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Molecular & Biochemical characterization of selected elite accessions of ginger

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ABSTRACT

Ginger (*Zingiber officinale* Roscoe) rhizomes have been widely used as a spice and flavoring agent in foods and beverages. Twenty ginger varieties which were selected for disease reaction, were also analyzed their biochemical properties such as protein, phenol polyphenol oxidase and fiber content to assess any relationship with disease incidence/disease reaction. The protein content ranged between 5.5 and 21.4 mg/g and fiber content between 4.5 and 9.9%. The total phenol content was also varied among the varieties ranging from 0.63 mg/g to 1.5 mg/g. The polyphenol oxidase range varied from 2.6 to 98.0 U/mg. These findings indicate that these 20 varieties of ginger contains phenolics in an appreciable amount so these plants can be commercially exploited. Molecular analyses of these varieties were also performed. ISSR markers were used to characterize these elite cultivars of ginger. Based on similarity coefficient analysis the dendogram showed two distinct clusters with 19 varieties in cluster I and only 1 in cluster 2. No correlation between the disease incidence with the biochemical and molecular analysis was seen.

1. Introduction

Ginger (*Zingiber officinale* Roscoe) is an economically important plant, valued all over the world for its medicinal properties. It has a respected history as a spice crop and is known to human generation since time immemorial (Anonymous, 1950). It is used in medicine as a carminative and an aromatic stimulant to the gastrointestinal tract and externally as a rubefacient and counter irritant (Rout and Das, 2002). The use of ginger oil and oleoresin in various food and drink items has increased its economic importance in the global market. The rhizome is also used as an antidiarrheal medicine in its powdered form. Phytochemical, pharmacological and toxicological properties of ginger have been reported by Ali et al., 2008. Many common ginger cultivars in India are mainly recognized by their locality of cultivation/ collection. The differentiation of the cultivars rather difficult due to absence of clear-cut morphological features coupled with the lack of specific characters (Shamina et al., 1997). So biochemical and molecular markers presume significance. The use of biochemical markers for germplasms characterization has been seen in several crops (Weeden and Weeden, 1985; Al-Jibojuri and Adham, 1990; Demiera and Vega, 1991; Bhat et al., 1992; Bult and Kiyangi, 1992). The chemistry of ginger is well documented with the respect to the oleoresin and volatile oil (Barnes et al., 2002; Sweetman, 2007). More than 400 chemicals have been identified in ginger rhizome (Garner-Wizard et al., 2006). The relative proportions of chemicals were determined by geographical location and age of rhizome during harvesting and extraction method. Chemical constituents of ginger categorised to volatile oils which constitutes (1–3%) mainly of zingeberene (Robbers et al., 1996) nonvolatile pungent compounds oleo-resin constitute (4–7.5%) mainly gingerols and other constituents with more than 50% of starch (Robbers et al., 1996), many fats, waxes, carbohydrates, vitamins and minerals. A variation of the phytoconstituent was observed when the cultivars were collected from various geographical region (Ravindran and Babu, 2004).

The available genetic resources can be utilized to improving the plant by diversity characterization. Conventionally, genetic diversity assessment was confined to morphological observations and progeny evaluation, but they had restriction of being plastic and environmentally-sensitive. During the past two decades, molecular markers have been widely used to overcome these deficiencies. At present the molecular marker techniques have revealed their potential and wide range of significance in recognizing genetic purity of germplasm stocks (Joshi et al., 2000), understanding genome organization, frequency and level of diversity in large and complex genomes (Blair et al., 1999), identifying genetic relationships (Tsumura et al., 1996), chromosome mapping

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(Giura and Saulescu, 1996), trait tagging and inheritance (Kelly et al., 2003) and molecular breeding (Gupta and Varshney, 2000). Inter-simple sequence repeat (**ISSR**) is a molecular marker which is a fast, inexpensive genotyping technique based on variation in the regions between microsatellites. ISSR markers are more reproducible than RAPD and have been proven to be a simple and reliable marker system for many organisms, especially plants, with highly reproducible results and abundant polymorphisms. ISSRs have been successfully used to estimate the extent of genetic diversity at inter- and intra-specific level in a wide range of crop species which include fingermillet, Vigna Savi, sweet potato, and Plantago major L. It has also been used for resolving problems relating to the phylogeny of Asian cultivated rice Oryza sativa L. (Joshi et al., 2000), wheat, and Diplotaxis DC. species. Literature states that there is a lack of information about the chemical and molecular characterization of ginger from Odisha.

The present work is an attempt to characterize 20 selected accessions of ginger germplasms available at HARS, Pottangi based on its Isozyme estimation and metabolite (protein, phenol and fiber) contents and their molecular characterization.

2. Materials and method

2.1. Screening of ginger germplasms

Survey for disease incidence was carried out at different locations as presented in the Table 1. In the field survey it was found that major portion of the cultivation uses the released varieties of Orissa (Suprava, Suravi and Suruchi) and a minor part is cultivated for local collected varieties (Pottangi local and Kalinga local). High Altitude Research Station (HARS), Pottangi maintained a large numbers (155) of filtered germplasms both from released varieties and locally collected varieties. All the filtered varieties were maintained in randomized block design (RBD) a plot size of $(1 \times 3)\ m^2$ with 30 cm spacing. Hence germplasm field of HARS, Pottangi, with geographical coordinates 18° 34′ 0″ North, 82° 58′ 0″ East was selected for screening of samples and collection of resistant varieties in the field condition. The cultivars were selected on the basis of yield ratio, resistance and degree of susceptibility (highly susceptible, susceptible and partially resistant) for comparative study. All the collected samples were high yielding varieties.

155 germplasms of ginger were screened against rhizome rot under field condition. The experiment setup established and carried out at HARS, Pottangi. Plantation was done in the month of April-May and was harvested after 11–12 months from the date of plantation. A total of 20 numbers of varieties were selected (Table 2) on the basis of percent disease incidence and maximum yield. The experiment was carried out in a randomized block design (RBD) with three replications (plot size 1 m × 3 m).

Table 2
Collection of 20 germplasms of Ginger from HARS Pottangi.

Sl. no.	Name of the germplasms	Source
P1	V1K1-1	Mutant from Rudrapur local (MH)
P2	Tura	Collection from Manipur
P3	Suvada	Mutant variety from Reo-de-jenero, HARS Pottangi
P4	Burdwan	Collection from Burdwan district (WB)
P5	Sleeva Local	Local collection from Africa
P6	Suravi	Mutant from Rudrapur local (MH)
P7	NO.12	Local selection Orissa
P8	Banspal	Local selection, Keonjhar, Orissa
P9	Suprava	Selection from Pottangi local, Orissa
P10	Jugijan	Local selection, Kerala
P11	Kalinga Local	Local selection, Kandhamal, Orissa
P12	Pottangi Local	Local selection, Koraput, Orissa
P13	Kuruppampadi	Local Selection from, Kerala
P14	ACC-60	Selection, ISSR, Calicut, Kerala
P15	Vaysay	Local Selection from, Kerala
P16	Sargiguda	Local selection, Koraput, Orissa
P17	China	Local collection from China
P18	Varada	Variety, ISSR, Calicut, Kerala
P19	Wild Ginger	Local Collection from Pottangi, Koraput, Orissa
P20	Singjhara	Local Collection from, Koraput, Orissa

Germination percentage counts at 45 DAP and percentages of rhizome rot incidence at 150 DAP during the harvesting period. In respect to rhizome rot the percent disease was calculated by using the formulae. Finally according to PDI, the germplasms were selected categorically and classified in to different types of reactions were determined (Table 3).

Percent Disease Incidence (PDI)=No. of plants infected/Total no. of plants × 100.

2.2. Biochemical analysis

Biochemical analysis of selected germplasms was undertaken by estimating total protein content, total fiber content, total phenol content and activity of polyphenolic oxidase.

2.2.1. Extraction and quantification of total protein from each variety

Buffer soluble protein was isolated in a buffer system suggested by Sengupta and Chattopadhyay (2000). Rhizome proteins were isolated from dried rhizome as per (Dadlani and Varier, 1994). For rhizome protein 100 mg of rhizome powder was used. The rhizome proteins were quantified following the protocol of (Bradford, 1976) using known concentration of Bovine Serum Albumin (BSA) as standard. The absorbance at 595 nm was measured in an UV–VIS Spectrophotometer (Specord, Analytica Jena, Germany) with Bradford reagent (0.01% Coomassie brilliant blue G 250, 4.7% ethanol, and 8.5% phosphoric acid). A standard curve was prepared taking known concentration of BSA protein and the protein

Table 1
Different locations under the research programme.

Sl. no.	Name of different location	Geographic position	Soil type
1.	HARS, OUAT, Pottangi, Koraput	Koraput is located at 18°49′N and 18.82°N latitude and 82°43′E and 82.72°E longitude. It has an average elevation of 870 m (2854 feet). Height from Sea Level is 300–900 m, Temperature Maximum- 30 °C to Minimum- 5 °C. Average Annual Rainfall – 1587 m m.	Sandy, loamy, lateraitic, Acidic soil pH – 6.2
2.	Farmers field, Semiliguda, Koraput		
3.	Farmers field, Nandapur, Koraput		
4.	Farmers field, Kalinga, Kandhamal	Kandhamal is located 19.34 and 20.50° North Latitude and 80.30 and 84.48° East Longitude. Height from Sea Level is 300–1100 m, Temperature Maximum – 40 °C to Minimum – 5 °C, Average Annual Rainfall – 1587 m m.	Lateritic, humic, Acidic soil pH – 5.8
5.	Farmer's field, Raikia, Kandhamal		
6.	Farmersfield, Daringibadi, Kandhamal		

Table 3
Selection of 20 numbers of varieties on the basis of disease reaction were determined on 1–5 scale.

Scoring scale	Percent disease incidence	Reaction
1	0	Resistant (R)
2	1–10	Tolerant/partially resistant (PR)
3	11–25	Partially susceptible (PS)
4	26–50	Susceptible (S)
5	> 50	Highly susceptible (HS)

contents of the unknown samples were measured using the standard curve.

2.2.2. Estimation of total fiber

Crude fiber consists of cellulose, variable proportions of hemicelluloses and highly variable proportions of lignin along with some minerals. The estimation is based on the percent (%) by weight of residue after successively treating the sample for moisture and fat with dilute acid and dilute alkali.

2 g of dry sample of each variety was taken and the fat was extracted for about 16 h with petroleum ether. Fat free samples were transferred to 1liter capacity spout less beaker and 200 ml 1.25% H_2SO_4 was added. The beaker was connected with a round bottom condenser for reflux and heat was applied to boil the content. The contents were frequently shaken such a way materials not sticked to the wall of the container and boiling was continued. Beaker was removed and filtered through the muslin cloth with the help of suction pump the residue was washed with hot water till it was free from acid. The material was added with 200 ml of 1.25% NaOH solution in a beaker and refluxed the contents for 30 min. The solution was filtered again through the sintered glass crucible with the help of suction pump and washed the residue with hot water till it was free from alkali. 1% HCl was used to quick removal of alkali. The sintered glass crucibles were kept in hot air oven at 100 °C for drying and then record the weight. The residue was ignited in muffle furnace at 55 °C for two hours and cooled. Final weight of residue was taken. The loss of weight was due to ignition of crude fiber.

$$\text{Crudefiber \% (onDMbasis)} = W_1 - W_2 / W_0 \times 100$$

where, W_0 = Weight of dried material taken.
W_1 = Weight of crucible and contents before ashing.
W_2 = Weight of crucible with ash.

2.2.3. Estimation of total phenol

The amount of total phenolics in the extracts was determined according to the Folin-ciacalteu procedure (Singleton and Rossi, 1965) modified by Negi and Jayaprakasha, 2003. Sample was dissolved in Methanol: water (6.4 V/V) to make volume 0.2 ml which was.

mixed with 1.0 ml 10 fold diluted Folin-ciacalteu reagent and 0.8 ml of 7.5% Sodium Carbonate solution. After 30 min at room temperature, the absorbance was measured at 705 nm using a UV–Vis Spectrophotometer. Total phenol content in the test sample was estimated from the standard curve and was expressed as Gallic acid equivalent/gm of sample in 0.2 ml of Methanol: water (6.4 V/V) was taken in blank. Standard curve was prepared by taking different concentration of Gallic acid (1 μg to 10 μg). The concentration of total phenolics was expressed as Gallic acid equivalent/gm of the dry material.

2.2.4. Estimation of isoenzyme (polyphenolic oxydase)

Five hundred milligram of fresh rhizome for each sample were ground to fine powder with 20 mg of high molecular weight insoluble PVPP in a cold mortar with repeated addition of liquid nitrogen avoiding thawing. PVPP was added to avoid phenolic compounds. The powder was then homogenized with 0.5 ml cold enzyme extraction buffer 50 mM Tris-HCl, pH 7.2; 6 mM Ascorbic acid; 6 mM Cystein-Cl and 500 mM Sucrose (Rychter and Levak, 1969). The homogenate was transferred to a 1.7-ml microcentrifuge tube. The mortar was again washed with 0.5 ml of the extraction buffer and the washing was collected. The entire operation was performed under diffused light inside the cold room at 4 °C. The homogenate was centrifuged at 4 °C at 10,000 rpm for 10 min. The supernatant was collected in aliquots and stored at −20 °C for subsequent use. The absorbance at 595 nm was measured in an UV–VIS Spectrophotometer (Specord, Analytica Jena, Germany) with by adding Odianisine for record the enzyme activity for 5 min at 1 min interval. Average activity was taken in unit value.

2.3. Molecular analysis

2.3.1. Extraction of genomic DNA

DNA was isolated following the protocol of (Doyle and Doyle, 1990) with little modification. Two-gram fresh, young leaf sample was ground with 2% insoluble PVPP to a fine powder in a cold mortar with repeated addition of liquid nitrogen. Thawing was avoided to reduce the shearing of DNA. The powder was transferred into a 50 ml centrifuge tube containing 10 ml of prewarmed (60 °C) CTAB-DNA extraction buffer (4% CTAB; 1.4 M NaCl; 20 mM Na2 EDTA, pH 8; 100 mMTris-HCl, pH 8; 2% β-Mercaptoethanol) and mixed vigorously. The mixture was incubated in a water bath for one hour at 65 °C with intermittent gentle mixing. Then it was centrifuged at 10,000 rpm for 20 min in a (Sigma K-50, Germany) at room temperature. The upper aqueous phase was pipetted out with the help of a micropipette into another 50 ml centrifuge tube and mixed with double volume of pre-chilled dehydrated ethanol. After quick inversion DNA, like a mass of cotton threads was precipitated. The precipitated nucleic acid was spooled out with a bend glass Pasteur pipette, washed twice with 70% ethanol, stored in a 1.7-ml micro centrifuge tube and dried in DNA-mini vacuum dryer (DNA Mini, Germany). The dried DNA was dissolved in excess amount of T_{10} E_1 buffer (Tris-Cl 10 mM, EDTA 1 mM pH 8).

2.3.2. Isolation and purification of genomic DNA

The dissolved DNA was impure with proteins, RNA and phenolics in some cases and the crude DNA was purified and RNA was removed. The RNA was removed by giving RNase-A treatment for one ml of crude DNA solution, 60 μg of RNase-A was added and the solution was incubated with continuous shaking in a water bath at 37 °C for 1 h. After 1 h it was removed from the water bath and equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and mixed thoroughly but gently. The solution was then centrifuged in 10,000 rpm for 10 min in Sigma centrifuge (Sigma K-13, Germany) and the upper aqueous phase was pipetted out. It was again washed with chloroform: isoamyl alcohol (24:1) twice and centrifuged at 10,000 rpm for 20 min at room temperature. The upper aqueous phase was separated after centrifugation (as described earlier) and mixed with 1/10th volume of 3 M sodium acetate (pH 4.8). DNA was precipitated by adding 2.5 vol of chilled absolute ethanol and pelleted by spinning. The pellet was washed twice with 70% ethanol, carefully and dried under vacuum. The dried DNA was dissolved in minimum amount of T10 E1 buffer (pH-8.0).

2.3.3. Test for quality and quantity of the purified DNA

The quality and quantity of DNA was measured by UV–VIS Spectrophotometer (Specord, Analytica Jena, Germany). The total

DNA quantity was measured by taking the absorbance at 260 nm wave length and the quality of the DNA was confirmed from the absorbance ratio at 260 nm and 280 nm. It was reported that if the ratio is about 1.8–2.00 then the quality of the DNA is good (Sambrook and Russell, 2001). The quantity of DNA was rechecked using the fluorometric method. The DNA was stained using Hoechst dye (33258), which binds only with double stranded DNA, and the quantity was measured with Versa Fluor Fluorometer (BioRad, USA). For final checking the quality as well as quantity of DNA, the DNA was loaded in 0.8% agarose gel alongside diluted uncut lambda DNA as standard and electrophoresed. It was observed that the DNA from all the samples were very good in quality. After quantification, the DNA was diluted with T10 E1 buffer to a working concentration of 100 ng/μl and 25-ng/μl for ISSR analysis.

2.3.4. Inter Simple Sequence Repeat (ISSR) analysis

Six numbers of ISSR primers were used for ISSR analysis. These simple sequence repeats were synthesized and procured from Genei (Bangalore GeneiPvt. Ltd., Bangalore, India). Those primers were $(GAC)_5$, $(GTGC)_4$, $(AGG)_6$, $(GA)_9T$, $T(GA)_9$, $(GTG)_5$. The ISSR analysis was implemented as per the methodology given by (Zietkiewicz et al., 1994). Each amplification reaction mixture of 25 μl comprised 20 ng of template DNA, 2.5 μl of 10X assay buffer (100 mM Tris-HClpH 8.3, 0.5 M KCl and 0.01%gelatin), 1.5 mM $MgCl_2$, 200 μm each of dNTPs, 44 ng of primer and 0.5 U Taq DNA polymerase. The amplification was undertaken in a thermal cycler. The first cycle consisted of denaturation of template DNA at 94 °C for 5 min, primer annealing at specific temperature for particular primer for 1 min and primer extension at 72 °C for 2 min. In the subsequent 42 cycles the period of denaturation was reduced to 1 min while the primer annealing and primer extension time was

Fig. 1. Selection of 20 varieties (Sl.1–20) of ginger germplasms on the basis of survey for artificial field screening against rhizome rot.

Table 4
Biochemical analysis for estimation of total protein, total fiber and total phenol content of the 20 germplasms.

Sl. no.	Name of the germplasm	Disease reaction (Pot)	Soluble protein content mg/g f wt	Fiber content (%) f wt	Phenol content mg/ g f wt	Polyphenolic oxydase (U/mg)
1	V1K1-1	HS	12.2	6.8	0.9	32.01
2	Tura	HS	05.7	7.5	0.7	35.6
3	Suvada	HS	14.5	8.5	0.6	12.03
4	Burdwan	HS	10.5	8.2	0.7	45.02
5	Sleeva Local	HS	06.03	7.6	0.7	08.5
6	Suravi	HS	09.6	7.5	0.8	07.05
7	NO.12	HS	15.5	8.5	0.6	06.6
8	Banspal	HS	14.3	9.4	0.7	19.6
9	Suprava	HS	16.4	9.8	0.6	22.02
10	Jugijan	PS	17.6	7.02	0.8	41.08
11	Kalinga Local	HS	13.2	5.9	0.7	06.04
12	Pottangi Local	PS	11.03	5.86	0.7	06.03
13	Kuruppampadi	PS	19.03	8.2	0.7	48.8
14	ACC-60	PS	14.7	7.5	0.8	42.05
15	Vaysay	HS	14.6	7.1	0.7	20.04
16	Sargiguda	R	08.5	4.4	1.5	98.03
17	China	PR	09.7	5.6	1.3	83.05
18	Varada	PR	13.00	7.9	0.9	83.04
19	Wild Ginger	HS	13.1	7.1	0.8	20.02
20	Singjhara	HS	16.3	7.5	0.7	05.06
SE(m) ±			0.1	0.03	0.01	0.4
CD (0.05)			0.3	0.07	0.03	1.2

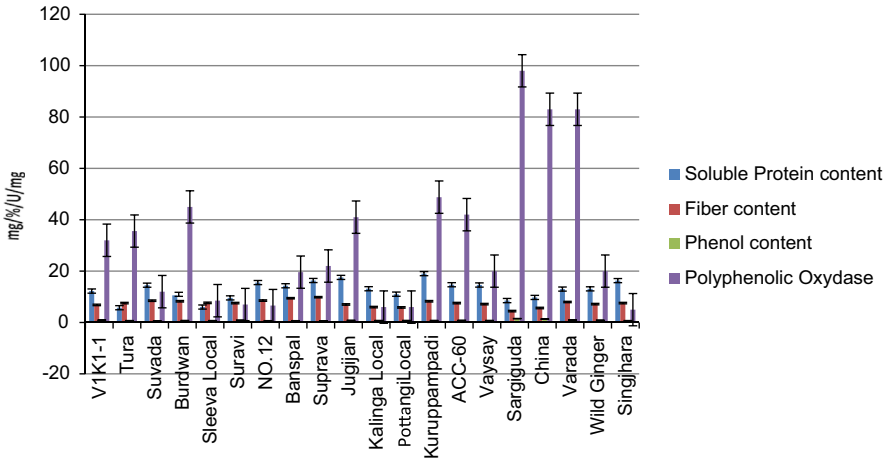

Fig. 2. Soluble protein, fiber, phenol and polyphenolic oxydase content of ginger varieties.

Table 5
ISSR analysis of 20 germplasms of Ginger from HARS Pottangi.

Primer	Sequence	Total band	Polymorphic	Polymorphism (%)	Monomorphic
GTGC4	GTGCGTGCGTGCGTGC	110	14	30.4	96
GTG5	GTGGTGGTGGTGGTG	100	0	0	100
GAC5	GACGACGACGACGAC	140	0	0	140
AGG6	AGGAGGAGGAGGAGGAGG	100	0	0	100
TGA9	TGAGAGAGAGAGAGAGAGA	120	0	0	120
GA9T	GAGAGAGAGAGAGAGAGAT	100	0	0	100

maintained same as in the first cycle. The last cycle consisted of only primer extension at 72 °C for 7 min the amplified products were resolved in 2% agarose gel stained with ethidium bromide.

2.3.5. Data analysis

After staining, the gels were photographed and data were recorded in binomial matrix form, i.e., presence and absence of band was denoted as '1' and '0', respectively. All the bands were taken into consideration to avoid over/underestimation of genomic relations. After scoring, the data were analyzed for similarity matrix (Jaccard, 1908) and Principal Co-ordinate Analysis (PCoA) using NTSYSPC 2.02e software (Rohlf, 1997).

3. Results

3.1. Biochemical analysis

The twenty ginger varieties which were selected for disease reaction, were also analyzed their biochemical properties such as protein, phenol polyphenol oxidase and its fiber content to find out any relationship with disease incidence/disease reaction (Fig. 1). Considerable variations were found between the varieties for total protein, total phenol content polyphenol oxidase content and total crude fiber content (Table 4)The disease reaction was

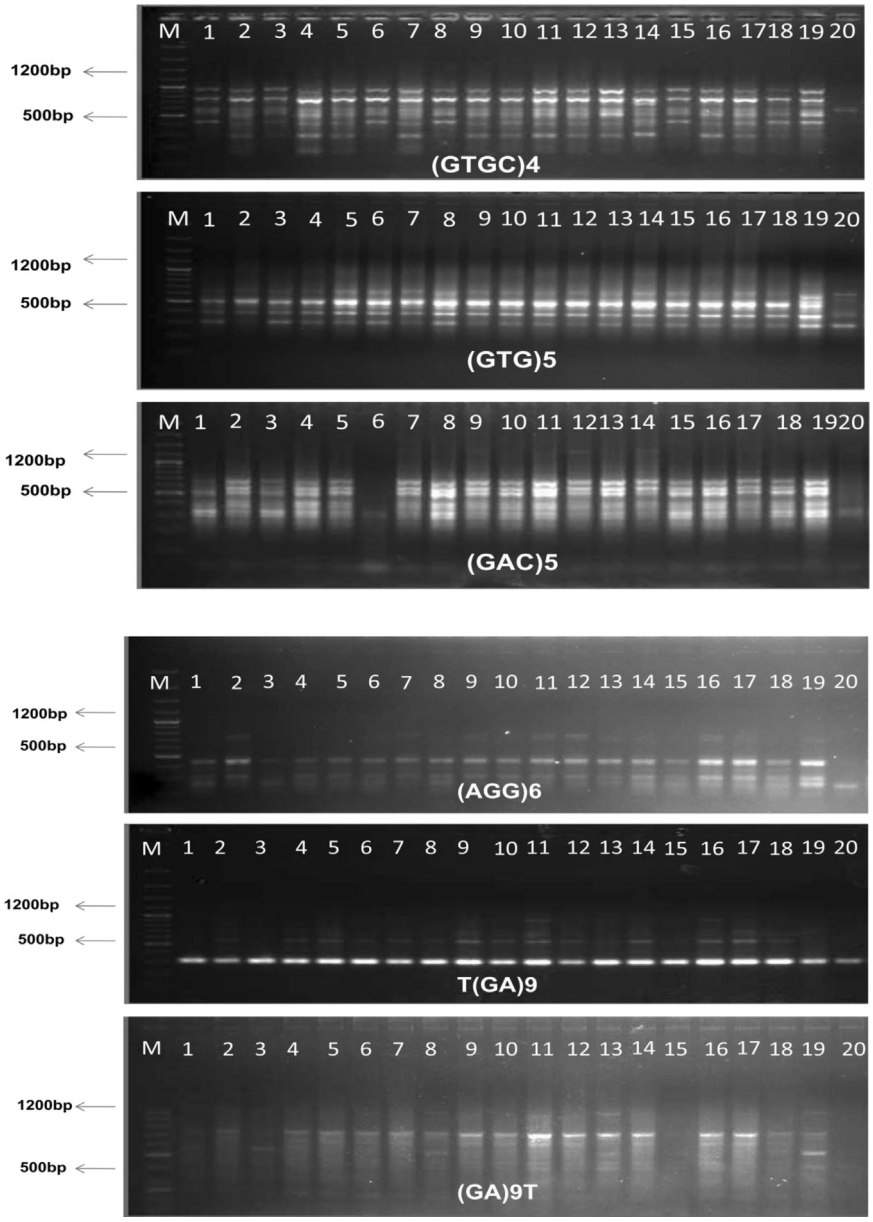

Fig. 3. DNA profiling by using six numbers of ISSR marker for 20 ginger samples.

different for different varieties and it was observed that Sargiguda has a resistant reaction whereas China and Varada produced partial resistant reaction. Whereas other all varieties showed partial susceptibility to highly susceptibility.

3.1.1. Protein estimation

Total buffer soluble protein was isolated from the selected cultivars of ginger but no such remarkable difference was observed among the samples. The protein content raised between 5.755 and 19.031 mg/g (Fig. 2). The minimum protein content was observed in Tura (5.755 mg/g) and Sleeva Local (6.034 mg/g). Maximum protein content was observed in Kuruppampadi (19.031 mg/g) followed by Jugijan (17.561 mg/g), Suprava (16.361 mg/g) and Singjhara (16.297 mg/g).

3.1.2. Estimation of total fiber

The total fiber content was different in all the cultivars. The fiber content ranges between 4.45% and 9.85% (Fig. 2). Maximum fiber content was observed on Suprava (9.85%) followed by Banspal (9.45%), No.12 (8.55%), Subhada (8.50%) and minimum was noted in Sargiguda (4.45%) followed by China (5.56%), Kalinga Local (5.48%) and Pottangi Local (5.86%).

3.1.3. Estimation of total phenol

The phenol content varied from 0.632 mg/g to 1.522 mg/g (Fig. 2). Maximum phenol content was noted on Sargiguda (1.522 mg/g) followed by China (1.324 mg/g) and Varada (0.965 mg/g) and minimum in Suprava (0.632 mg/g) followed by Subhada (0.645 mg/g), No.12 (0.665 mg/g) and Banspal (0.675 mg/g).

3.1.4. Estimation of polyphenolic oxidase

The polyphenolic oxidase varies from 5 to 98.0 unit/mg (Fig. 2). The minimum polyphenol oxidase was noted on variety Singjhara (5.0 unit/mg) followed by Pottangi Local (6.0 unit/mg), Kalinga Local (6.0 unit/mg), No.12 (6.6 unit/mg) enzyme. The maximum polyphenol oxidase was observed on Sargiguda (98.0 unit/mg) followed by China (83.0 unit/mg) and Varada (83.0 unit/mg).

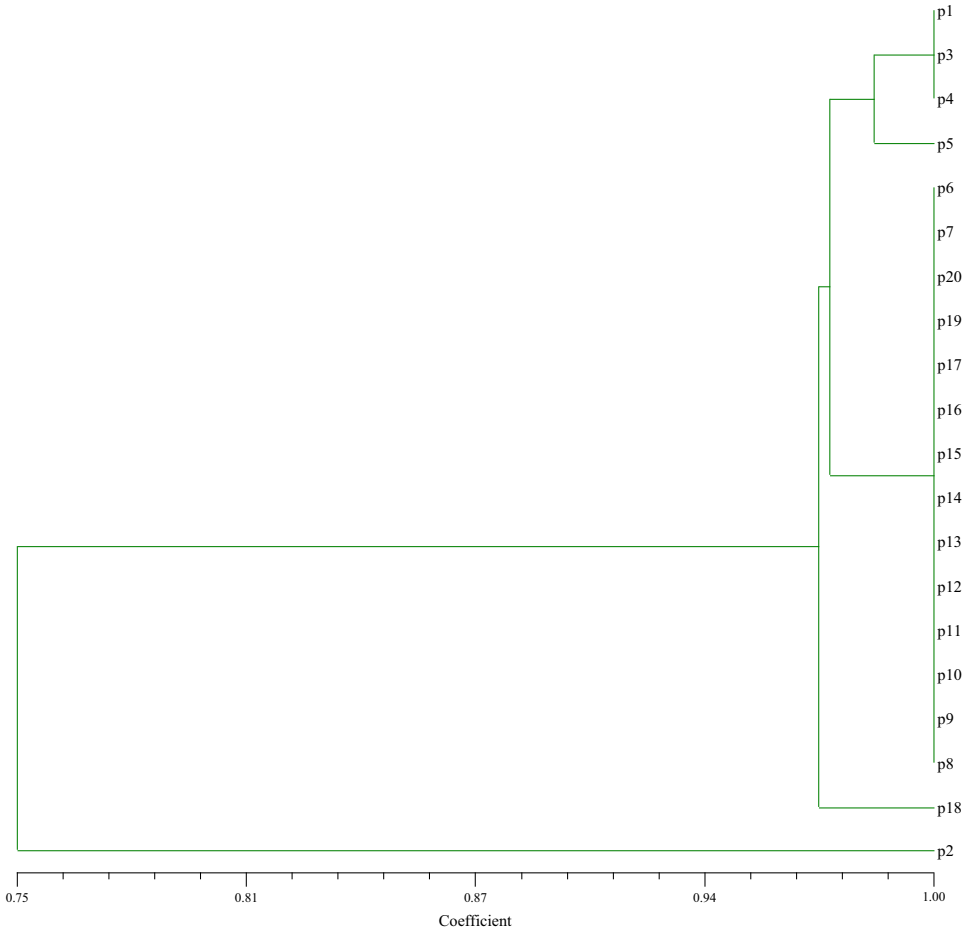

Fig. 4. Jaccards similarity coefficient index dendrogram of 20 ginger varieties based on ISSR profiles.

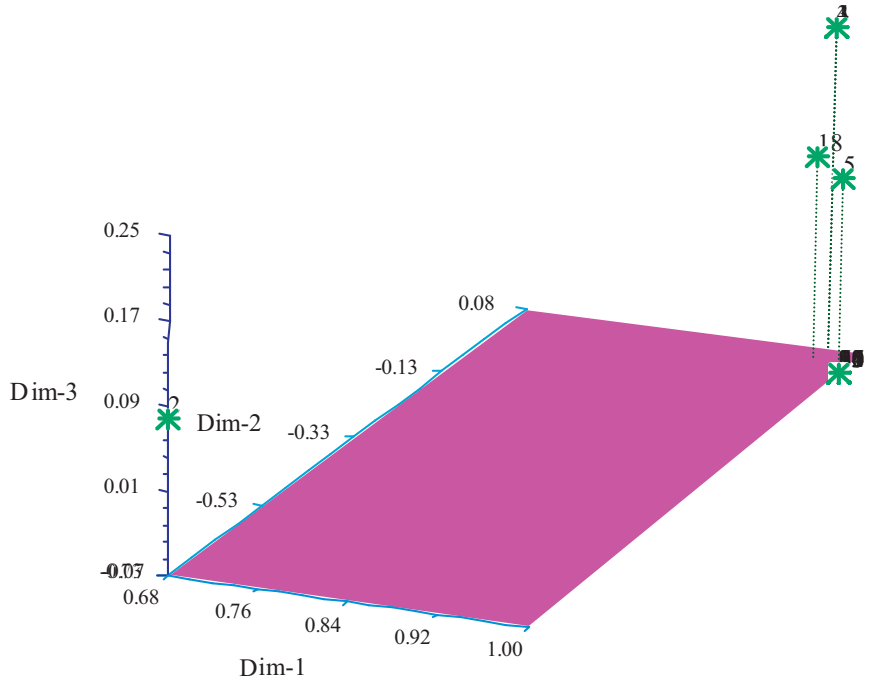

Fig. 5. Principal coordinate analysis of 20 ginger varieties.

3.2. Inter Simple Sequence Repeats (ISSR) analysis of ginger varieties

It is difficult to distinguish the 20 varieties of ginger in relation to the disease reaction using traditional morphological differences. The suitability of inter simple sequence repeats (ISSR) was used to detect the variation among the different varieties of ginger. Six different primers i.e., GTGC4, GTG5, AGG6, GAC5, TGA9 and GA9T were used to determine the genetic relationship (Table 5) (Fig. 3).

3.2.1. Jaccard's similarity among different varieties as revealed from combined data

The sequence of the ISSR primers was used for the genetic finger printing of the 20 varieties and the total number of bands produced by each primer. Number of polymorphic bands and percentage of polymorphism produced by each primer present in Table 5. Among the six primers one primer is polymorphic. Maximum polymorphism i.e. 30.43% was observed in case of primer, GTGC4. Among the six primers used no primers produced any unique bands. So no ISSR primers produced any unique band in the 20 varieties tested.

The dendogram was build using SAHN clustering (Fig. 4). The dendogram consists of two major clusters: cluster I contain 19 varieties and the rest 1 variety was classified under cluster-2. Cluster-I was further grouped in to two sub-clusters, IA consists of 18 varieties and IB with 1 species. Cluster-IA was further subdivided into two sub-clusters, IA1 presenting 14 varieties and IA2 with 4 species. Cluster-IA1 was further subdivided in to two sub-clusters, IA1A presenting 3 varieties and IA2A with 1 species. The dendogram based on similarity coefficient showed two distinct clusters. In cluster I, 19 varieties and in cluster 2 only 1 varieties were grouped. Principal coordinate analysis of the 20 varieties of ginger is given in Fig 5.

4. Discussion

Biochemical qualities of 10 elite accessions of ginger were studied by Eleazu et al. (2012). Results showed that these accessions of ginger could possess considerable economic, nutritive and medicinal potentials. In our research protein content raised between 5.50–21.35 mg/g, the fiber content varied between the varieties were4.45–9.85%, the range of total phenol varied from 0.632 mg/g to 1.522 mg/g and polyphenol oxidase varied from 2.6 to 98.0 unit/mg. Results attained from Odebunmi et al. (2009) showed that on dry basis ginger have moisture content, crude protein, crude fiber, fat and ash as 76.86 ± 1.43, 76.86 ± 1.43, 2.93 ± 0.05, 5.62 ± 0.75 and 2.54 ± 0.20% respectively.

EL-Ghorab et al. (2010) investigate the chemical composition of ginger and observed that ginger contain 88.5 ± 0.39%, moisture tracked by 0.2 ± 0.01% crude fat, 1.1 ± 0.16% crude fiber, 1.5 ± 0.07% ash, 1.2 ± 0.17% protein. Similarly, Nwinuka et al. (2010) determined that on dry basis ginger contain moisture (76.67 ± 0.01), crude protein (8.25 ± 0.01), fat (5.35 ± 0.15) and ash (6.40 ± 0.15). For protein content this results were in accordance with our research work. According to Latona et al. (2012) ginger contains moisture content 76.53%, crude protein 9.13%, crude fiber 3.07%, fat content 5.09% and ash 2.64%. Results relating to protein are in agreement to our findings while fiber contents are highly inconsistent. Additionally, Okolo et al. (2012) concluded that the moisture, protein fiber, fat and ash contents of ginger are 74.72 ± 1.32, 7.57 ± 0.59, 6.07 ± 0.64, 4.92 ± 0.61 and 2.92 ± 0.14% respectively on dry basis. Results relating to protein and fiber contents are close agreement with our result. Phenolics are important plant secondary metabolites with antioxidant activity owing to their redox potential, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Mishra et al., 2011).

Inter Simple Sequence Repeats (ISSR) was used to characterize 20 varieties of the family Zingiberaceae. Previous results proved that RAPD, ISSR, and AFLP are very powerful methods for identifying genetic relationships between species of Zingiberaceae. However, ISSR (98.55%) marker showed a different polymorphic capability in comparision to RAPD (93.22%) and AFLP (97.27%) and was found to be most explanatory in characterizing closely related *Curcuma* species from northeast India (Das et al., 2011). Yang et al. (1994) also found that ISSR assay can give more informative data than other techniques. Syamkumar and Sasikumar (2007) exhibit that of eight ISSR primers, six (75%) gave 100% polymorphic bands among the 15 species of *Curcuma* and two primers namely (TCC)5 AG and (GACA)3 showed 70% and 90.91% polymorphism, respectively. All the resistant varieties & susceptible varieties of ginger were grouped under a single cluster.

Different hierarchical positions of the Ginger varieties in the dendrograms showed that genomes of each variety are not exactly the same. Also the dendrogram tree indicated that 19 varieties may have a common ancestor which is different from that of the one variety. Group constellations were also independently developed by using principal coordinate analysis (PCA) to verify grouping obtained through the dendrogram. Principle coordinate analysis provides a field representation of the variability in 2D or 3D set of axes. It is a very useful analysis for inspecting visually the similarity of samples since dissimilar samples will appear to be further apart than highly similar samples. No specific clustering of the 19 cultivars was observed indicating a wide genetic variation among themselves.

These findings indicate that these 20 elite germplasms of ginger contains phenolics in an substantial amount so these plant can be economically exploited. The results acquired a high yield of total phenolics from the ginger plant pointing that it can be utilized as a wonderful source for the preparation of not only neutraceuticals as potent antioxidants but also for the treatment of other major health problems. But there is no correlation between the diseases incidence and biochemical, molecular analysis. Molecular analysis indicated that 19 varieties may have a common ancestor which is different from that of the one variety.

Conflict of interest

There is no conflict of interest between the authors.

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