Journal of Spices and Aromatic Crops 6 (2) : 119-127 (1997)

Biochemical variability in selected ginger (*Zingiber officinale* Rosc.) germplasm accessions

A SHAMINA, T JOHN ZACHARIAH, B SASIKUMAR & JOHNSON K GEORGE

Indian Institute of Spices Research
Marikunnu P.O., Calicut - 673 012, Kerala, India.

Abstract

Twenty five accessions of ginger (*Zingiber officinale*) germplasm were studied for variations in total free amino acids, proteins, total phenols and isozymes. Considerable variations were observed for total free amino acids, proteins and total phenols. The variability for the isozyme loci in the population was generally low. Dendrograms were prepared based on the average similarity of the accessions with respect to the isozyme profiles and accessions collected from the same geographical area had a tendency to cluster together.

Key words: biochemical variation, cluster analysis, ginger, isozyme, *Zingiber officinale*.

Abbreviations

PAI : Paired affinity index

PPO : Polyphenol oxidase

PRX : Peroxidase

SOD : Superoxide dismutase

Introduction

Ginger (*Zingiber officinale* Rosc.) is considered to have originated in South East Asia. Many ginger cultivars, mainly identified by their locality of cultivation/ collection, are prevalent in India. Lack of clear-cut morphological features coupled with the absence of cultivar specific characters, make discrimination of the cultivars rather difficult. Biochemical and molecular markers assume siginificance in this context. The use of biochemical markers in germplasm characterization has been demonstrated in several crops (Weeden & Lamb 1985; Al-Jibojuri & Adham 1990; de Miera & Vega 1991; Bhat *et al.* 1992; Bult & Kiyangi 1992).

Isozymes being multiple forms of enzyme proteins, are primary gene prod-

ucts; variation in their structure should give reliable information about the variability in the genome as they are less susceptible to environmental influence than the secondary products of metabolism which are formed as a result of enzyme activity. This technique of isozyme electrophoresis is being widely used to study genotypic variation in living organisms. Isozymes are theoretically well suited to identify closely related individuals or clones, simply by comparison of 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). The present work is an attempt to characterize 25 accessions of ginger germplasm available at Indian Institute of Spices Research (IISR), Calicut, based on its isozyme profile and metabolite (total free amino acids, proteins and total phenols) contents.

Materials and methods

Plant material

Twenty five accessions (Table 1) of ginger, obtained from the germplasm collection of IISR, Calicut, were planted in pots (12" size) and maintained for the study.

Estimation of total free amino acids, proteins and total phenols

Rhizome bits of 0.5 g were extracted in 10 ml of cold 80% ethanol and cenrifuged. The pellet was taken for protein assay (Lowry *et al.* 1951), while the supernatant was used to estimate total phenol and free amino acid contents. Phenol was estimated using the method of Sadasivam & Manikam (1992) and total free amino acids by the method of Moore & Stein (1948). Each accession was replicated thrice and the data statistically analysed.

Electrophoresis assay

Young leaves, including the first un-

Table 1. Ginger accessions screened for biochemical parameters

Acc. No.	Popular name	Location
2	Baharica	Assam
11	Edapalayam	Kerala
25	Maran	Assam
51	PGS 37	Pottangi collection
53	PGS 39	Pottangi collection
63	Saw Thing	Mizoram
65	Saw Thingpui	Manipur
73	Thingpui	Mizoram
74	Thinladium	Mizoram
101	Baratisogaon	Assam
106	Bokalia	Assam
110	Machiplavu local	Kerala
141	PGS 43	Pottangi collection
151	VI-SI-2	Pottangi collection
222	Tetraploid Maran	Kerala
226	Arakuzha	Kerala
231	Angamaly	Kerala
233	Thodupuzha	Kerala
238	Pottangi	Orissa
249	Jorhat	Assam
250	Himachal	Himachal Pradesh
251	Waynadan	Kerala
252	Muvattupuzha local	Kerala
293	Suprabha	Orissa
295	Maran	Assam

furled leaf and two to three leaves just below (mature leaves near the base were not used), were found most suitable for isozyme studies based on a preliminary standardization using rhizomes, pseudostems and leaves of different maturity. Enzyme extracts were prepared by throughly homogenizing 3.0-3.5 g of the tissue in 5 ml of prechilled extraction buffer (0.05 M Tris-HCl, pH 7.4, containing 0.1% cysteine HCl, 0.1% ascorbic acid and 17% sucrose) by the method of Bhat *et al.* (1992). The homogenate, after filtering through a muslin cloth, was centrifuged at 15,930 x g for 20 min at 4°C. The supernatant was kept frozen until use.

Polyacrylamide gel electrophoresis (PAGE) was done using the mini-dual model of the Genei vertical slab gel electrophoresis system (Genei, India). The dimensions of the gel were 8.0 x 7.0 x 0.1 cm. The separations were performed on a 2.5% stacking gel in 0.5 M Tris-HCl, pH 6.8 stacking gel buffer and 7.5% resolving gel in 3 M Tris-HCl, pH 8.8 resolving gel buffer. The reservoir buffer contained 0.025 M Tris and 0.19 M glycine, pH 8.3, according to the method of Hames (1994) and 25-50 µl of the extract was loaded. 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 & Hopkinson (1976) for catalase (EC 1.11.1.6) and esterase (EC 3.1.1.2); of Ravindranath & Fridovich (1975) for SOD (EC 1.15.1.1); of Shimoni & Reuveni (1988) for PRX (EC 1.11.1.7); of Mahadevan & Sridhar (1986) for PPO (EC 1.14.18.1) and of Sadasivam & 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 values calculated for each band and zymograms constructed. The analysis was replicated twice for each accession.

The isozyme profiles were compared and the PAI for the 25 accessions were calculated from the ratio between the number of shared bands between two accessions and the total number of bands (Payan & Dickson 1990). The per cent similarity between the accessions based on isozymes of all the six enzymes, 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

Categorization based on total free amino acids, protein and total phenol contents

Considerable variations were found between the accessions for total free amino acids, proteins and total phenol contents (Table 2). The total amino acid content ranged between 0.378 (Acc. No. 249) to 4.037 mgg^{-1} fresh weight (Acc. No. 2); the protein content ranged between 8.125 (Acc. No. 73) to 36.419 mgg^{-1} fresh weight (Acc. No. 222). The range was narrower in the case of total phenol content - 0.576 (Acc. No. 295) to 1.297 mg g^{-1} fresh weight (Acc. No. 101). Based on these values, an arbitrary categorization was resorted to (Table 3). The following accessions were found grouped together when these three parameters were taken into consideration:

Table 2. Biochemical constituents of ginger accessions

Acc. No.	Amino acids (mg/g fw*)	Protein (mg/g fw*)	Phenol (mg/g fw*)
2	4.037	24.387	1.197
11	1.142	15.193	0.667
25	1.737	29.335	0.825
51	2.480	20.689	1.010
53	0.754	10.729	0.658
63	0.830	10.353	0.766
65	1.258	10.128	0.855
73	2.553	8.125	0.827
74	1.138	8.230	0.788
101	1.178	28.919	1.297
106	1.088	28.125	1.112
110	1.868	26.864	1.057
141	2.443	32.673	1.091
151	3.624	29.090	0.943
222	1.822	36.419	1.229
226	1.192	29.263	1.027
231	1.399	28.725	1.021
233	1.889	18.491	0.706
238	2.601	17.720	0.741
249	0.378	13.635	0.792
250	1.898	17.592	0.603
251	2.210	17.472	0.726
252	2.580	20.000	0.674
293	1.788	19.404	0.625
295	2.815	17.587	0.576
CD at 5%	0.149	1.438	0.079
PV	0.000	0.000	0.000
CV%	14.04	12.18	15.94

*fw = fresh weight

(1) 53, 63 & 249 (2)11, 65, 233, 250 & 293 (3) 238, 251, 252 & 295 (4) 101, 110, 223, 226 & 231 (5) 101, 106 & 110.

Categorization based on isozyme profiles

Among the six isozymes, acid phosphatase showed the most number of bands (3 to 13) followed by SOD (6 to 7) and esterase (3 to 7); catalase isozymes numbered from 1 to 3. Isozymes of PPO (3 to 6) and PRX (3 to 5) were the most consistent and reproducible between replicates.

The PAI and per cent similarity for the accessions are presented in Table 4. The per cent similarity ranged between a maximum of 100% between accessions 2 and 11, and to a minimum of 50% between accessions 2 and 73. The groups derived from cluster analysis and their average similarity are presented in Table 5. Accessions 2 and 11 were found to be 100% similar and can be considered as a single group. Most groups which included 18 accessions (up to node 11), showed more than 90% average similarity, while Nodes 12-20, including the remaining 7 accessions, had an average similarity of 80-90%.

The dendrograms constructed from the above data are presented in Fig. 1. The actual lengths of the branches of this dendrogram is inversely proportional to the average similarity of each group as they are fused. Thus accessions included in Node 21 were the most distant in similarity from those in Node 23, and these two formed distinct groups. One group consisted of the Accessions 2, 11, 25, 51, 63, 65, 53, 141 and 106, while the other group consisted of the rest of the accessions.

In general, with the exception of Accessions 2 and 11, the accessions collected from neighbouring localities showed a

Table 3. Categorization of ginger accessions based on biochemical constituents

Group	Grouping of accessions based on values of		
	Amino acids	Protein	Phenols
Group I	(<1 mg/g fw)	(<10 mg/g fw)	(0.5-1.0 mg/g fw)
	53, 63, 249	73, 74	11, 25, 53, 63, 65, 73, 74, 151, 233, 238, 249, 250, 251, 252, 293, 295
Group II	(1-2 mg/g fw)	(10-20 mg/g fw)	(1.0-1.5 mg/g fw)
	11, 25, 65, 74, 101, 106, 110, 222, 226, 231, 233, 250, 293	11,53, 63, 65, 233, 238, 249, 250, 251, 252, 293, 295	2, 51, 101, 106, 110, 141, 222, 226, 231
Group III	(2-3 mg/g fw)	(20-30 mg/g fw)	
	51,73, 141,238, 251, 252, 295	2, 25, 51, 101, 106, 110, 151, 226, 231	-
Group IV	(>3 mg/g fw)	(>30 mg/g fw)	
	2, 151	141, 222	-

fw = fresh weight

high per cent of similarity, so the chances of duplication of the same accession under different popular names are high. Accessions 2 and 11 (Baharica from Assam and Edapalayam from Kerala, respectively) were totally similar. The other pairs of accessions showing very high similarity were from adjoining areas, as in Accessions 63 (Saw Thing from Mizoram) and 65 (Saw Thingpui from Manipur); 222 (Maran, an induced tetraploid derivative of Maran) and 226 (Arakuzha), both from Kerala; 238 (Pottangi from Orissa) and 249 (Jorthat from Assam); 74 (Thinladium from Mizoram) and 101 (Baratisogaon from Assam) and 53 and 141, both Pottangi collections.

The groups derived from the amino acids, protein and phenol contents do not necessarily fall under those based on isozyme profilies. However, most show a high per cent of similiarity (80% and above) as in the pairs of accessions: (1) 53 & 63 - 94.2% (2) 226 & 231 - 94.2% (3) 233 & 250 - 91.6% (4) 110 & 226 - 88.2% (5) 101 & 106 - 88% (6) 251 & 252 - 84.2% (7) 106 & 110 - 84% (8) 233 & 293 - 83.4% (9) 251 & 295 - 82.8% (10) 11 & 233 - 82.4 % (11) 238 & 252 - 82.2% (12) 250 & 293 - 82% (13) 11 & 65 - 81.2% (14) 11 & 250 - 81.2% (15) 238 & 295 - 80.8 % (16) 238 & 251 - 80.0 %.

Accessions 25 and 295, which are both popularly known as Maran showed relatively lower levels of similarity - 77.4%. However, Accession 222 (tetraploid Maran) showed higher levels of similarity with Accessions 25 (81.2%) and 295 (86.2%), the diploid lines.

In general, the variability for isozyme polymorphism was low in the population studied. This may be due to the limited number of polymorphic isozyme loci in the population. However, the

Table 4. Paired affinity indices (per cent similarity) of ginger accessions based on six isozyme profiles

Acc. No.	2	11	25	51	53	63	65	73	74	101	106	110	141	151	222	226	231	233	238	249	250	251	252	293	295
2	-	10/20 50%	9/20 45%	9/22 41%	9/23 39%	9/23 39%	9/23 39%	3/12 25%	3/11 27%	3/11 27%	9/25 36%	9/23 39%	9/22 41%	9/24 38%	9/23 39%	9/23 39%	9/23 39%	9/25 36%	3/11 27%	3/11 27%	3/11 27%	8/22 36%	8/21 38%	8/22 36%	8/21 38%
11		-	14/30 47%	14/32 44%	12/31 39%	12/31 39%	13/32 41%	10/32 31%	11/32 34%	11/32 34%	13/34 38%	13/32 41%	12/30 40%	12/32 38%	14/32 44%	14/32 44%	14/32 44%	14/34 41%	13/32 41%	13/32 41%	13/32 41%	12/31 39%	12/30 40%	12/31 39%	12/31 39%
25			-	14/32 44%	12/31 39%	12/31 39%	13/32 41%	11/32 34%	12/32 38%	12/32 38%	13/34 38%	13/32 41%	12/30 40%	12/32 38%	13/32 41%	13/32 41%	13/32 41%	13/34 38%	13/32 41%	13/32 41%	13/32 41%	12/31 38%	12/30 40%	13/31 42%	12/31 39%
51				-	23/49 47%	23/52 44%	22/53 42%	21/56 38%	19/55 35%	19/54 35%	23/57 40%	23/57 40%	22/55 40%	23/58 40%	19/53 36%	19/53 36%	19/49 39%	20/52 39%	18/52 35%	19/53 36%	19/54 35%	19/53 36%	17/54 32%	17/52 33%	19/55 35%
53					-	24/51 47%	24/52 46%	21/55 38%	18/54 33%	18/53 34%	21/56 38%	21/56 38%	26/54 48%	22/57 39%	17/52 33%	17/51 33%	17/48 35%	18/54 33%	16/51 31%	17/52 33%	17/53 32%	17/52 33%	15/53 28%	16/51 31%	17/54 32%
63						-	27/55 49%	23/58 40%	21/57 37%	22/56 39%	25/59 42%	21/59 36%	22/57 39%	24/60 40%	19/55 35%	19/54 35%	17/51 33%	19/57 33%	19/54 35%	19/55 35%	19/56 34%	19/55 35%	17/56 30%	18/54 33%	20/63 32%
65							-	24/64 38%	22/58 38%	23/57 40%	26/60 43%	22/60 37%	23/58 40%	25/61 41%	20/56 36%	20/55 36%	18/52 35%	20/58 35%	20/55 36%	20/56 36%	20/57 35%	20/56 36%	18/57 32%	19/55 35%	21/58 36%
73								-	27/61 44%	27/60 45%	26/63 41%	25/63 40%	24/61 39%	25/64 39%	21/59 36%	20/58 35%	17/55 31%	23/61 38%	21/58 36%	21/59 36%	21/60 35%	23/59 39%	22/60 37%	23/58 40%	23/61 38%
74									-	29/59 49%	26/62 42%	22/62 36%	21/60 35%	23/63 37%	21/58 36%	21/57 37%	19/54 35%	24/60 40%	22/57 39%	23/58 40%	23/59 39%	22/58 38%	22/59 37%	23/57 40%	24/60 40%
101										-	27/61 44%	23/61 38%	22/59 37%	24/62 39%	21/57 37%	21/56 38%	19/53 36%	24/59 41%	22/56 39%	23/57 40%	23/58 40%	23/57 40%	22/58 38%	23/56 41%	23/59 39%
106											-	27/64 42%	26/62 42%	29/65 45%	23/60 38%	23/59 39%	21/56 38%	26/62 42%	24/59 41%	25/60 42%	25/61 41%	25/60 42%	23/61 38%	23/59 39%	27/62 44%
110												-	27/62 44%	28/65 43%	26/60 43%	26/59 44%	22/56 39%	24/62 39%	24/59 41%	24/60 40%	26/61 43%	25/60 42%	22/61 36%	22/59 37%	27/62 44%
141													-	30/63 48%	23/58 40%	22/57 39%	19/54 35%	23/60 38%	21/57 37%	22/58 38%	22/59 37%	25/58 43%	23/59 39%	22/57 39%	24/60 40%
151														-	24/61 39%	24/60 40%	20/57 35%	25/63 40%	24/60 40%	24/61 39%	24/62 39%	27/61 44%	23/62 37%	24/60 40%	24/63 38%
222															-	27/55 49%	24/52 46%	27/58 47%	24/55 44%	24/56 43%	24/57 42%	24/56 43%	22/57 39%	22/55 40%	25/58 43%

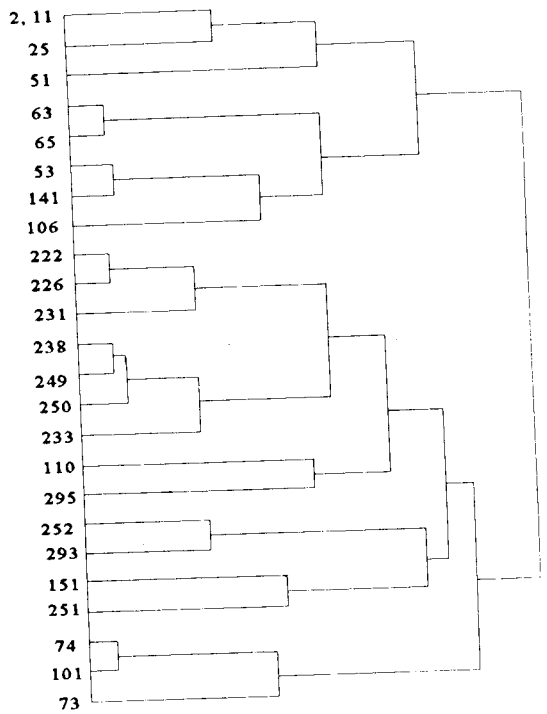

Fig. 1. Cluster analysis dendrogram of ginger accessions obtained from six isozyme profiles

trend obtained after cluster analysis based on isozyme profiles, gives important clues for germplasm organization in this clonally propagated crop. Generally, accessions collected from one geographical area showed more similarity and clustered together. This implies that ginger cultivars within a particular geographical area, though may have a different cultivar name, may not be genetically distinct. However, this problem may extend to cultivars collected from far flung areas as well, as is seen in the case of Accessions 2 and 11, collected from Assam and Kerala, respectively. This could probably be due to the spread of cultivars under a different name in a new place. Since germplasm collections are generally done based on

Table 5. Cluster groups of ginger accessions

Node	Group I	Group II	Average similarity	No. of objects in fused group
1	Acc. No.2	Acc. No. 11	100.0	2
2	Acc. No.74	Acc. No. 101	98.4	2
3	Acc. No. 63	Acc. No. 65	98.2	2
4	Acc. No. 222	Acc. No. 226	98.2	2
5	Acc. No. 238	Acc. No. 249	98.2	2
6	Acc. No. 53	Acc. No. 141	97.8	2
7	Node 5	Acc. No. 250	97.3	3
8	Node 4	Acc. No. 231	93.3	3
9	Acc. No. 233	Node 7	93.2	4
10	Acc. No. 252	Acc. No. 293	92.8	2
11	Node 1	Acc. No. 25	91.7	3
12	Node 6	Acc. No. 106	89.4	3
13	Acc. No. 73	Node 3	89.3	3
14	Acc. No. 151	Acc. No. 251	88.6	2
15	Acc. No. 110	Acc. No. 295	87.0	2
16	Node 12	Node 3	85.8	5
17	Node11	Acc. No. 51	85.7	4
18	Node 8	Node 9	85.6	7
19	Node 15	Node 18	82.3	9
20	Node 14	Node 10	80.5	4
21	Node 17	Node 16	80.0	9
22	Node 19	Node 20	79.3	13
23	Node 13	Node 22	75.7	16
24	Node 21	Node 23	74.1	25

vernacular names and lack any distinct morphological features, the same material may have been collected from different places. The present study, which can provide only a broad grouping, calls for refinement of the organizing of the germplasm based on molecular markers, including DNA markers.

Acknowledgments

We thank the Indian Council of Agricultural Research, New Delhi, for funding the project, under which the work was undertaken. We are also grateful to Prof. (Dr.) K V Peter, Director, Indian Institute of Spices Research, Calicut, for providing facilities and to Mr. José

Abraham, Senior Scientist (Statistics), for his assistance in statistical analysis.

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