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Short communication

Genetic diversity analysis of ginger (*Zingiber officinale* Rosc.) germplasm based on RAPD and ISSR markers

Jaleel Kizhakkayil[1], B. Sasikumar*

Division of Crop Improvement and Biotechnology, Indian Institute of Spices Research, P.O. Marikunnu, Calicut, Kerala 673012, India

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ABSTRACT

A global collection of ginger germplasm consisting of 46 accessions were characterized using two types of molecular markers, RAPD (Random Amplified Polymorphic DNA) and ISSR (Inter Simple Sequence Repeats). UPGMA dendrograms constructed based on three similarity coefficients, i.e., Jaccard's, Sorensen–Dice and Simple Matching using the combined RAPD and ISSR markers placed the accessions in four similar clusters in all the three dendrograms revealing the congruence of clustering patterns among the similarity coefficients and a rather less genetic distance among the accessions. Improved varieties/cultivars are grouped together with primitive types. Moreover, in the clustering pattern of the accessions, a geographical bias was also evident implying that germplasm collected from nearby locations especially with vernacular identity may not be genetically distinct. The clustering of the accessions was largely independent of its agronomic features.

1. Introduction

Ginger (*Zingiber officinale* Rosc.), a pan tropical crop, propagated exclusively through rhizome, is valued as a spice and medicinal plant besides its use as a food additive. Most of the ginger cultivars are named in vernacular or after a particular trait of the cultivar or a place. This cultivar identity coupled with the lack of clear cut morphological features among the accessions have lead to considerable confusion in the gene pool conservation and exploitation.

The molecular approach for identification of plant genotypes seems to be more effective as it allows direct access to the hereditary material (Paterson et al., 1991) unlike the morphological markers. Though RAPD markers are reported to be more suitable for genetic diversity analysis of clonal organisms (Bardakei, 2001), the ISSR markers are more reproducible than RAPD markers (Goulao and Oliveira, 2001). Surprisingly very few reports are there using molecular markers in germplasm characterization of ginger barring the few RAPD reports (Nayak et al., 2005; Palai and Rout, 2007).

The present study uses two types of molecular markers, RAPD and ISSR, for the first time, to characterize a world sample of ginger germplasm, conserved at the Indian Institute of Spices Research (IISR), Calicut, Kerala, India.

2. Materials and methods

2.1. Genomic DNA extraction and RAPD and ISSR analysis

Good quality genomic DNA was extracted from two grams young leaf of 46 ginger accessions (Table 1) following the CTAB method (Syamkumar et al., 2003) and quantified in 0.8% agarose according to a standard DNA marker.

RAPD PCR reactions were performed in a thermal cycler (Eppendorf, Germany) using 30 decamer primers (OPA 01, OPA 04, OPA 05, OPA 07, OPA 08, OPA 11, OPA 12, OPA 13, OPA 15, OPB 01, OPB 05, OPB 06, OPB 07, OPB 11, OPB 18, OPB 19, OPB 20, OPC 10, OPC 15, OPD 01, OPD 02, OPD 03, OPD 04, OPE 06, OPE 09, OPE 11, OPE 14, OPE 15, OPJ 07, and OPJ 08) as described by Williams et al., 1990. ISSR PCR analysis was performed with 17 ISSR primers (Bangalore Genei, Bangalore and Integrated DNA technologies, USA) (ISSR01($(CA)_7AG$), ISSR02($(AGTG)_3$), ISSR03($(GACA)_4$), ISSR04($(AC)_8C$), ISSR05($(CT)_7TG$), ISSR06($(GA)_8C$), ISSR07($(GA)_8G$), ISSR08($(GA)_8T$), ISSR09($(CT)_8G$), ISSR10($(AC)_8G$), ISSR11($(GACA)_3$), ISSR12($(CAC)_3GC$), ISSR13($(AGTG)_3GG$), ISSR14($(AGC)_4GT$), ISSR15($(TCC)_5$), ISSR16($(GAGAGA)_2$ GAGAT), and ISSR17$(CACACA)_2$ CACAG). Except for the annealing temperature of 50 °C for 1 min for IISR, other reaction components were same for both RAPD/IISR PCRs. Electrophoresis was performed in 1.5% agarose gel.

2.2. Statistical analysis

RAPD and ISSR products were scored for the presence (1) and absence (0) of bands between 250 and 2500 bp. Data were

* Corresponding author.
E-mail address: bhaskaransasikumar@yahoo.com (B. Sasikumar).
[1] Present address: Cell Signaling Lab, Department of Biochemistry, Faculty of Medicine and Health Science, UAE University, Al-Ain, United Arab Emirates. Tel.: +971 37137633; fax: +971 37672033.

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Table 1
Ginger accessions studied and their salient features.

Sl. No.	Name	Acc No.	Remark	Sl. No.	Name	Acc No.	Remark
1	Varada	64	Released variety from Indian Institute of Spices Research (IISR), Kerala, India.	24	China	9	Originally from China.
2	Mahima	117	Released variety from IISR, Calicut, Kerala, India.	25	Juggigan	18	Originally from Nigeria.
3	Rejatha	35	Released variety from IISR, Calicut, Kerala, India.	26	Acc. No. 50	50	Kerala, India. Zero farnesene, oil contains relatively more constituents
4	Suruchi	714	Released variety from Orissa University of Agriculture & Technology (OUAT), Orissa, India.	27	Pulpally	56	Collected from Pulpally, Kerala, India.
5	Suprabha	293	Released variety from Orissa University of Agriculture & Technology (OUAT), High Altitude Research Station, Pottangi, Koraput, Orissa, India	28	Acc. No.95	95	Kerala, India.
6	Himachal	294	Land race from Himachal Pradesh, India.	29	Ambalawayalan	109	Collected from Wynad, Kerala, India.
7	Maran	295	Land racefromAssam, India.	30	Kozhikkode	162	Collected from Kozhikkode, Kerala, India.
8	Nadia	27	Land race from West Bengal, India. Low fiber	31	Thodupuzha-1	204	Collected from Thodupuzha, Kerala, India.
9	Karakkal	20	Land race from Pondicherry, India.	32	Konni local	206	Collected from Konni, Kerala, India.
10	Mananthody	244	Land race from Wynadu, Kerala, India.	33	Angamali	214	Collected from Angamali, Kerala, India.
11	Sabarimala	246	Primitive type collected from Sabarimala forests, Western Ghats, Kerala.	34	Thodupuzha-2	217	Collected from Thodupuzha, Kerala, India.
12	Kozhikkalan	537	Primitive type collected from Nedumangad, Kerala, India.	35	Kottayam	225	Collected from Kottayam, Kerala, India.
13	Ellakallan	463	Primitive type collected from Idukki, Kerala India, slender rhizome.	36	Palai	228	Collected from Palai market, Kerala, India.
14	Kakakkalan	558	Very primitive type collected from Nedumangad, Kerala, India.	37	Silent valley	240	Collected from Silent valley forests of Western Ghats, India.
15	Pakistan	733	From Pakistan.	38	Wyanad local	251	Collected from Wynad, Kerala, India.
16	Oman	734	From Oman.	39	Vizagapatnam-1	411	Collected from Vizagapatnam, Andhrapradesh, India.
17	Brazil	736	From Brazil.	40	Vizagapatnam-2	420	Collected from Vizagapatnam, Andhrapradesh, India.
18	Jamaica	17	From Jamaica originally.	41	Fiji	430	From Queensland.
19	Rio-de-Janeiro	59	From Brazil originally.	42	Gorubathani	515	Collected from Sikkim, India. Bold type
20	Pink ginger	731	Collected from Meghalaya state, India.	43	Bhaise	552	Collected from Kalimpong, West Bengal, India.
21	Bakthapur	563	From Nepal.	44	Naval parasi	569	Collected from Nepal.
22	Kintoki	648	From Japan.	45	Neyyar	650	Collected from Neyyar, Kerala, India.
23	Nepal	575	Collected from Nepal.	46	Jolpaiguri	654	Collected from Jalpaiguri, West Bengal, India.

analysed based on the Jaccard's, Sorensen–Dice and Simple Matching similarity coefficients for binary data via SIMQUAL of the Numerical taxonomy and Multivariant Analysis System program package for PC (NTSYS-pc ver. 2.02i Package) (Rohlf, 1993). UPGMA dendrograms were constructed based on the analysis of the data (Sokal and Sneath, 1963).

3. Results and discussion

Out of 60 RAPD primers screened, the 30 which gave consistent pattern were used for further amplification. A total of 269 scorable bands were produced, out of which 126 were polymorphic. Seventeen ISSR primers produced 160 scorable bands out of which 76 were polymorphic.

The genetic similarity coefficients (Jaccard's) obtained by the RAPD and ISSR markers were in the range of 0.76–0.97. Though good morphological genetic variability is reported in ginger, the present molecular diversity study revealed a rather low genetic variability as reported earlier using isozyme markers (Sasikumar et al., 2000). The cluster analysis using the combined data of RAPD and ISSR has revealed four clusters in Jaccard's, Sorensen and Simple Matching coefficient dendrograms (Fig. 1) (latter two dendrograms not shown, being similar).

Dendrograms drawn based on Jaccard's, Sorensen–Dice and Simple Matching coefficients showed similar clustering pattern indicating congruence of clustering patterns among the three similarity coefficients, though the entities in one of the clusters differed slightly. The congruence in grouping patterns of different similarity indices, was reported earlier too in egg plant (Mace et al., 1999) and grape vine (Vidal et al., 1999). Most of the improved varieties grouped distinctly from the land races/cultivars in all the clusters. Primitive type gingers such as 'Sabarimala' (Acc. No. 246), 'Kozhikkalan' (Acc. No. 537), 'Ellakallan' (Acc. No. 463) etc. are grouped in the first cluster and showed close similarity with the landraces, Acc. No. 27, Acc. No. 20 and Acc. No. 295 as well as the improved varieties, 'Varada' (Acc. No. 64), 'Mahima' (Acc. No. 117), and 'Rejatha' (Acc. No. 35) indicating that the primitive types may be the progenitor of the present day ginger varieties. In the first cluster some exotic gingers such as Acc. No. 733, Acc. No. 734, Acc. No. 736 and Acc. No. 17 too are grouped together with the other accessions thus making the cluster a group of diverse entities. Continuous selection for yield and/or quality is known

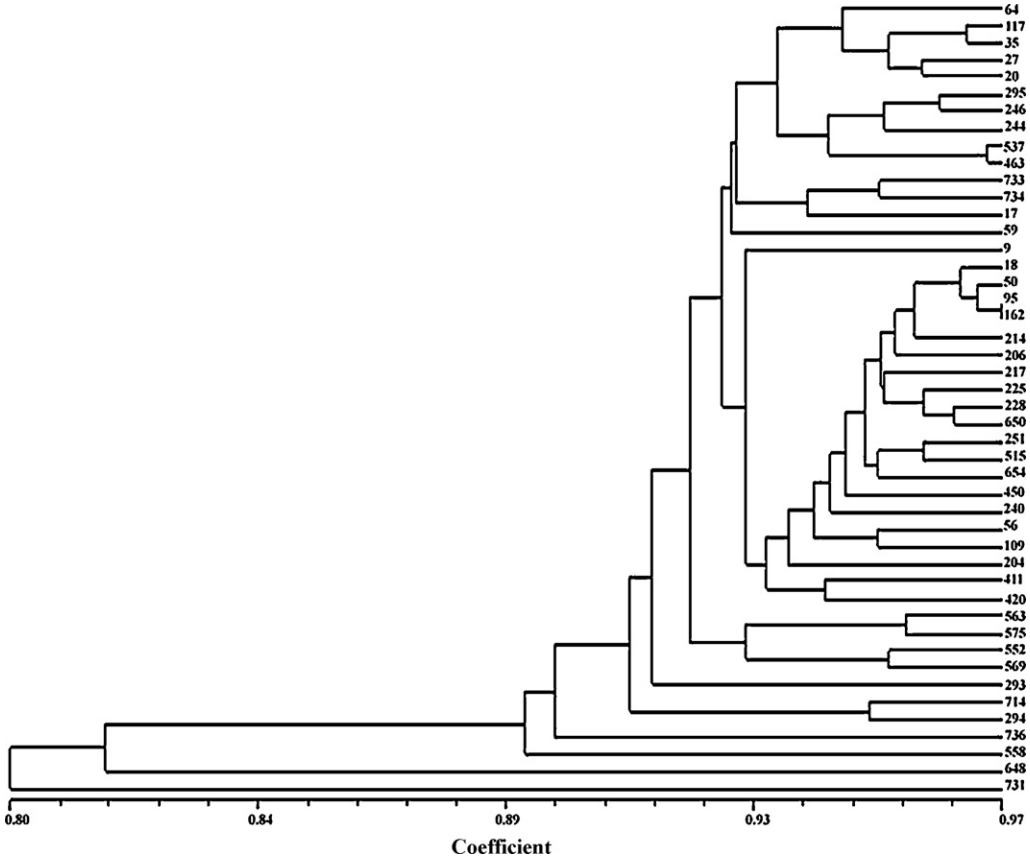

Fig. 1. UPGMA dendrogram based on RAPD and ISSR markers using Jaccard's similarity coefficients.

for fixing of genetic variability in crop plants (Desclaux, 2005). This would have been responsible for clustering together of the three improved varieties ('Varada', 'Mahima' and 'Rejatha') from IISR, Calicut, Kerala, India. The primitive types being the progenitor of the present day ginger varieties/cultivars, the clustering of the primitive accessions ('Sabarimala', 'Kozhikkalan' and 'Ellakallan') with the improved varieties is also of sense. However, the grouping of the exotic introductions with the other accessions in the cluster is rather intriguing.

Accessions collected from same geographical areas such as Acc. No. 411 and Acc. No. 420 (from Vizagapatnam) as well as Acc. No. 204 and Acc. No. 217 (from Thodupuzha), have clustered together in the cluster two of all the dendrograms, indicating that accessions collected from nearby localities need not be genetically distinct. Similarly, the Nepal collections such as 'Bakthapur' (Acc. No. 563), 'Naval Parasi' (Acc. No. 569) and 'Nepal' (Acc. No. 575) are included in the third cluster along with 'Bhaise' (Acc. No. 552). 'Bhaise' is an accession collected from Sikkim, India adjoining region of Nepal.

In the last cluster an improved variety, 'Suruchi' (Acc. No. 714) and another good yielder, 'Himachal' (Acc. No. 294), were grouped together. The accessions that stood singly in the dendrograms were rather unique entities in one or other aspects. Three primitive gingers, 'Pink ginger' (Acc. No. 731), Kintoki' (Acc. No. 648) and 'Kakakalan' (Acc. No. 558) clustered singly in all the three dendrograms. 'Pink ginger' is indigenous to Meghalaya state in the North East India and 'Kintoki' to Japan. 'Pink ginger' is characterized by pink coloured rhizome scales whereas 'Kintoki' (*Zingiber officinale* var. *rubens*) is characterized by dwarf stature, profuse tillering, small globose rhizomes and very less fiber. These two accessions showed highest variability when compared with the other ginger accessions in all the dendrograms. 'Kakkakalan', a very primitive type ginger almost extinct from cultivation is a very poor yielder but rich in oil, especially citral. The fourth single cluster entity, 'Brazil' (Acc. No. 736) is the only high farnesene type accession in the germplasm studied. A geographical bias coupled with less genetic distance was the main feature of the study. Collection of the accessions based vernacular identity irrespective of the geographical proximity may be the probable reason for this behaviour. No clear cut clustering based on yield or quality too could be discerned in the present study. Though some of the high oil and low yielding primitive gingers as well as some improved varieties or some semi bold ones clustered together, it was but with other accessions implying that genes amplified by the markers need not be strictly linked with any agronomic traits.

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