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Assessment of genetic diversity by using RAPD and ISSR markers in ginger (*Zingiber officinale* Rosc.) genotypes

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Abstract
Ginger (*Zingiber officinale* Rosc.) is an herbaceous perennial, the rhizomes of which are used as a spice. Several promising cultivars released in ginger are characterized on the basis of morphological and biochemical data, oleoresin content and yield potential. However these characters differ under varying environmental conditions making characterization of different ginger cultivars a complicated task. In addition to this practical concern the continued release of new cultivars makes the development of new techniques for genetic purity determination even more essential. Due to resurgence of interest in the commercial development of different cultivars of ginger as new spice crops, it has become necessary to precisely characterize the genetic diversity that exists in cultivars, advanced selections and native population. This is one step towards providing accurate genetic information for future breeding and germplasm collection efforts. The present study was carried out with an aim to characterize 31 different ginger genotypes using molecular markers *viz*., RAPD and ISSR. RAPD amplification of genomic DNA using 34 primers amplified total 9210 scorable bands with 475 loci, among them, 370 loci were found polymorphic, showing 76.83% polymorphism. The PIC values ranged from 0.74 (OPE- 09) to 0.93 (OPG-07, OPG-09 and OPH-12) with an average of 0.88. The highest (100%) polymorphism was exhibited by primers *viz*., OPA 16, OPG-07, OPG-10, OPG-11, OPG-19, OPD19 and OPE-09 while the lowest polymorphism (38.46%) was observed with primer OPA-01. PCR amplification of 14 ISSR primers generated a total of 4076 scorable bands with an average of 291 bands per primer. The PIC values ranged from 0.77 (UBC-845) to 0.94 (ISSR-10). The highest PIC value of ISSR-10 indicated its usefulness for genetic diversity analysis in ginger. The Jaccard's similarity values for RAPD, ISSR and pooled data of RAPD+ISSR markers ranged from 0.60 to 0.92, 0.39 to 0.95 and 0.55 to 0.92, respectively. The dendrogram constructed using RAPD and ISSR markers distinguished all the ginger genotypes geographically, in which genotypes collected from Gujarat showed high level of variations as compared to IISR, Calicut accessions.

Keywords: Ginger, genetic diversity, molecular marker, ISSR, RAPD

Introduction
Ginger (*Zingiber officinale* Rosc.) is an important perennial horticultural herb belongs to the family *Zingiberaceae.* Ginger having valued for its aromatic and pungent rhizome all over the world as a spice and vegetable. It has large genome of 23618 Mbp distributed in 2n=22 chromosomes **(**Arumugam *et al*. 2009) [2]. It has intense medicinal properties which played an important role in primary health care in ancient India and China as carminative, stimulant, anti-inflammatory and antiemetic in ayurvedic system of medicine (Purseglove *et al*. 1981) [20]. The ginger rhizome contains various active compounds and these constituents may vary depending on the place of origin and whether the rhizomes are fresh or dry such as gingerol, shogaol, ginger protease, capsaicin and several sesquiterpenes like zingiberol, zingiberenol (Ali *et al*. 2008) [1]. Identification and characterization of germplasm is an important link for diversity study. Limitation in ginger productivity is due to narrow genetic base (Ghosh *et al.* 2015) [8]. Breeding of ginger is difficult due to lack of variability, absence of natural seed set and exclusive vegetative propagation. Broadening the genetic base can be possible through mutagenesis or tissue culture techniques that provided a way for isolation of variable plant types with desirable characters. Morphological traits, as modulated by environment will not always guide to fulfil this perspective because morphological traits are highly influenced by environment (Last *et al*. 2014) [15].

While, molecular diversity assessed by using molecular markers is independent of the influence of environment and estimated by using DNA from any growth stage (Khanam *et al*. 2012) [13]. Moreover a large number of polymorphic markers are required to measure genetic relationships and genetic diversity. Molecular marker is the powerful tool for determining genetic variation in ginger genotypes as they can reveal abundant difference among genotypes at the DNA level and provide a more direct, reliable and efficient tool for germplasm characterization, conservation, management. Molecular markers have played a leading role in delineating the diversity in most living organisms. Each marker has its own advantages and disadvantages but the proper selection of molecular marker and stringent experimentation can produce useful information (Idrees and Irshad. 2014) [11].

Being a poorly studied crop, literature on molecular characterization of ginger is limited, where molecular information is limited. Random Amplified Polymorphic DNA markers are the markers of choice because of their simplicity in use and low cost. DNA markers have been widely used to assess the genetic diversity and phylogenetic relationship in different crop species (Da Costa. 2006) [4]. In the field of molecular genetics, various techniques have been emerged to analyze genetic variation. The DNA fingerprinting techniques Random Amplified Polymorphic DNA (RAPD) (Paterson *et al*. 1991). Amplified Fragment Length Polymorphism (AFLP) (Kavitha *et al*. 2010) [12]. Simple Sequence Repeat (SSR), Inter Simple Sequence Repeat (ISSR) etc. are widely used to assess genetic diversity and to understand genetic relationship among different genotypes in ginger and other crops. In many spice crops species PCR-based molecular markers are used for identification, phylogenetic analyses, population studies and genetic linkage mapping (Williams, 1990) [26]. Both RAPD and ISSR markers have proved to be a reliable, easy to generate, inexpensive and versatile set of markers that rely on repeatable amplification of DNA sequence using single primer. The RAPD and ISSR markers have been used to study the genetic diversity analysis of *Zingiber officinale* cultivars, reported by (Gao *et al*. 2006; Nayak. 2005; Mohd *et al*. 2004; Wahyuni *et al*. 2003 and Rout *et al*. 1998) [7, 18, 17, 25, 21]. Diversity analysis and identification of genetically distant clones or genotypes are vital to the ginger improvement program, because most of the crop improvement programs of ginger are restricted to the assessment and selection of naturally occurring clonal variations (Rout *et al*., 1998; Palai and Rout., 2007) [21, 19]. Literature survey revealed lack of information on the molecular characterization of ginger from Gujarat region. The genetic diversity studies on ginger populations growing in Gujarat will helps to understand the diversity study of ginger of this region and conservation of gene pool for breeding purposes.

Materials and methods

List of clones and their known sources of collection is mentioned in Table 1.

Isolation of genomic DNA

Genomic DNA from frozen rhizome samples was isolated by modified cetyl trimethyl ammonium bromide (CTAB) extraction method (Doyle and Doyle, 1990) [5]. 500 milligrams of rhizome sample was triturated in liquid nitrogen to fine powder. One ml of 5% CTAB (1 M Tris–HCl buffer pH 8.0, 5 M NaCl, 0.5 M EDTA, 1.5% mercaptoethanol) buffer was added. This mixture was incubated at 65 ºC for about 60 min with intermittent shaking. The suspension was then cooled to room temperature and equal volume of (C:I-24:1) was added. The mixture was then centrifuged at 10,000 rpm for 18 min. The aqueous phase was collected, and to it 2/3 volume of isopropanol was added for precipitation of DNA followed by Incubation. Centrifuged the sample at 10,000 rpm for 18 min at 4ºC. The isopropanol was carefully drained and washed the pellet with 500 µl of 80% ethanol and centrifuged for 7 min at 8,000 rpm at 4ºC. The pellet was dissolved into 100 µl TE buffer. For RNase treatment 2 µl of RNase A (Fermentas, USA) was added, incubated it for 35ºC for 50 min followed by 65ºC for 10 min for denaturation of enzyme. The DNA quality of isolated genomic DNA was checked using 0.8% aga rose gel and quantified on nano drop spectrophotometer 1000 (V.3.3.0, Thermo Scientific, USA).

Table 1: List of clones and their known sources of collection

Sr. No	Genotypes collected from IISR-Indian Institute of Spice Research, ICAR (Calicut)	Sr. No	Genotypes collected from Agriculture Research Station, AAU, Dahod.
1	Athira	16	DGN12-6
2	Rio de Janerio	17	DGN14-22
3	IISR Mahima	18	DGN12-25
4	IISR Varada	19	DGN12-2
5	IISR Rejatha	20	DGN12-23
6	Suprabha	21	DGN12-48
7	Nadia	22	DGN14-23
8	Gourubathani	23	DGN14-20
9	Thinladium	24	DGN12-12
10	Thingpui	25	DGN12-11
11	Suruchi	26	DGN12-5
12	Bhaise	27	DGN12-3
13	Himachal	28	DGN12-1
14	Aswathi	29	DGN14-43
15	Mahim	30	DGN12-21

Genetic diversity analysis using RAPD and ISSR

Bulked DNA samples were prepared by pooling equal amount (20ng) of genomic DNA extracted from 31 genotypes. These bulked DNA were used in Polymerase chain reaction reaction for screening of RAPD, ISSR primers for genetic diversity study.

RAPD

Total of 180 primers of OPA, OPB, OPC, OPD, OPE, OPG, OPH, OPO and OPN Series were used for the screening by bulked DNA sample using end point PCR assay. Polymerase chain reactions (PCR) with single primer were carried out in a final volume of 25 µl containing 20 ng template DNA, 2.5

mM of each deoxyribonucleotide triphosphate, 10 p moles/µl primer (MWG Biotech, Germany), Dream *Taq* PCR buffer (10 x) with 20 mM $MgCl_2$ and 5 U/µl Taq DNA polymerase (Fermentas, USA). Amplification was performed in a thermal cycler (Eppendorf, Germany) programmed for a preliminary 7 min denaturation step at 95°C, followed by 40 cycles of denaturation at 94°C for 1 min., annealing at 38°C for 1 min and extension at 72°C for 1 min, finally at 72°C for 10 min for RAPD amplification. Amplification products were separated in agarose gel of 1.8% concentrations was prepared in 1XTBE. Standard DNA ladders *viz.*, 100 bp and 1 kb (Fermentas, USA) were also run along with the samples. The electrophoresis was carried out at 5 V/cm current (constant) for 1.5 h to separate the amplified bands. The separated bands were visualized under UV transilluminator (Genie, Bangalore, India) and photographed using gel documentation system (Alpha InfoTech, USA).

ISSR

ISSR PCR reactions were performed in a thermal cycler (Eppendorf) using 40 ISSR (MWG Biotech, Germany) primers. The reaction components and PCR condition for ISSR were same as of RAPD. Except for the annealing temperature of 50 ºC to 55.9 ºC for 1min.

Data collection and statistical analysis

Clear and distinct bands amplified by ISSR and RAPD primers were scored for the presence or absence of the corresponding band among the 31 ginger genotypes. The scores '1' or '0' indicates the presence or absence of bands, respectively. The data was entered into binary matrix and subsequently analysed using NTSYSpc (Numerical Taxonomy System Applied Biostatistics, Setauket, New York) version 2.02. UPGMA dendrograms were constructed based on the similarity matrix. Various components were calculated which included, number of monomorphic and polymorphic loci, polymorphism information content (PIC), marker index (MI), polymorphism% and resolving power (Rp).

Result and discussion

RAP analysis

RAPD analysis of the 180 primers tested 34 showed reproducible polymorphic bands. Out of 475 loci produced, 370 were polymorphic recording polymorphism percentage ranging from 38.46% (OPA-01) to 100% (OPA-16, OPD-19, OPE-09, OPG-07, OPG-10, OPG-11, OPG-19) with an average of 84.27% per primer. The average number of polymorphic loci obtained per primer was found to be 11. OPH-12 produced maximum number of 25 loci while minimum number of loci i.e. 4 was produced by primer OPE-09. Maximum number of 22 polymorphic loci was produced by primer OPH-12 while, primer OPE-09 produced minimum number of 4 polymorphic loci. The molecular size of amplified PCR products ranged from 118 bp (OPN-17) to 3132bp (OPC-18) with an average range of 273-1600bp per primer (Table 2). Rp values in the current study ranged from 6.96 (OPN-17) to 27.41 (OPG-09) indicating the variability in the discriminating capacity of a primer. The PIC values ranged from 0.74 to 0.93 with average of 0.88 per primer indicating hyper variability among the accessions studied. The results obtained in the present investigation were almost in agreement with the discovery of Sajeev *et al*. (2011) [24] where they were found out the Jaccard's similarity coefficient ranged from 0.57 and 0.96. The level of polymorphism detected in the species was dependent on a wide range of factors like breeding system, habitat specialization, intensity and direction of selection, type of the genetic material and the type of molecular marker used. Moreover higher value of PIC was an indication of the high amount of diversity present among the accessions. Marker index value was 18.8. Jaccard's similarity coefficient of RAPD showed that Gourubathani and Suprabha, Nadia and Mahim were most closely related with a similarity value of 0.92 and DGN12-6 and DGN12-23 were most remotely placed with a similarity coefficient of 0.60. Between any two genotypes the average similarity coefficient was calculated to be 0.76. r=0.96 indicated a very high correlation between the similarity matrix and dendrogram pattern. The dendrogram based on UPGMA analysis grouped all the genotypes into two major clusters A and B (Fig.1) with similarity coefficient ranging from 0.65 to 0.92. Cluster B contained only one genotype DGN12-23 which is the high yielding variety. Major Cluster A was again divided into two subminor clusters A1 and A2. The subminor cluster A2 contains only one genotype DGN12-6 while, sub minor cluster A1 was further divided into two minor subclusters A1a and A1b. The minor sub-cluster A1b contained only single high yielding genotype DGN12-3. The minor sub cluster A1a was further divided into two clusters A1a1 and A1a2. The cluster A1aii contains only single genotype DGN14-22. The cluster A1ai was further divided into two subcluster. The cluster A1ai1 included all the genotypes collected from IISR along with Gujarat local variety. In cluster A1ai1 Bhaise, IISR Varada, Himachal, IISR Mahima, Athira were grouped together on the basis of bold rhizome, high yielding, high dry recovery content, while Thingpui and Aswathi were grouped together, due to high dry recovery and low fibre content. Rio-de-janeiro, Gourubathani and suprabha were grouped together contain high oleoresin, high yielding variety. Suruchi and Nadia contain slender rhizome while Mahim is the low yielding variety. IISR Rejatha is the improved variety with high oleoresin and high essential oil content, while Thinladium is the variety with high dry recovery content. Local variety showed 0.90 highest similarity to Rio-de-Janeiro while lowest similarity of 0.67 with DGN12-23. Thinladium and IISR Rejatha were highly diverse than all the other varieties in this cluster and formed single cluster. Cluster A1ai2 contain all the remaining genotypes. The dendrogram constructed involving 31 ginger genotypes using pooled RAPD data distinguished all genotypes based on geographical collection. In the clustering pattern of the accessions/germplasm collected from Gujarat locations showed highly diverse than the germplasm collected from Calicut.

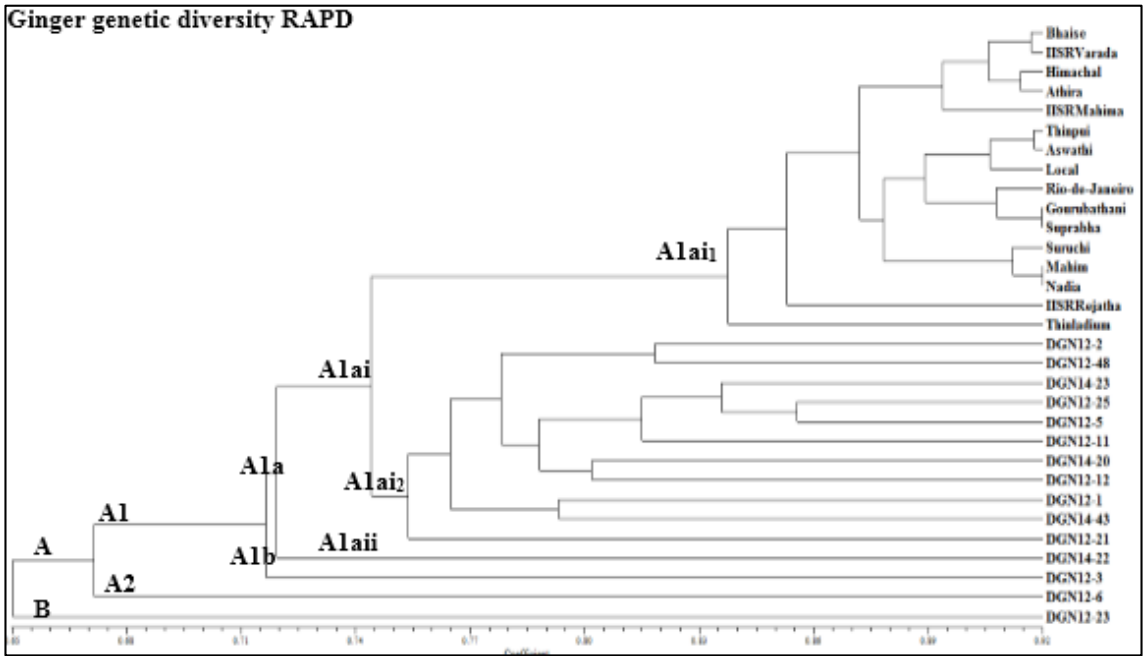


Fig 1: Dendrogram showing clustering of 31 ginger genotypes constructed using UPGMA based on Jaccard's coefficient obtained from RAPD analysis

Table 2: Results of randomly amplified polymorphic DNA analysis

Sr. No.	Prime Name	Range of molecular weight (bp)	Total No. of bands	Total No. of Loci	No of polymorphic loci	No of monomorphic loci	Percent Polymorphism (%)	PIC value	Rp Value
1	OPA-01	126-1335	317	13	5	8	38.46	0.9	20.45
2	OPA-02	237-1963	274	13	9	4	69.23	0.89	17.67
3	OPA-03	321-1504	245	11	8	3	72.72	0.89	15.77
4	OPA -15	174-1253	250	10	6	4	60	0.88	16.12
5	OPA-16	240-1442	202	12	12	0	100	0.84	13.03
6	OPA-19	167-1913	245	15	13	2	86.66	0.89	15.8
7	OPA-20	342-2825	286	11	7	4	63.63	0.9	18.45
8	OPB-06	215-1529	215	10	5	5	50	0.87	13.87
9	OPB-07	362-1613	308	13	8	5	61.53	0.91	19.87
10	OPB-12	356-966	132	7	6	1	85.71	0.78	8.51
11	OPC-01	192-915	283	11	8	3	72.72	0.89	18.25
12	OPC-02	688-1509	213	12	11	1	91.66	0.88	13.74
13	OPC-14	174-1252	259	15	12	3	80	0.89	16.7
14	OPC-18	238-3132	286	15	11	4	73.33	0.9	18.45
15	OPD-19	109-1853	339	19	19	0	100	0.93	21.87
16	OPD-20	307-1525	400	20	12	8	60	0.92	25.8
17	OPE-06	300-1732	347	14	11	3	78.57	0.92	22.38
18	OPE-07	240-1279	307	13	6	7	46.15	0.9	19.8
19	OPE-08	144-1361	202	11	8	3	72.72	0.87	13.03
20	OPE-09	349-1191	116	4	4	0	100	0.74	7.48
21	OPE-11	250-1300	200	9	6	3	66.66	0.86	12.9
22	OPE-18	325-1335	367	17	9	8	52.94	0.92	23.67
23	OPG-07	153-2830	361	21	21	0	100	0.93	23.29
24	OPG-09	201-1266	425	19	17	2	89.47	0.93	27.41
25	OPG-10	308-1353	304	14	14	0	100	0.91	19.61
26	OPG-11	504-1309	207	16	16	0	100	0.88	13.35
27	OPG-19	424-1252	231	17	17	0	100	0.9	14.9
28	OPG-20	267-1285	305	17	15	2	88.23	0.91	19.67
29	OPH-12	264-1702	405	25	22	3	88	0.93	26.12
30	OPK-15	401-2264	228	11	5	6	45.45	0.87	14.7
31	OPN-06	304-1860	313	18	14	4	77.77	0.91	20.16
32	OPN-17	118-1174	108	13	12	1	92.3	0.82	6.96
33	OPO-13	234-1365	377	18	12	6	66.66	0.92	24.32
34	OPO-19	258-1720	153	11	9	2	81.81	0.84	9.87
Total		9292-54410	9210	475	370	105	2613	31.02	594
Mean		273-1600	270.88	14	11	3	76.83	0.88	17.46

PIC = Polymorphism information, **Rp**- Resolving power

ISSR analysis

Out of 40 ISSR primers, 14 primers generated a total 4076 scorable bands with 245 loci, among them 213 loci were found to be polymorphic showed 87.10% polymorphism. The minimum sized amplified fragment (138 bp) was obtained by primer ISSR-15, whereas maximum sized fragment (4307 bp) was amplified by primer ISSR-06. The highest (100%) polymorphism were exhibited by primers ISSR-01, ISSR-10, ISSR-15, ISSR-20 while lowest polymorphism (44.44%) was observed with primer ISSR-06. Maximum number of 25 polymorphic loci was produced by primer ISSR-01 while, primer ISSR-21 produced minimum number of 5 polymorphic loci. The molecular size of amplified PCR products ranged from 138 bp (ISSR-15) to 2863 (ISSR-20) with an average range of 243-2104bp per primer (Table 3). Primers produced bands specific to a particular clone or a group of clones (Table 3). The resolving power of the primers was varied from 6.7 (UBC 845) and 28.12 (ISSR-06) and the ISSR marker index was 8.38. The maximum PIC value was observed from 0.77 (UBC-845) to 0.94 (ISSR-10, ISSR-22). Moreover higher value of PIC was an indication of the high amount of diversity present among the accessions. The highest similarity index value of 0.95 was found between Suruchi and Mahim. While the least similarity index value of 0.39 was found between DGN12-6 and DGN12-25. The results obtained in the present investigation were in agreement with Saha *et al.* (2016) [23] in which they have studied the diversity in different species of Curcuma with 0.47 to 0.97 similarity matrices. The cluster diagram showed in Fig 2 revealed the presence of two major groups A and B, which showed the existence of high amount of genetic diversity among accessions. Cluster B contained only one genotype DGN12-6. Major Cluster A was again divided into two minor clusters A1 and A2. The minor cluster A2 contain three genotypes DGN12-25, DGN12-48, DGN14-23. The minor cluster A1 was further divided into two minor sub clusters A1a and A1b. The minor sub-cluster A1b contained four genotypes DGN14-20, DGN12-12, DGN12-1 and DGN12-3. The minor sub cluster A1a was further divided in to two clusters A1a1 and A1a2. The cluster A1a2 contained DGN14-22, DGN12-2, DGN12-23, DGN12-11, DGN14-43, DGN12-21 and DGN12-5. While A1a1 cluster contained all the genotypes collected from IISR, Calicut with DGN14-22 and Local of Gujarat Dahod collection. In cluster A1a1 Bhaise, IISR Varada, Himachal, Athira, Thingpui, Aswathi, Gourubathani, suprabha, Suruchi and Nadia were grouped together based on bold rhizome, high yielding and high dry recovery content. While IISR Mahima, Rio-de-Janeiro and IISR Rejatha were high yielding, high oleoresin content varieties and they were distantly related to the other varieties and form a separate group in these cluster. While Gujarat local and DGN 14-22 were also distantly related to the other varieties. The highly diverse genotypes were observed on basis of clustering pattern and genotype DGN12-6, DGN12-25 with least similarity coefficient 0.39. Similar to RAPD, clustering pattern generated by ISSR detected relatively higher genetic diversity within the genotypes from Gujarat as compared to Calicut accessions. Cophenetic correlation was high (r = 0.95) showed a good fit of the similarity matrix with the dendrogram.

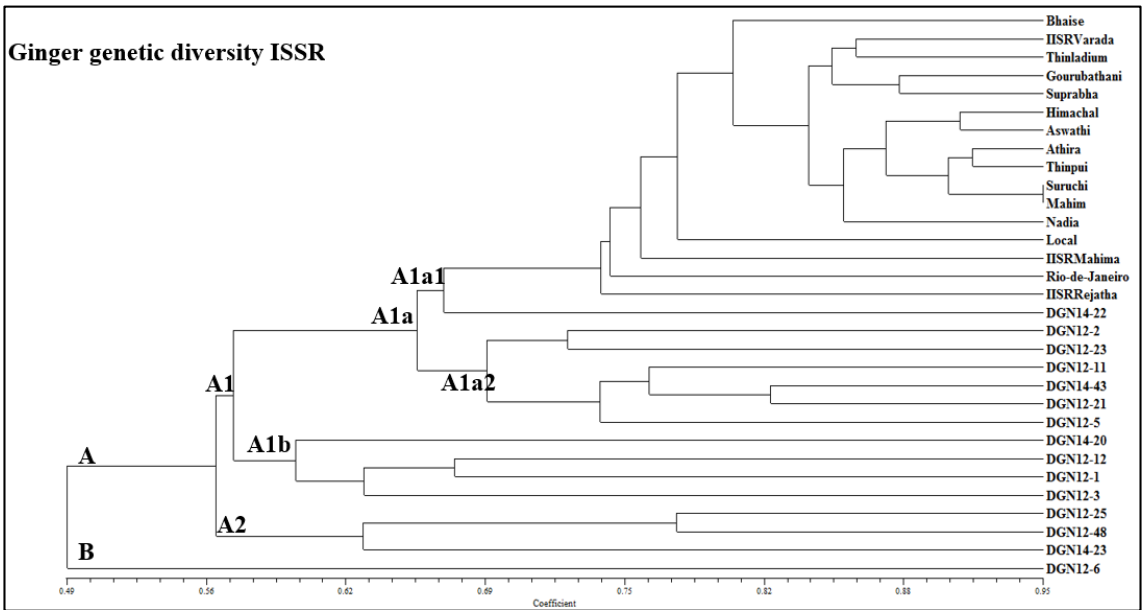


Fig 2: Dendrogram showing clustering of 31 ginger genotypes constructed using UPGMA based on Jaccard's coefficient obtained from ISSR analysis.

Table 3: Results of inter simple sequence repeat analysis

Sr. No.	Primer Name	Range of molecular weight (bp)	Total No. of bands	Total No. of Loci	No of polymorphic loci	No of monomorphic loci	Percent Polymorphism (%)	PIC value	Rp Value
1	ISSR-01	226-2218	180	25	25	0	100	0.91	11.61
2	ISSR-02	227-1299	275	14	11	3	78.57	0.91	17.74
3	ISSR-05	265-2240	351	21	18	3	85.71	0.93	22.64
4	ISSR-06	222-2293	436	18	8	10	44.44	0.93	28.12
5	ISSR-07	186-2077	390	19	17	2	89.47	0.93	25.16
6	ISSR-10	214-2296	387	25	25	0	100	0.94	24.96
7	ISSR-15	138-1760	308	23	23	0	100	0.93	19.87
8	ISSR-20	171-2863	298	20	20	0	100	0.92	15.09
9	ISSR-21	319-1593	234	9	5	4	55.55	0.88	19.22

10	ISSR-22	178-2253	432	19	15	4	78.94	0.94	27.87
11	ISSR-35	218-2271	268	13	10	3	76.92	0.9	17.29
12	UBC-815	214-1786	103	11	10	1	90.90	0.78	6.64
13	UBC-845	528-1685	104	13	12	1	92.30	0.77	6.7
14	UBC-888	294-2823	310	15	14	1	93.33	0.91	20
Total		3400-29457	4076	245	213	32	1186	12.58	262.91
Mean		243-2104	291	18	15	2	84.72	0.89	18.77

PIC = Polymorphism information, **Rp**- Resolving power

Combined marker analysis

Total number of bands amplified with two markers in 31 genotypes is 13286 with an average value of 16 per primer. Polymorphism percentage when compared with two markers RAPD & ISSR, ISSR scored the highest with polymorphism of 84.72% whereas RAPD showed least polymorphism of 76.83% (Table 4). In combination studies, RAPD+ISSR showed almost equal polymorphism with ISSR of approximately 80.77%. Average number of polymorphic loci was highest in ISSR that is 15 than 11 in RAPD while combined showed 13 loci. Marker index, average number of polymorphic loci were also found to be the highest for RAPD as compared to ISSR. This may be due to more number of RAPD primes used in the present study than the ISSR primers employed. The Jaccard's similarity coefficient values for pooled ISSR and RAPD data indicated that, Suruchi was more genetically similar to Mahim with 0.92 similarity. Whereas, DGN12-6 was genetically dissimilar to DGN12-23 with 0.55 with average value 0.73.

The dendrogram constructed through pooled RAPD and ISSR divided it into two main clusters consisting cluster A and B. Cluster B included only single genotype DGN12-6 (Fig 3.). Cluster A was further divided into two minor clusters A1 and A2. Cluster A2 contained single genotype, DGN12-23. The minor clusterA1 was further divided into two sub minor clusters. The cluster A1b contained genotypes of Gujarat Dahod collections while cluster A1a contained all the genotypes collected from IISR, Calicut. In cluster A1a Bhaise and IISR Varada (bold rhizome, high yielding varieties) were grouped together with improved variety IISR Mahima. Himachal, Athira, Aswathi, Thingpui and Nadia were grouped together because of high dry recovery content. Suruchi and Mahima are low yielding varieties which showed highest similarity than all the varieties in this cluster. Rio-de–Janeiro, Gourubathani and Suprabha formed the same group due to high yielding, high oleoresin content. DGN14-22, Thinladium and IISR Rejatha were distantly related to all other varieties and cluster singly in these dendrograme. In this A1a cluster Mahim and Suruchi showed maximum similarity to each other as compared to all other varieties in this cluster. Genotypes, DGN12-6 and DGN12-23 were highly diverse than all other genotypes with lowest similarity value 0.55. Similar to RAPD and ISSR, pooled data of RAPD and ISSR showed that the genotypes collected from nearby regions in Gujarat were more genetically distant as compared to genotypes collected from geographically different area i.e. IISR, Calicut. The results obtained in the present investigation were in agreement with sera *et al*. (2003) [22] in coffee cultivar.

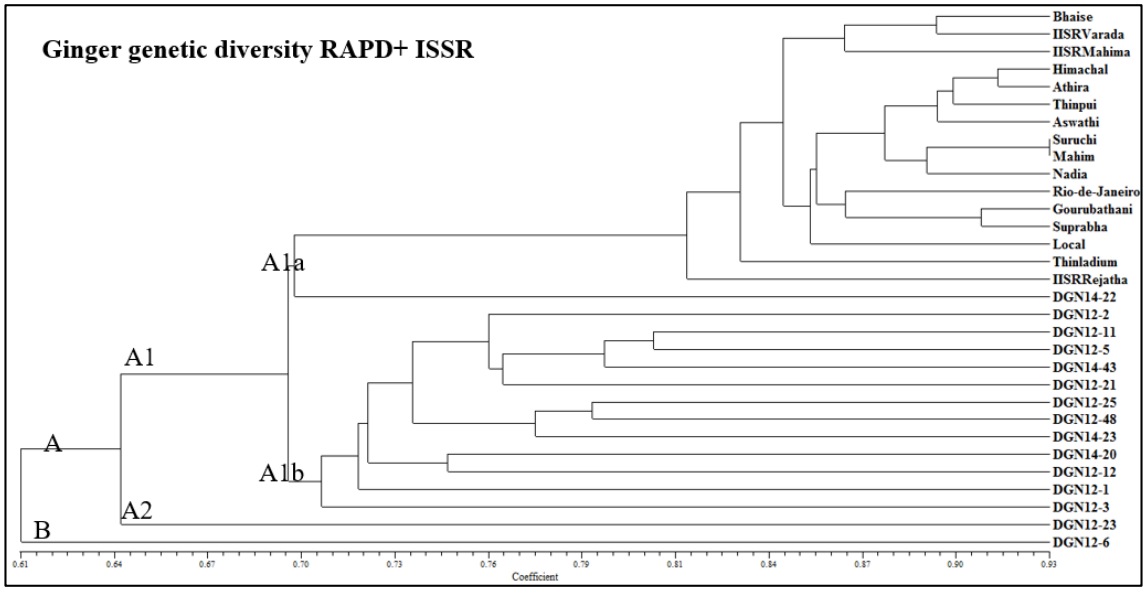


Fig 3: Dendrogram showing clustering of 31 ginger genotypes constructed using UPGMA based on Jaccard's coefficient obtained from pooled RAPD+ISSR analysis

Table 4: Pooled RAPD and ISSR analysis

Parameters	RAPD	ISSR	RAPD+ISSR
Primers Used	34	14	48
Total amplicons obtained	9210	4076	13286
Average bands obtained	271	291	281
Average No of bands/primer	14	18	16
Total loci analysed	475	245	720
Total No of monomorphic loci	105	32	137

Total No of polymorphic loci	370	213	583
Average no of polymorphic loci	11	15	13
Average Polymorphism%	76.83	84.72	80.77
Average Polymorphism Information Content(PIC value)	0.88	0.89	0.88
Average genetic similarity coefficient	0.76	0.67	
Resolving Power (Rp)	17.46	18.77	18.11
Marker Index(MI)	18.8	8.38	13.59

Genetic variability parameter comparison between RAPD and ISSR

Various statistical parameters for genetic variability calculated for each marker and accordingly the efficiency of marker for its suitability for diversity analysis was ascertained. In comparision between Polymorphism percentages of two markers, ISSR scored the highest polymorphism of 84.72% whereas RAPD showed least polymorphism of 76.83% (Table 4). In combination studies, RAPD+ISSR showed almost equal polymorphism with ISSR of approximately 80.77%. Average number of polymorphic loci was highest in ISSR that is 15 than 11 in RAPD while combined showed 13 loci. Marker index value was the highest for RAPD marker (18.8) as compared to ISSR (8.38). Resolving power (Rp) value was found to be highest in ISSR (18.77) than the RAPD (17.46). Thus by considering the polymorphism percentage and resolving power, it can be concluded that ISSR markers was found to be more efficient as compared to RAPD markers. Other parameters calculated i.e. marker index, average number of polymorphic loci were also found to be the highest for RAPD as compared to ISSR. This may be due to more number of RAPD primes used in the present study than the ISSR primers employed.

Discussion

In this report, two RAPD and ISSR PCR-based markers have been used to characterize 31ginger genotypes. Molecular markers are a powerful tool for identification and characterization, cultivar development, certification and breeder's right's protection of different germplasm resources at the genetic level (Hale *et al.* 2006) [9]. The marker choice is depends on the purpose of which the marker system will be used to study germplasm characterization, varietal identification, clonal fidelity testing, assessment of genetic diversity, validation of genetic relationship, phylogenetic and evolutionary studies, marker-assisted selection and gene tagging. However a marker system in one species does not give same applicable specify to another species. As per the stability, plasticity, specificity, ubiquity DNA markers RAPD and ISSR are easier, efficient and less time consuming, compare to morphological markers. The level of polymorphism in a ginger species is dependent on a wide range of factors like breeding system, habitat specialization, intensity and direction of selection, genetic material and molecular marker used (Chandrade, 2017) [3]. The highest polymorphism detected by ISSR in this study displayed the resolving power of this marker for diversity study. Due to little information is available on genome and the molecular characterization of ginger, there is need for the studies underlying the genetic diversity of ginger for breeding and conservation purposes. In different species molecular characterization had been carried out by many researchers using PCR-based molecular markers like RAPD, ISSR, SSR and AFLP (Fu *et al*. 2008; Lu *et al*. 2009: Kizhakkayil and Sasikumar, 2010) [6, 16]. In the present study, the molecular marker data separated the clones into two groups Gujarat accession and Kerala accession based on geographical bias because of their habitat homogeneity. Kizhakkayil and Sasikumar (2010) also reported a similar results and geographically grouped the *Z. officinale* clones from India.

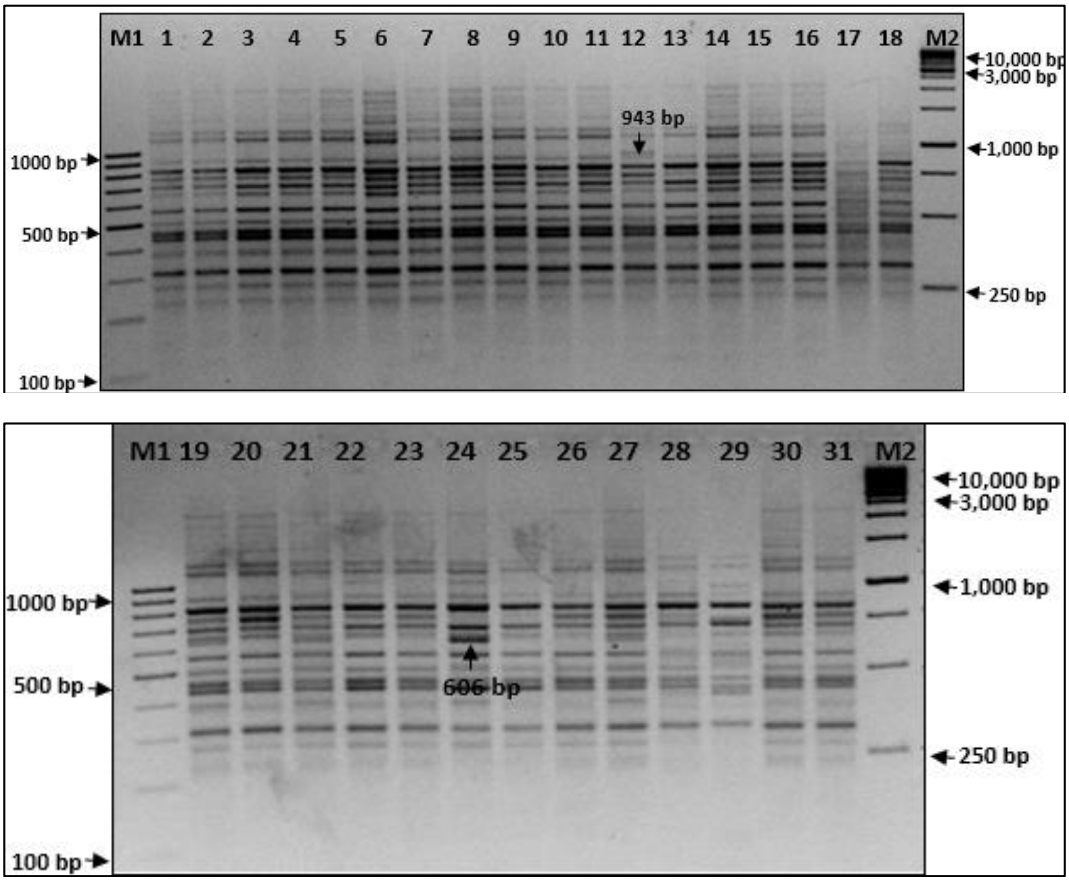
Conclusion

In Zingiberaceae family low genetic variation were observed because of asexually propagation and the lack of genetic recombination. The species specific bands amplified in different species (Table 5), would help in further development of varietal specific SCAR marker, certification of species as well as in protection of breeder's right. Our Results showed that the accessions collected from nearby regions shows high genetic variability than the accessions collected from farther apart. The main cause of polymorphism could be intraspecific variation among different cultivars. These findings will also provide an important contribution in determining resourceful management strategies for breeders for ginger improvement program.

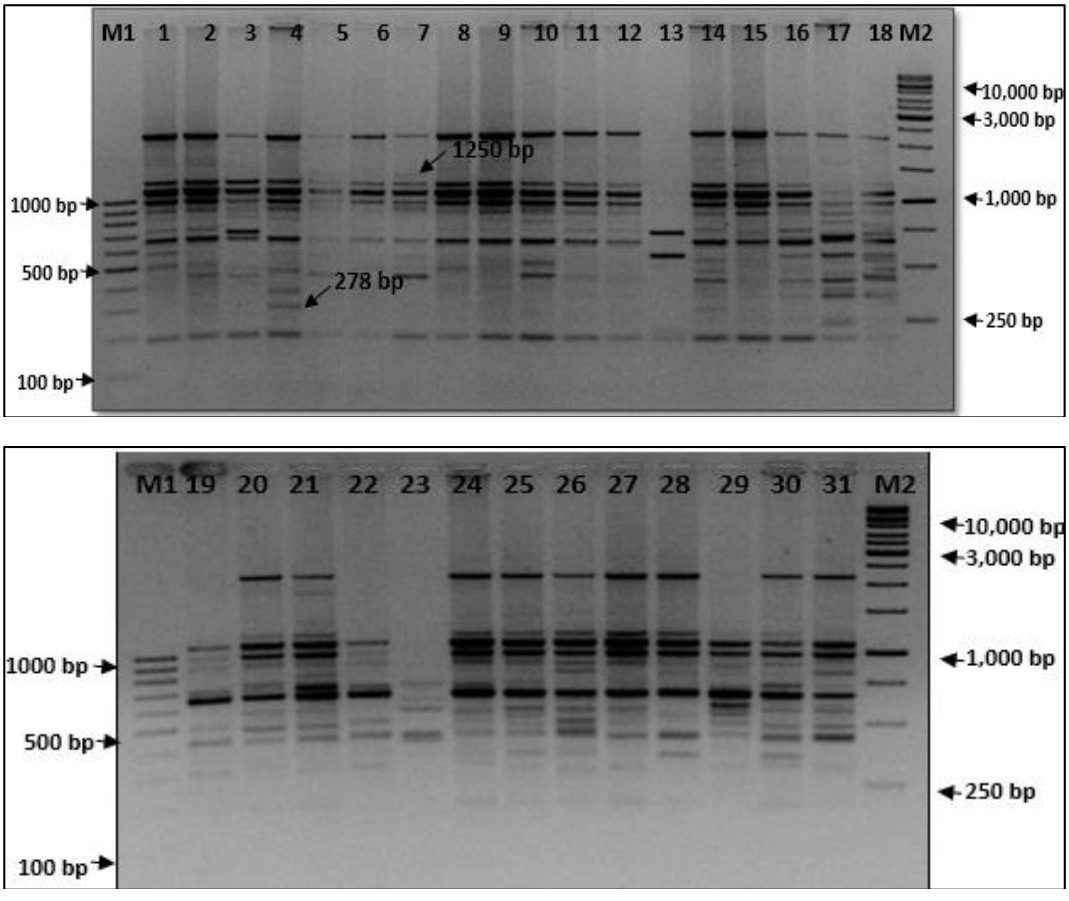
Table 5: RAPD and ISSR primers and their unique bands in different ginger genotypes

Sr. No.	Ginger genotypes	primers	Size of bands
1.	IISR Rejatha	OPE-18(RAPD)	463 bp
		ISSR-06	943 bp
2.	IISR Varada	OPC-18(RAPD)	517 bp
3.	Aswathi	ISSR-20	1250 bp
4.	DGN14-20	ISSR-06	606 bp
		ISSR-07	1166bp
5.	Himachal	ISSR-20	278 bp
6.	Mahim	OPG-19(RAPD)	1347 bp
7.	Bhaise	OpO19(RAPD)	353 bp
8.	DGN12-3	OPO19(RAPD)	1084 bp
9.	DGN12-11	OPN17(RAPD)	569 bp
10.	DGN12-23	OPC02(RAPD)	791 bp
11.	DGN12-12	OPC02(RAPD)	1023 bp
		OPG-20(RAPD)	721 bp
12.	DGN12-6	OPG-19(RAPD)	1347 bp
13.	DGN12-5	ISSR-35	1468 bp

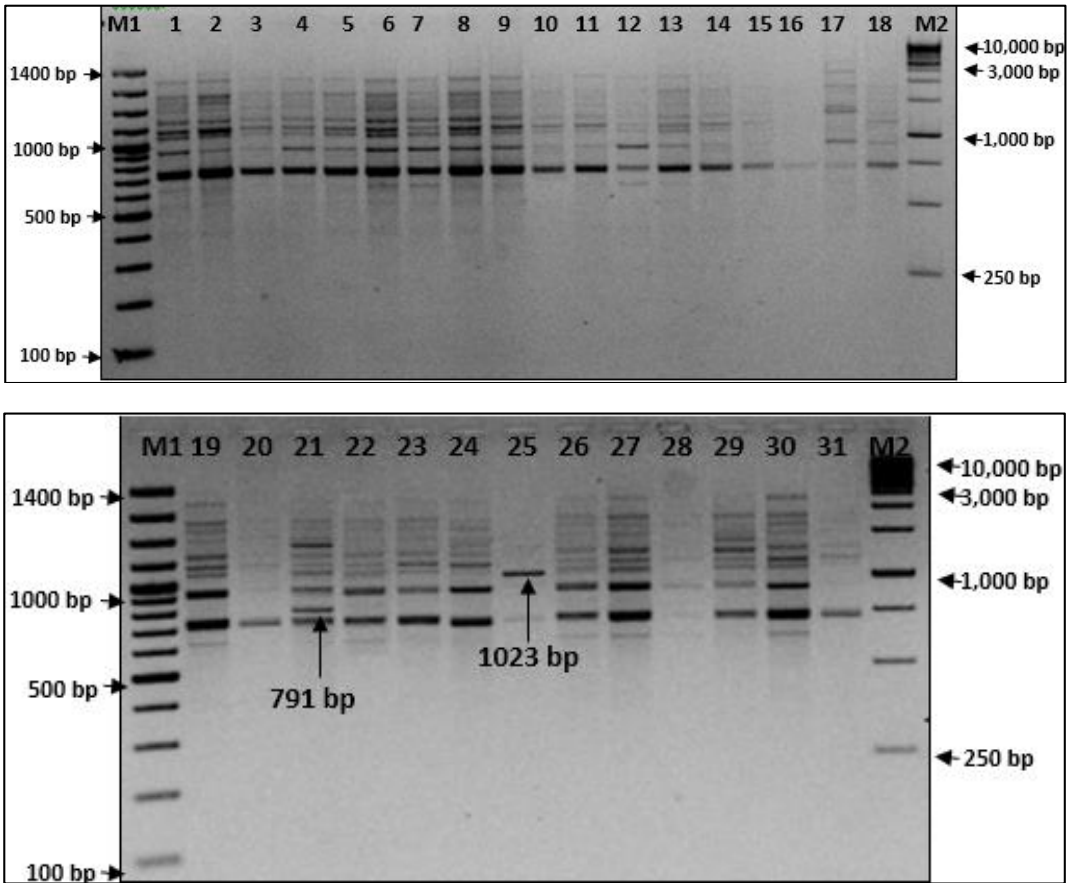
RAPD- Random Amplified Polymorphic DNA, ISSR- Inter Simple Sequence Repeat



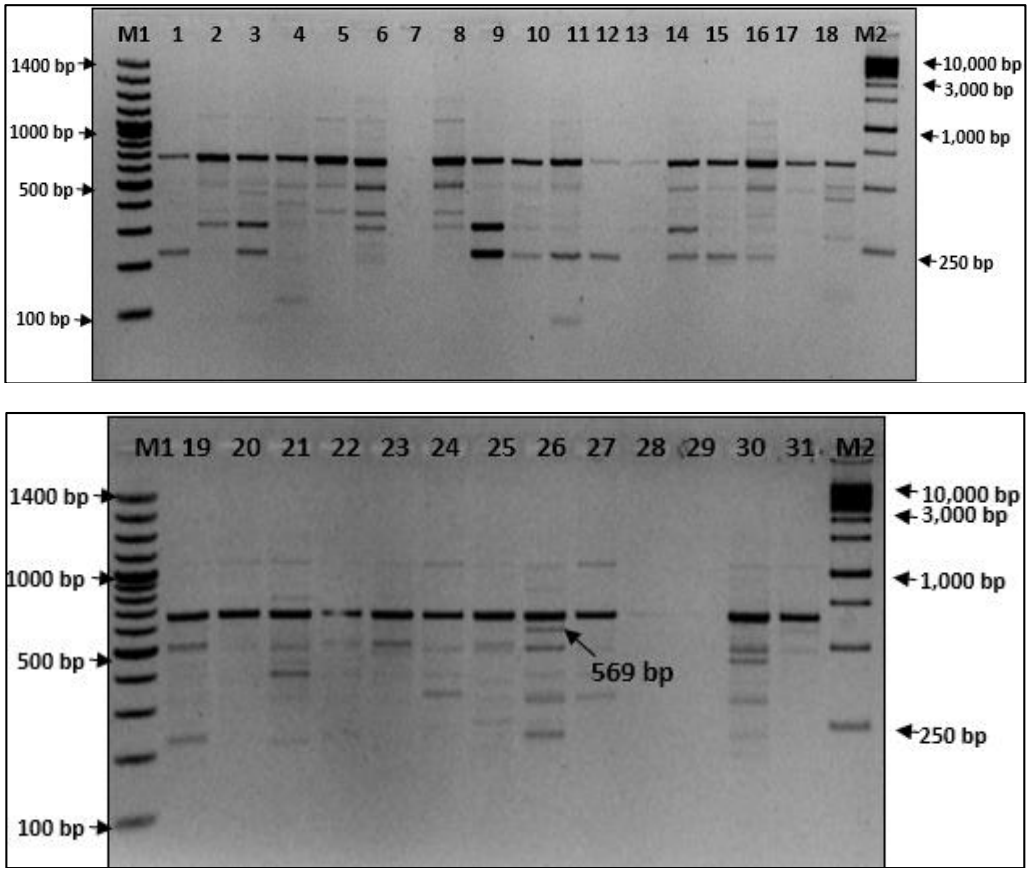
ISSR profile of ginger genotypes generated by primer ISSR-06



ISSR profile of ginger genotypes generated by primer ISSR-20



RAPD profile of ginger genotypes generated by primer OPC-02



RAPD profile of ginger genotypes generated by primer OPN-17

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