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CHARACTERISATION OF GINGER GERMPLASM BASED ON ISOZYME POLYMORPHISM

B. SASIKUMAR, JOHN T. ZACHARIAH and A. SHAMINA

Indian Institute of Spices Research
Marikunnu Post, Calicut 673 012, Kerala

Fourteen accessions of ginger collected from Kerala and north eastern states were studied for variations in the pattern of four isozyme viz., acid phosphatase, polyphenol oxidase (PPO), superoxide dismutase (SOD) and peroxidase (PRX). Among them, acid phosphatase showed maximum number of bands followed by SOD while PRX had very few bands. Though the variability in the population under studies was low, the accessions collected from Kerala were distinct from those collected from northeastern states. Dendrograms were prepared on the basis of average similarity of the accessions in isozyme profile.

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) is considered to have originated in the southeast Asia. Many ginger cultivars, identified only by their locality of cultivation/collection are available in the country. Lack of clearcut morphological features coupled with the cultivar specific characters makes the identification of the cultivars rather difficult. Biochemical and molecular markers assume significance in this context. Use of biochemical markers in germplasm characterisation/organisation has been demonstrated in certain plantation crops (Al-Jibouri and Adham 1990; Shamina *et al.*, 1997, 1998). Isozyme being multiple forms of enzyme proteins, are primary gene products; variation in their structure should give reliable information on the variability in the genome. The present work is an attempt to characterise 14 accessions of ginger conserved at the National Ginger Germplasm Repository of the Indian Institute of Spices Research, Calicut based on their isozyme profile.

MATERIALS AND METHODS

Fourteen accessions of pot grown ginger plants were used for this study (Table 1). Preliminary screening of rhizomes, pseudostems and leaves of different maturity revealed that young leaves including the first unfurled leaf and two to three expanding leaves from top downward were found suitable for isozyme studies. Enzyme extracts were prepared with three to 3.5 g of leaf tissues in five ml of prechilled extraction buffer (Bhat *et al.*, 1992). Filtered homogenate then centrifuged was at 15, 930 x g for 20 minutes at 4^{0} C. The supernatant was kept frozen until use.

The procedure outlined by Hames (1994) was followed for polyacrylamide gel electrophoresis (PAGE) using mini-dual model

Table 1. Ginger accessions collected for isozyme polymorphic studies

Acc. No	Popular name/details	Location
235	Palai Local	Palai, Kerala
237	Konni Local	Konni, Kerala
262	Bisalgarh	Tripura
263	G.B. market collection	Tripura
265	G.B. market collection	Tripura
267	Lembucherra market	Tripura
271	Gole market collection	Tripura
276	Agarthala market	Tripura
284	Kamalgat market	Tripura
285	Kamalgat market	Tripura
286	G.B market collection	Tripura
289	Ranibazar	Tripura
283	ICAR Res. Complex, Shillong	Assam
291	Jorhat	Assam

vertical slab gel electrophoretic system (Genei, India). About 25 to 50 µl of extract was loaded on top of the gels and the electrophoresis was carried out at a constant 70 V until proper stacking was achieved followed by 150 V until the tracing dye reached the other end of the gel. The activity staining was performed for SOD, PRX, PPO and acid phosphotase as detailed by Ravindranath and Fridovich (1975), Shimoni and Reuveni (1988), Mahadevan and Sridhar (1986) and Sadasivam and Manickam (1992), respectively. After staining, the gels were fixed in seven per cent acetic acid and bands were recorded. Electrophoretic mobility (Em) values were calculated for each band and zymogram was constructed. Electrophoretic analysis was repeated twice for each accession. Isozyme profiles were compared and the paired affinity indices (PAI) were calculated as described by Gayon and Dickson (1970). Based on the per cent similarity, dendrogram was prepared as per hierarchical cluster analysis using SPSS package (SPSS Professional Statistics version 6.1).

RESULTS AND DISCUSSION

Among the four isozymes studied acid phosphatase had maximum number of bands (9-11) followed by SOD (5-6), polyphenol oxidase (3-4) and PRX (1-3). Per cent similarity index (PAI) for the accessions ranged between a maximum of 100 and a minimum of 85 indicating the narrow range of variability. Many accessions have maximum PAI value (Table 2). Results on the cluster analysis in the agglomeration schedule identified the clusters as being combined at each stage (Table 3). The dendrogram (Fig. 1) indicated not only the linkage groups of the cluster but also the affinity between the accessions. Actual length of the branches in the dendrogram was inversely proportional to the average similarity of each group as they were fused. Thus accessions included in stage 13 (Acc. 235 and 283) represent the most distant clusters. One cluster consisted of Accessions 283, 285 and 284 whereas the other group consisted of the remaining accessions.

In general, the variability for the four isozymes was low in the population studied. But the accessions collected from nearby geographical areas tend to cluster together. Thus accessions (Acc.235 and 237) had 100 per cent similarity and they formed one cluster. Both these accessions were collected from Kerala. Similarly, the other accessions which had 100 per cent similarity were collected from West Tripper district. However, Accession 262, though collected from Bisalgarh area (farmer's plot) in Tripura had high similarity (100%) with the accessions collected from Kerala (Acc.235 and 237). One possibility of this unexpected similarity is that the accession collected from Tripura may be a duplicate collection of the accession collected from Kerala. Large-scale introduction of planting material from Kerala to Tripura assumes significance, in this context.

Further, the accessions collected from Kerala had minimum similarity with the accessions collected from Tripura and other northeastern states. Average similarity between Accession 235 and 284 and Accessions 237 and 284 collected from Kerala and Tripura had the least similarity. Accession 262 showed less similarity with other collections from Tripura.

Table 2. Paired affinity indices of ginger accessions using four isozyme profiles

Acc. No	235	237	262	263	265	267	271	276	283	284	285	286	289	291
235	-	20/40	20/40	19/39	19/39	19/40	18/39	18/39	18/41	17/40	18/40	19/42	18/39	19/41
		100	100	97.4	97.4	95	92.3	92.3	87.8	85	90	90.5	92.3	92.7
237	-	-	20/40	19.39	19/39	19/39	18/39	18/39	18/41	17/40	18/40	19/42	18/39	19/41
			100	97.4	97.4	97.4	92.3	92.3	87.8	85	90	90.5	92.3	92.7
262	-	-	-	19/39	19/39	19/40	18/39	18/39	18/41	17/40	18/40	19/42	19/39	19/41
				97.4	97.4	95	92.3	92.3	87.8	85	90	95.5	92.3	92.3
263	-	-	-	-	19/38	19/39	18/38	18/38	18/40	17/39	18/39	19/41	18/38	19/40
					100	97.4	94.7	94.7	90	87.2	92.3	92.7	94.7	95
265	-	-	-	-	-	19/39	18/38	18/38	18/40	17/39	18/39	19/41	18/38	19/40
						97.4	94.7	94.7	90	87.8	92.3	92.7	94.7	95
267	-	-	-	-	-	-	18/38	18/38	19/41	18/40	19/40	20/42	18/38	20/41
							94.7	94.7	92.7	90	95	95.2	94.7	97.6
271	-	-	-	-	-	-	-	20/40	18/38	17/37	18/38	20/40	20/40	19/39
								100	94.7	91.9	94.7	100	100	97.4
276	-	-	-	-	-	-	-	-	18/38	17/37	18/38	20/40	20/40	19/39
									94.7	91.9	94.7	100	100	97.4
283	-	-	-	-	-	-	-	-	-	20/41	20/41	20/43	18/38	19/42
										97.6	97.6	93	94.7	90.5
284	-	-	-	-	-	-	-	-	-	-	19/40	19/42	17/37	18/41
											95	90.5	91.9	87.8
285	-	-	-	-	-	-	-	-	-	-	-	20/42	18/38	19/41
												95.2	94.7	92.7
286	-	-	-	-	-	-	-	-	-	-	-	-	20/40	21/43
													100	97.7
289	-	-	-	-	-	-	-	-	-	-	-	-	-	19/39
														97.4
291	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3. Agglomeration schedule using average linkage between groups

Stage	Clusters combined Cluster-1	Cluster-2	Coefficient
1	Acc.276	Acc.289	0.000
2	Acc.271	Acc.276	0.000
3	Acc.263	Acc.265	0.000
4	Acc.235	Acc.262	0.000
5	Acc.235	Acc.237	5.760
6	Acc.271	Acc.286	22.022
7	Acc.283	Acc.285	58.349
8	Acc.263	Acc.267	67.230
9	Acc.271	Acc.291	79.910
10	Acc.235	Acc.263	124.369
11	Acc.283	Acc.284	186.735
12	Acc.235	Acc.271	427.099
13	Acc.235	Acc.283	669.442

Variability for the polymorphic isozyme loci was low in the population studied. However, the trend obtained after cluster analysis based on isozyme profile gave certain indications for germplasm organisation. Accessions collected from adjacent locations showed more similarity and clustered together. As a result ginger cultivars within a particular geographical area may not be genetically distinct. However, this problem may extent to cultivars collected from far away places. Similar observations were reported earlier in ginger (Shamina *et al.*, 1997). Germplasm collections of ginger are generally based on vernacular names and/or geographical location. In the absence of any distinct morphological markers, the chances of duplication will be more in ginger germplasm.

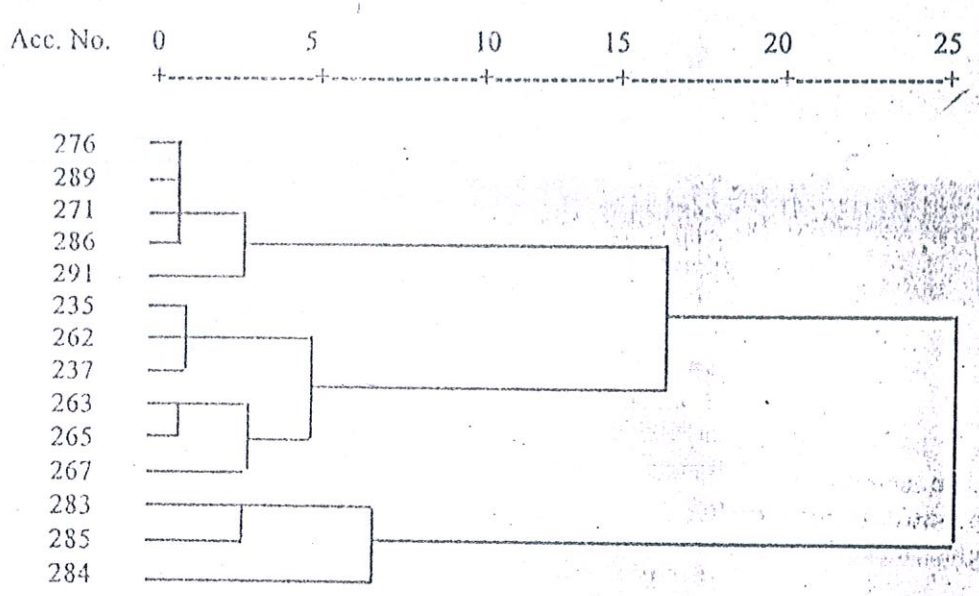

Fig. 1. Dendrogram using average linkage between groups

Present study warranted germplasm organisation based on the molecular markers in ginger.

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