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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		