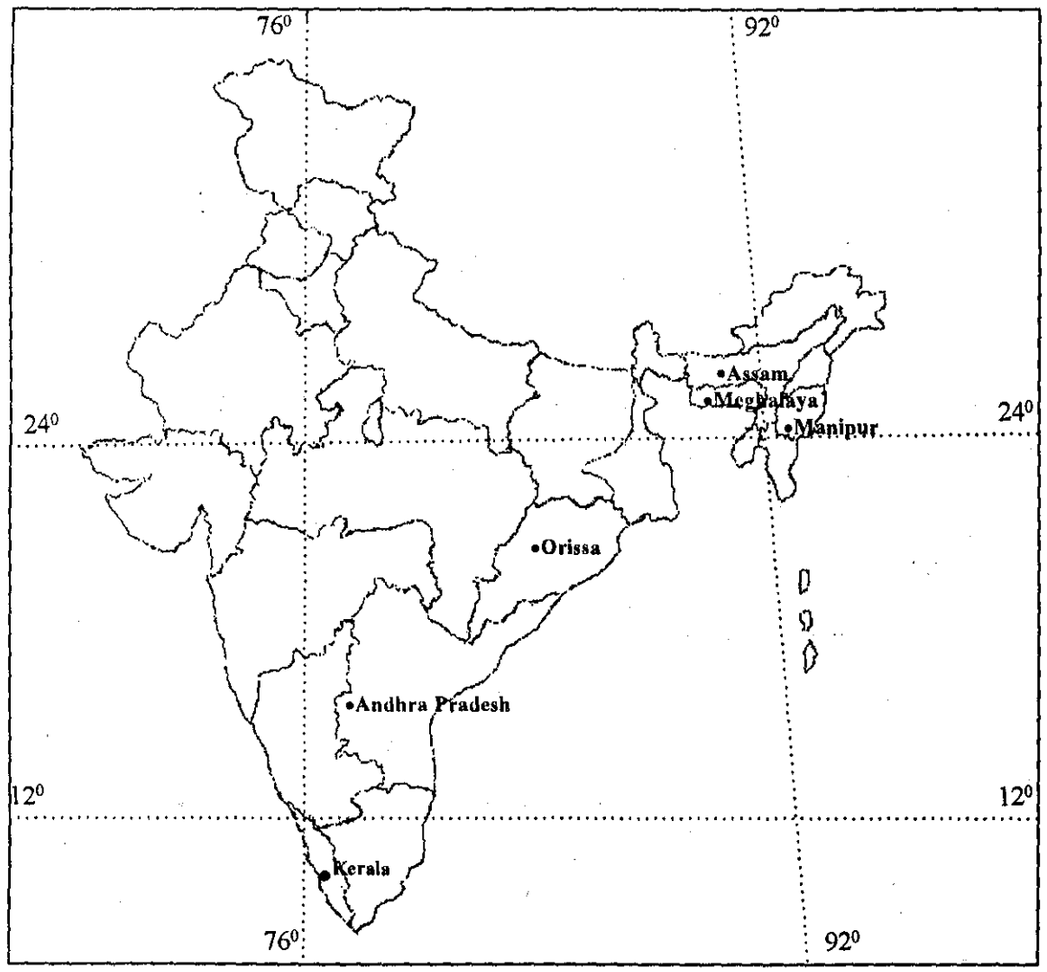

FIG. 2
Map of India, indicating relative geographical distances between locations. States from where turmeric germplasm was collected are indicated and named.

from most of the other Accs such as 37, 38, 88, 98, 42, 64, 366, 119, 143, 109 and 364. That the Accs 37 and 38, both 'Sugandham', fell under the same cluster gave further credibility to the clustering method based on isozymes, as both the Accs are the same. 'Sugandham' is a traditional cultivar of Andhra Pradesh. The same cultivar is also available in the neighbouring state of Orissa, from where it was collected again. Studies by Prober and Brown (1994) on the population genetics and fragmentation of *Eucalyptus albens* have similarly revealed that patterns of genetic variation were consistent with geographical relationship.

Considerable variability was noticed for polymorphic isozyme loci in the present material studied. This is interesting considering the clonal propagation of the crop. It implies that natural selection and conscious selection by man over years have helped to evolve locally adapted cultivars (genotypes). However, in Accs collected from within or nearby geographical areas, there is every possibility that such Accs will not be genetically distinct as exemplified by the Accs 88 and 98 and Accs 42 and 64. Further, it would be prudent to avoid collecting the same cultivar (identified by traditional names) even from different regions because it could lead to duplication as is seen with the 'Sugandhams' collected from Andhra Pradesh and Orissa.

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