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RESEARCH ARTICLE

# EVALUATION OF GENETIC RELATIONSHIPS AND CHEMICAL ASSAY OF KAEMPFERIA GALANGA L. CULTIVARS FOUND IN MANIPUR, NORTH-EAST INDIA

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**ABSTRACT**

*Kaempferia galanga* L., a member of the family Zingiberaceae is used as a medicinal plant for remedy of nasal blocks, asthma, hypertension, rheumatism etc. Eight cultivars from Manipur, North-East India were subjected to genetic diversity estimation using random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) analyses. Twenty five RAPD primers were used for amplification of the genomic DNA. Out of these, thirteen primers yielded polymorphic bands generating distinct bands in all the cultivars. Twenty ISSR primers were used out of which eleven produced prominent and scorable bands. On average, 5.38 bands per primer were observed by RAPD and 6.54 bands per primer by ISSR markers. The polymorphic bands were used to estimate genetic variability with Dice Similarity Co-efficient using NTSYSpc2.2 software. A dendrogram was constructed using unweighted pair group method with arithmetic mean (UPGMA) algorithm based on the similarity index for the taxa. Cluster analysis by RAPD and ISSR markers revealed clear distinct diversity between cultivars. The chemical analyses revealed that Tamenglong cultivar has the highest vitamin C content. Highest phenol was found in Ukhrul cultivar. Imphal-east cultivar has maximum value of kaempferol content.

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## INTRODUCTION

Zingiberaceae, found mainly in tropical and sub-tropical regions, is the largest family of monocots in Zingiberales and contains more than 1300 species worldwide (Wu & Larsen 2000), including many important medicinal plants, perfume plants and ornamental flowers. In India there are 170 species belonging to 20 genera in Zingiberaceae, representing a diverse treasure house of gingers. The north-east region of India is considered as one of the centre of diversity of gingers having a total account of 24 species out of the 65 valid species found in the world (Wood *et al*. 2000). Manipur is located in one of the biodiversity rich region of the world i.e, Indo-Burma Biodiversity Hotspot, 22,327 sq km stretching between latitude 23º50´ and 25º42´ N and longitude 92º59´ and 94º46´ E and this hotspot is home to 13,500 plant species of which 7000 are endemic constituting 2.3% of the plant endemic species of the world. It falls under the Eastern Himalayan agro climatic zones with two broad topographic divisions viz., plains and hills. It is within the monsoon belt of the country with sub-tropical to semi temperate climate in valley and semi temperate to temperate climate in the higher altitudes. Distinct forms of physical regions and different climatic zones of Manipur might have led to high divergence of *Kaempferia galanga* cultivars.

*Kaempferia galanga* L., a member of the family Zingiberaceae is an acaulescent perennial that grows in Southern China, South-East Asia and Malaysia. It is best known for its medicinal properties and chemical constituent. Extracts of the plant have shown remarkable resistance to asthma, epilepsy, fever and splenic disorders. Rhizomes and root-stocks are bitter, thermogenic, carminative and aromatic and are used as expectorant, digestive and stimulant. They are good for leprosy, skin diseases, cough, bronchitis, jaundice, ulcer, fever and nasal obstruction (Rahman *et al*. 2004). Chan *et al*. (2009) reported that *K. galanga* showed that fresh weight of the plant exhibited total antioxidant activities better than freeze dried and air dried plant extract (fresh weight > freeze dried > air dried). Essential oil obtained from rhizomes of micropropagated and conventionally grown *K. galanga* was analysed to evaluate its detailed chemical composition (Mohanty *et al*. 2011). The essential oil of *K. galanga* also has antimicrobial activities and hence could be used for the treatment of microbial infections and skin diseases. The anti-proliferative activity of kaempferol inhibits cancer cell growth in various cells (Bestwick *et al*. 2007).The methanol extract of the rhizome contains ethyl-p-methoxy cinnamate and trans-cinnamate which are highly cytotoxic to HeLa cells (Kosuge *et al*.1985)

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Local genotype within the same climatic conditions and same locations are easier to utilize for breeding purposes than those available in long distances. These precious genetic resources are to be conserved for plant breeding (Vijayan *et al.* 2006). Complexity in morphological variations and interbreeding between cultivars make it difficult to discern the direction of morphological changes and these rules seldom hold true. Cultivars with similar morphology do not necessarily have the closest evolutionary relationship. The knowledge of genetic variability is a pre-requisite to study the evolutionary history of a species as well as for other studies like intraspecific variations, genetic resources conservation, etc (Islam *et al.* 2007). The usefulness of molecular markers in genetic diversity studies has been convincingly established. DNA based markers are not affected by physiology or the environment and have been widely used in cultivar identification and seed purity testing (Hu and Quiros, 1991; Hale *et al.* 2006; Liu *et al.* 2007; Louarn *et al.* 2007). Due to their economy, simplicity of use and high throughput, random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) markers have been used for cultivar identification (Hu and Quiros, 1991; Yonemoto *et al.* 2006). Das *et al.* (2011) reported that RAPD and ISSR provide a rapid and sensitive method for detection of genetic variations among the different species of Zingiberaceae. Genetic diversity within cultivars based on RAPD and ISSR fingerprinting techniques could be of practical application in classic breeding to produce better varieties with high yielding characters (Kalpana *et al.* 2012). Developing a systematic strategy for collection of representative sample covering large part of the variability and re-establish them in the area requires information regarding existing diversity for nutritional parameters as well as at molecular level (Vyas *et al.* 2012).Molecular characterization and biochemical assay of *Kaempferia galanga* cultivars will help in the analysis of taxonomic relationships and assessment of intraspecific diversity of the cultivars. It would represent an important step in future breeding programmes. The aim of the present study was to genetically characterize and assay the chemical content of the cultivars of *Kaempferia galanga* from Manipur, North East region of India using RAPD and ISSR markers and biochemical assay.

## MATERIALS AND METHODS

Surveys were conducted in eight districts of Manipur viz., Imphal-East, Imphal-West, Thoubal, Bishnupur, Senapati, Ukhrul, Tamenglong and Churachandpur during the flowering season of *Kaempferia galanga* (Fig 1).

The materials for the present study consist of eight cultivars of *Kaempferia galanga*. The cultivars were collected and maintained in the departmental green house of Institute of Bioresources and Sustainable Development, Takyelpat, Imphal. Fresh, young and tender green leaves of these samples were used for DNA extraction and subsequent fingerprinting and chemical analyses.

### *DNA Extraction*

Genomic DNA was extracted from young leaves using the CTAB method following the procedure of Doyle and Doyle (1987) with minor modifications. DNA quality was checked on 0.8% agarose gel stained with ethidium bromide. DNA quantitation was done with the help of a *Nanodrop Spectrophotometer (ND2000)*. The DNA was stored at -20ºC, for further use as templates for polymerase chain reaction (PCR) amplification.

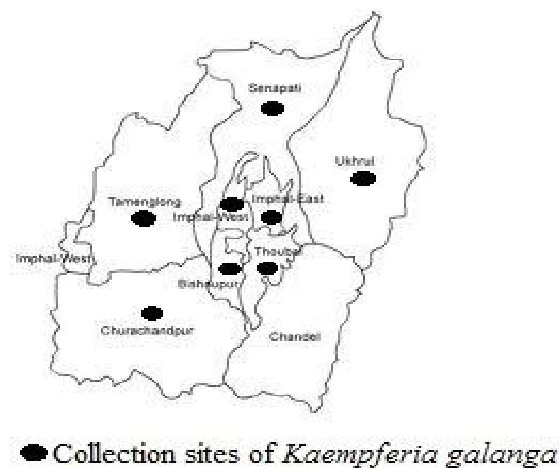


**Fig.1** Manipur map showing collection sites of *Kaempferia galanga*

### *RAPD Analysis*

PCR amplification of the genomic DNA was carried out using 25 random decamer oligonucleotide primers (Table 1) which were synthesized by *Sigma Aldrich Chemicals Pvt.Ltd., India* as per the sequence of *Operon Technologies, Inc., USA*. RAPD amplifications were performed routinely using PCR mixture.

The reaction mixture of 25 µl contained 50 ng of template DNA, 10X PCR buffer, 1.5 mM of magnesium chloride ($MgCl_2$) 200µM of deoxynucleotide triphosphates (dNTPs), 10 picomol of each primer, and 1 U of *Taq* polymerase. PCR amplification was carried out in a thermal Cycler *(Eppendorf Mastercycler pro S)*. Thermal cycling conditions were as follows: initial denaturation step for 5 min at 94 ºC, followed by 35 cycles each of 1 min at 94ºC (denaturation), 1 min at 37 ºC (annealing), 2 min at 72ºC (extension) followed by one final extension of 7 min at 72 ºC. The amplification products were electrophoresed in 1.8% agarose gels in 0.5X TBE (10X stock contained 1M Tris, 0.8 M boric acid, 0.5 M EDTA) and stained with ethidium bromide(0.5µg/ml). The gels were photographed under a Gel Documentation system *(Perkin Elmer Geliance 200)*.

### *ISSR Analysis*

PCR amplification was carried out using 20 ISSR primers. The PCR composition was same as that used for RAPD analysis. PCR conditions were identical with that of RAPD except that the annealing temperatures were different for each primer depending upon the melting temperature. The annealing temperatures are given at Table 2.

The amplified products were visualized in a 1.8% agarose gel stained with ethidium bromide (0.5µg/ml) and photographed for further analysis.

***Preparation of extracts***

Fresh rhizomes were washed and cleaned thoroughly in running tap water. Rhizomes were sliced into small pieces and dried at the room temperature separately.

**Table 1** List of RAPD primers used in the present study

| Sl no. | Primer | Sequence 5'-3' | Total no. of bands | Total no. of monomorphic bands | Total no. of polymorphic bands | % of polymorphism |
|---|---|---|---|---|---|---|
| 1 | OPA-01 | CAGGCCCTTC | 4 | 2 | 2 | 50 |
| 2 | OPA-14 | TCTGTGCTGG | 2 | 1 | 1 | 50 |
| 3 | OPA-18 | AGGTGACCGT | 8 | 1 | 7 | 87.5 |
| 4 | OPB-01 | GTTTCGCTCC | 6 | 3 | 3 | 50 |
| 5 | OPB-07 | GGTGACGCAG | 7 | 0 | 7 | 100 |
| 6 | OPC-05 | GATGACCGCC | 4 | 0 | 4 | 100 |
| 7 | OPC-07 | GTCCCGACGA | 5 | 2 | 3 | 60 |
| 8 | OPC-09 | CTCACCGTCC | 8 | 0 | 8 | 100 |
| 9 | OPC-11 | AAAGCTGCGG | 4 | 1 | 3 | 75 |
| 10 | OPC-13 | AAGCCTCGTC | 5 | 1 | 4 | 80 |
| 11 | OPD-20 | ACCCGGTCAC | 9 | 1 | 8 | 88.8 |
| 12 | OPAM-01 | TCACGTACGG | 4 | 1 | 3 | 75 |
| 13 | OPAM-18 | ACGGGACTCT | 4 | 2 | 2 | 50 |
| | Grand Total | | 70 | | 55 | 78.57 |

**Table 2** List of ISSR primers used in the present study

| Sl no. | Primer Name | Primer sequence 5'-3' | Annealing temperature | Total no. of bands | Total no. of monomorphic bands | Total no. of polymorphic bands | % of polymor-phism |
|---|---|---|---|---|---|---|---|
| 1 | ISSR-6 | CACACACACACAAG | 45 ºC | 10 | 1 | 9 | 90 |
| 2 | UBC-811 | GAGAGAGAGAGAGAGAC | 50 ºC | 11 | 1 | 10 | 90.9 |
| 3 | IG-10 | AGAGAGAGAGAGAGAGC | 56.4ºC | 13 | 0 | 13 | 100 |
| 4 | ISSR 10/15 | CCCGTGTGTGTGTGT | 52.2 ºC | 3 | 1 | 2 | 66.6 |
| 5 | IG-19 | TGGAACACACACACACAC | 55.1 ºC | 5 | 2 | 3 | 60 |
| 6 | IG-09 | AGAGAGAGAGAGAGAGT | 52.2 ºC | 6 | 2 | 4 | 66.6 |
| 7 | IG-14 | GAGAGAGAGAGAGAGAT | 50.1 ºC | 5 | 1 | 4 | 80 |
| 8 | IG-05 | GACAGACAGACAGACA | 49 ºC | 6 | 2 | 4 | 66.6 |
| 9 | ISSCR-16 | GAGAGAGAGAGAGAGAGAT | 57 ºC | 5 | 1 | 4 | 80 |
| 10 | ISSCR-10 | GAGAGAGAGAGACC | 45.6 ºC | 2 | 1 | 1 | 50 |
| 11 | UBC-826 | ACACACACACACACACC | 50 ºC | 6 | 1 | 5 | 83.3 |
| | Grand total | | | 72 | | 59 | 81.94 |

The dried plant material about 250g was extracted in a soxhlet apparatus successively with 4 times the amount of materials (V/W) of methanol each time until no more colored matter was extracted. Solvent was evaporated to dryness in a rotavapour *(Buchi)* and freeze dried respectively.

***Total phenol assay***

The total phenol content of different extracts of rhizomes was determined by using Folin- Ciocalteu method (Singleton *et al.* 1965). An aliquot (1ml)of the extract (V/W) and standard solution of gallic acid (1,2,4,5,6,8 and 10 mg) were taken in different test tubes and made up to the volume of 1ml with distilled water respectively.

Then, 0.5 ml of Folin-Ciocalteu reagent (1:1 with distilled water) from amber colored bottle and 2.5 ml of 20% sodium carbonate solution were added to the test tubes sequentially. A blank was prepared using distilled water. The reaction mixtures were mixed well and kept for incubation in dark at room temperature for 1h and the absorbance against the prepared reagent blank was determined at 725nm with a *100-bio Cary UV-Visible spectrophotometer*. The total phenol content was calculated and expressed as mg gallic acid equivalents (GAE)/100g weight. The experiments were carried out twice.

***Calculation***

$C = c\ (V/m')$

Where,

C = Total content of phenolic compounds, mg/g rhizome extract, in GAE.

c = the concentration of gallic acid established from the concentration curve mg/ml.

V = the volume of the extract, ml

m' = the weight of pure rhizome extract, g.

***Anti-oxidant activity:*** The anti-oxidant properties of different extracts obtained consequently were measured by using 1, 1-diphenyl, 2-picrylhydrazyl radical (DPPH) (Okada and Okada, 1998). 1ml of different concentration of extracts (2W/V) (10, 20, 40, 60, 80 and 100µl/ml) prepared in respective solvents were added to 4ml of a 0.01 mm (1.97165mg of DPPH in 50ml of ethanol in amber colored bottle) chloroform solution. The mixtures were shaken vigorously and left standing at room temperature in dark for 30 min. The absorbances of the resulting solutions were measured at 517nm after 30 min using *100 Bio Cary UV-Visible spectrophotometer*. The antioxidant activities (three replicates per treatment) were expressed as IC50 (mg/ml), the concentration required to cause a 50% DPPH inhibition. The anti-oxidant activities of the different rhizome extracts were estimated by comparing with the standard ascorbic acid.

The ability to scavenge the DPPH radical = $[(A0 - A1)/A0] \times 100$

Where, A0 = Absorbance of the control at 30 minutes.

A1= Absorbance of the sample at 30 minutes.

***Determination of Kaempferol content***

Kaempferol in *Kaempferia galanga* was determined using Okada and Okada method (1998). A sample of dried powder (1g) was extracted in 10 ml acetonitrile overnight and centrifuged for 10 min at 5000rpm *(Remi)*. The supernatant was filtered through Whatmann paper No. 2 and then filtrate was filtered through 0.45µm nylon filter. Reverse-phase high performance liquid chromatography (HPLC) was used for the determination of kaempferol in the extract of *Kaempferia galanga*. 20 µl of the extract was injected into the HPLC system (*Waters*) was used. The column was reverse phase *XbridgeTM* C18 id 5µm (4.6mm x 250mm) *(Waters)*. The mobile phase was acetonitrile (HPLC grade): double deionized water (HPLC grade) (80:20 v/v) at 360nm. The solvent flow rate was 1.0 ml/min. Area under curve of sample was determined the kaempferol content using the external standard curve. Standard kaempferol was purchased from *Sigma-Aldrich Co.*.

***Data analysis:*** The amplified bands of two independent replicates were scored for their presence (1) or absence (0). The genetic relationship among the cultivars was evaluated with phylogenetic trees constructed by UPGMA, performed to show the multiple distributions by the NTSYS-pc2.2 software. The similarity was estimated using the Dice Co-efficient of Nei and Li (1979). Chemical data were analyzed for significance using ANOVA and the differences contrasted using Tukey's comparison tests at 5% probability test. Statistical analysis was performed using the SPSS statistical software package.

## RESULTS AND DISCUSSION

The present work evaluates the genetic diversity and relationships among eight *Kaempferia galanga* cultivars found in Manipur, North East region of India using RAPD and ISSR markers. The wide variation in genetic distance among eight cultivars revealed by both RAPD and ISSR techniques reflected a high level of polymorphism at DNA level. Generally, at intra-specific level the rate of polymorphism is dependent on various factors viz. the breeding system, habitat specialization, impact of human communities, intensity & direction of selection, the type of genetic material & the type of molecular marker used. High allelic diversity is generally associated with the extensive range of genetic diversity represented in the ginger germplasm (Nayak *et al.* 2005). Accurate identification and characterization of different germplasm resources is important for cultivar development & certification and breeders' right protection (Hale *et al.* 2006). The genetic diversity among the eight cultivars was evaluated by using twenty-five random primers out of which thirteen primers produced distinct polymorphic pattern in all the cultivars examined. A total of 70 polymorphic markers were generated by 13 primers, at a rate of 5.38 markers per primer which was comparable to an earlier study of Nayak *et al.* (2005) with ginger (5.05). The amplicons ranged between 300bp-1800bp in size with 2-8 bands per primer. Polymorphism also varied with different cultivars of *Kaempferia galanga* with maximum eight bands for the primers OPC-09, OPD-20 and minimum of 2 bands in the primer OPA-01. RAPD profile of eight cultivars of *Kaempferia galanga* analyzed showed the polymorphic index value of 78.57% across all the cultivars in the present study. The percentage of RAPD polymorphism ranged from 50 to 100% (Fig. 2a; Table 1).

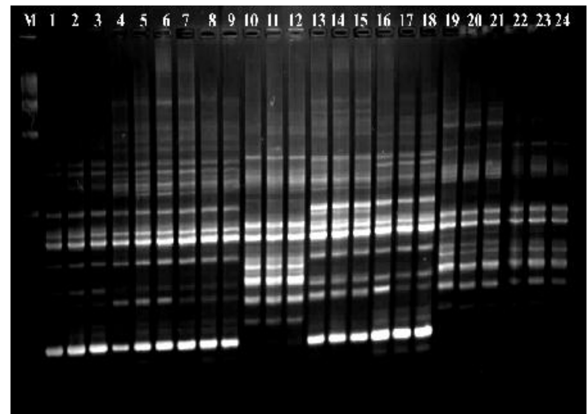


**Fig 2a)** RAPD profile of eight cultivars of *Kaempferia galanga* using primer OPA-18 M- DNA ladder; 1 to 3-Thoubal cultivar; 4 to 6- Imphal-east cultivar; 7 to 9- Imphal- West; 10 to 12-Tamenglong cultivar;13 to15- Bishnupur cultivar; 16 to18-Ukhrul cultivar; 19 to 21- Churachandpur cultivar; 22 to 24- Senapati cultivar.

ISSR analysis was carried out using twenty ISSR primers to characterize the genetic diversity present among the eight cultivars of *Kaempferia galanga.* Eleven of these primers showed a total of 72 reproducible fragments that ranged from 200bp-1000bp in size. High percentage of polymorphism with all the 11 primers (81.94%) was displayed among the eight cultivars of *Kaempferia galanga* with 59 polymorphic bands. An average of 6.54 amplified fragments was produced per primer. The percentage of ISSR polymorphism ranged from 50 to 100% (Fig. 2b; Table 2).

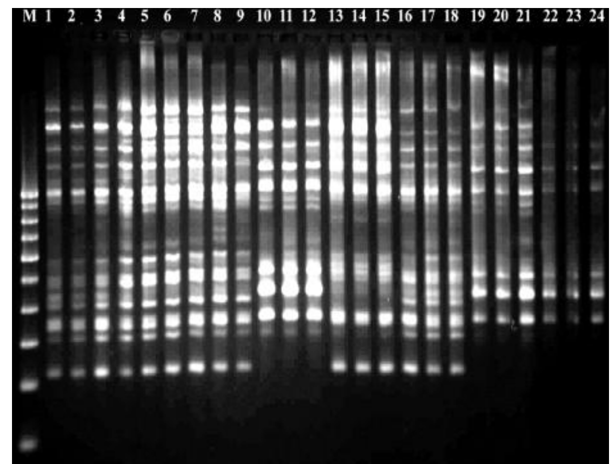


**Fig 2b)** ISSR profile of eight cultivars of *Kaempferia galanga* using IG-10 M- DNA ladder; 1 to 3-Thoubal cultivar; 4 to 6- Imphal-east cultivar; 7 to 9- Imphal- west; 10 to 12-Tamenglong cultivar;13 to15- Bishnupur cultivar; 16 to18-Ukhrul cultivar; 19 to 21- Churachandpur cultivar; 22 to 24- Senapati cultivar.

The genetic similarity (GS) matrices were calculated with Dice coefficient with NTSYSpc 2.2 software (Rohlf 2004) by pair wise comparison of eight cultivars following the method of Nei & Lei (1979). Using RAPD profile, the GS co-efficient ranged from 0.381 to 0.988. The highest GS co-efficient was obtained between Imphal-East cultivar (ST_CV2) and Imphal- West

(ST_ CV3) and the lowest between Imphal-east cultivar (ST_CV2) and Tamenglong cultivar (KM_ CV1) (Table 3). With ISSR markers, the GS ranged from 0.359 to 0.949 for all the cultivars (Table 4). The lowest GS (0.359) occurred in case of Imphal-east cultivar (ST_CV2) and Senapati cultivar (SL_CV) and the highest in case of between Imphal-east cultivar and Imphal-West cultivar.

major groups consisting each of four, one, one and two cultivars (Fig.5). With UPGMA cluster analysis, the RAPD as well as ISSR dendrogram clustered Thoubal, Imphal-East, Imphal-West and Ukhrul in one group which indicates that they may have similar genetic background. Churachandpur and Tamenglong formed one sud-group in ISSR dendrogram but in RAPD dendrogram Churachandpur is grouped with Senapati.

**Table 3** Genetic Similarity coefficients among the eight *Kaempferia galanga* cultivars generated by RAPD markers

| | Thoubal | Imphal-East | Imphal-West | Tamenglong | Bishnupur | Ukhrul | Churachandpur | Senapati |
|---|---|---|---|---|---|---|---|---|
| Thoubal | 1.00 | | | | | | | |
| Imphal-east | 0.965 | 1.00 | | | | | | |
| Imphal-West | 0976 | 0.988 | 1.00 | | | | | |
| Tamenglong | 0.386 | 0.381 | 0.386 | 1.00 | | | | |
| Bishnupur | 0.894 | 0.930 | 0.918 | 0.452 | 1.00 | | | |
| Ukhrul | 0.952 | 0.941 | 0.952 | 0.410 | 0.918 | 1.00 | | |
| Churachandpur | 0.438 | 0.459 | 0.466 | 0.806 | 0.459 | 0.493 | 1.00 | |
| Senapati | 0.417 | 0.411 | 0.417 | 0.817 | 0.411 | 0.417 | 0.885 | 1.00 |

**Table 4** Genetic Similarity coefficients among the eight *Kaempferia galanga* cultivars generated by ISSR markers

| | Thoubal | Imphal-East | Imphal-West | Tamenglong | Bishnupur | Ukhrul | Churachandpur | Senapati |
|---|---|---|---|---|---|---|---|---|
| Thoubal | 1.00 | | | | | | | |
| Imphal-east | 0.854 | 1.00 | | | | | | |
| Imphal-west | 0.860 | 0.949 | 1.00 | | | | | |
| Tamenglong | 0.487 | 0.462 | 0.477 | 1.00 | | | | |
| Bishnupur | 0.778 | 0.835 | 0.800 | 0.587 | 1.00 | | | |
| Ukhrul | 0.889 | 0.872 | 0.879 | 0.458 | 0.779 | 1.00 | | |
| Churachandpur | 0.466 | 0.442 | 0.458 | 0.907 | 0.621 | 0.487 | 1.00 | |
| Senapati | 0.431 | 0.359 | 0.373 | 0.806 | 0.532 | 0.429 | 0.839 | 1.00 |

**Table5** Genetic Similarity coefficients among the eight *Kaempferia galanga* cultivars generated by RAPD+ISSR markers

| | Thoubal | Imphal-East | Imphal-West | Tamenglong | Bishnupur | Ukhrul | Churachandpur | Senapati |
|---|---|---|---|---|---|---|---|---|
| Thoubal | 1.00 | | | | | | | |
| Imphal-east | 0.908 | 1.00 | | | | | | |
| Imphal-west | 0.918 | 0.967 | 1.00 | | | | | |
| Tamenglong | 0.435 | 0.423 | 0.433 | 1.00 | | | | |
| Bishnupur | 0.834 | 0.878 | 0.854 | 0.523 | 1.00 | | | |
| Ukhrul | 0.921 | 0.905 | 0.914 | 0.434 | 0.844 | 1.00 | | |
| Churachandpur | 0.438 | 0.438 | 0.449 | 0.857 | 0.534 | 0.477 | 1.00 | |
| Senapati | 0.409 | 0.371 | 0.381 | 0.812 | 0.461 | 0.408 | 0.862 | 1.00 |

Using RAPD and ISSR profiles together, the highest GS co-efficient was obtained between Imphal-east cultivar and Imphal-west cultivar (0.967) and the lowest between Imphal-east cultivar and Senapati cultivar (0.371) (Table 5). The genetic similarity co-efficient obtained by RAPD, ISSR & RAPD + ISSR were in the range of 0.359 to 0.949, 0.381 to 0.988 and 0.371 to 0.967 respectively.

The present molecular diversity study revealed a higher genetic variability among the cultivars even though the cultivars are morphologically similar. Kizhakkayil and Sasikumar (2010) reported a geographical bias in the *Z. officinale* clones from India. Because of the lack of genetic recombination, asexually reproducing species should, theoretically, show low genetic variation (Hangelbroek *et al.* 2002); however, this is not necessarily true in practice. Jatoi *et al.* (2008) reported high degree of genetic variation in Asian collection of ginger. UPGMA clustering method was used to generate a dendrogram for the eight cultivars of *Kaempferia galanga* in the present study by computing the genetic similarity values with Dice coefficient in NTSYSpc 2.2 program. With RAPD data the eight cultivars were divided into four major groups as depicted in the dendrogram (Fig.3). The dendrogram obtained for ISSR markers divided the eight cultivars into four major clusters (Fig.4). The dendrogram constructed by combining together RAPD and ISSR using UPGMA resulted in formation of 4

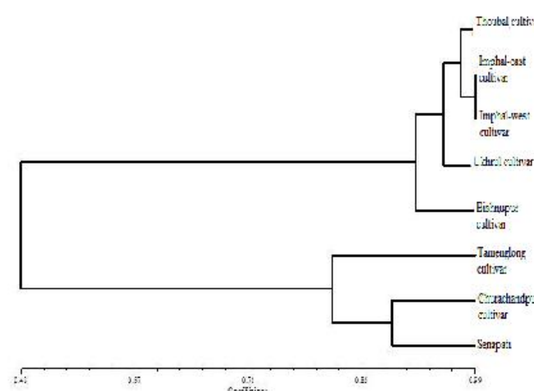

**Fig.3** Dendrogram derived from UPGMA cluster analysis using Dice co-efficient of RAPD

The discrimination and grouping among the cultivars in dendrograms derived from RAPD and ISSR marker systems were found to be different. These differences may be attributed to marker sampling error and/or the level of polymorphism detected, reinforcing again the importance of the n umber of loci and their coverage of the overall genome in obtaining reliable estimates of genetic relationships among cultivars (Loarce *et al.* 1996). So to have a clear and concise idea about genetic diversity among the eight cultivars a dendrogram was constructed using pooled data of both RAPD and ISSR

markers. The study revealed that Churachandpur and Senapati are closely related, though the cultivars have different origins. It can be inferred that the parental lines in these two cultivars may share a similar genetic background which was concordant to the study of Lu *et al*. (2009) with the broccoli cultivars.

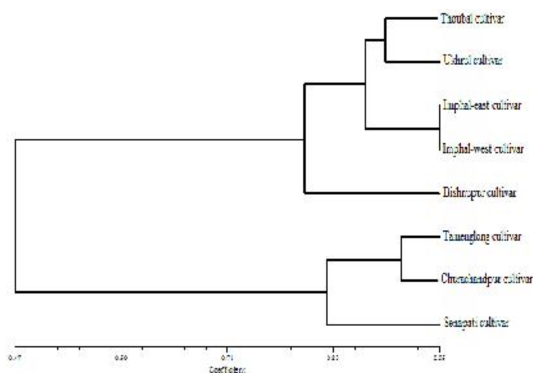

**Fig.4** Dendrogram derived from UPGMA cluster analysis using Dice co-efficient of ISSR

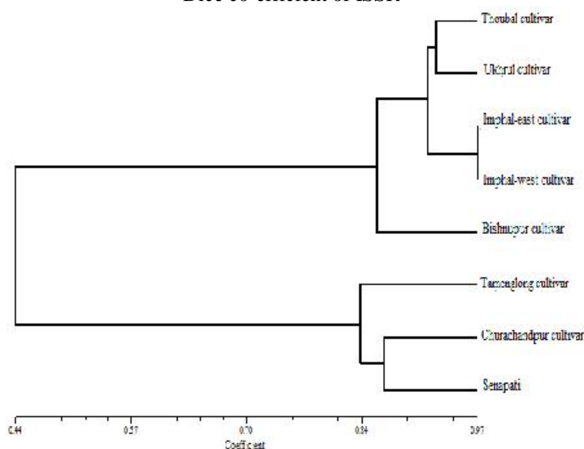


**Fig.5** Dendrogram derived from UPGMA cluster analysis using Dice co-efficient of RAPD+ISSR.

The two cultivars Tamenglong and Bishnupur showed highest variability as they stood singly in all the dendrograms. High genetic/molecular variance within population/group in comparison to among population/group has been reported in both sexually and asexually reproducing crops, including ginger (Haldimann *et al*. 2003; Hangelbrook *et al*. 2002; Sreekumar and Renuka, 2005; Jatoi *et al*. 2008).Genotypes of ginger that form separate groups or operational taxonomic units (OTUs) dissimilar to remaining cultivars are potential germplasm that may be exploited to broaden the genetic base which is in high accordance with Sajeev *et al*. (2011). In this regard Tamenglong and Bishnupur cultivars are most important in that they are genetically distant.

Extensive work has been done to understand whether PCR-based data are useful for estimating the chemical characteristics of *K. galanga* samples or not. With the advent of DNA fingerprinting, the phylogenies originating from DNA data against those derived from presence/absence matching of selected terpene compounds has been exploited (Adams, 2000a). Antioxidants provide chemical protection for biological systems against harmful effects of reaction or processes that cause excessive oxidation, protein and DNA damage and cell death (Papas 1999; Arnao *et al*. 2001). In the present study, variance analysis indicated significant difference ($p<0.05$) in anti-oxidant activities and phenol content of different *Kaempferia galanga* cultivars. The highest anti-oxidant activity was recorded in Tamenglong cultivar which is significantly different ($p<0.05$) from other cultivars (Table. 6). The mean anti-oxidant activity was maximum in Tamenglong cultivar followed by Bishnupur cultivar.

The highest phenol content was found in Ukhrul cultivar (20.58 ± 0.20) while the lowest in Tamenglong cultivar (13.26 ±0.25) (Table.7). Kaempferol is a flavonoid which a plant secondary metabolite characterized by a diphenylpropane structure. They are widely distributed in the plant kingdom and are common constituents of vegetables and fruits. A positive relationship between the ingestion of foods containing flavonoid and reduced risk of developing cancer and cardiovascular diseases has been observed in some epidemiological studies. Some of this evidence comes from the study of plants used in traditional medicine to treat a wide range of pathologies (Lopez-Lazaro 2009). Among the 8 different cultivars of *Kaempferia galanga* L., Imphal-East cultivar has maximum value of kaempferol content (1.480939%) while the minimum is found in Senapati cultivar (0.00004%) (Table. 8).

**Table 6** Anti-oxidant activities and phenol content of different cultivars of *Kaempferia galanga* L.

| Cultivar | Anti-oxidant activities (mg/100g) Mean ± S.E* |
|---|---|
| Tamenglong | 124.98$^{a}$ ± 0.22 |
| Bishnupur | 121.97$^{b}$± 0.13 |
| Thoubal | 98.17$^{d}$± 0.22 |
| Imphal-East | 102.33$^{c}$ ± 0.21 |
| Imphal-West | 100.09$^{e}$ ± 0.18 |
| Senapati | 99.13$^{d}$± 0.10 |
| Ukhrul | 93.18$^{f}$ ± 0.19 |
| Churachandpur | 99.23$^{d}$ ± 0.18 |

*Means followed by same letters are not significantly different at $p < 0.05$, according to Tukey's comparison test

**Table 7** Phenol content of different cultivars of *Kaempferia galanga* L.

| Cultivar | Phenol content (mg/100g) Mean ± S.E* |
|---|---|
| Tamenglong | 13.26$^{a}$ ±0.25 |
| Bishnupur | 15.38$^{b}$ ± 0.21 |
| Thoubal | 18.09$^{d}$ ± 0.16 |
| Imphal-East | 16.44$^{c}$ ± 0.27 |
| Imphal-West | 18.30$^{d}$± 0.12 |
| Senapati | 19.11$^{e}$ ± 0.07 |
| Ukhrul | 20.23$^{f}$± 0.20 |
| Churachandpur | 18.44$^{d}$± 0.15 |

*Means followed by same letters are not significantly different at $p < 0.05$, according to Tukey's comparison test

**Table 8** Kaempferol content of different cultivars of *Kaempferia galanga* L.

| Name of cultivar | Retention time | Peak Area | Ppm | %(mg) |
|---|---|---|---|---|
| Standard(10ppm) | 2.735 | 56606 | 10 | |
| Tamenglong | 2.782 | 5123 | 5.165683 | 0.516568 |
| Bishnupur | 2.770 | 9641 | 10.7796 | 1.07796 |
| Thoubal | 2.744 | 9492 | 11.56451 | 1.156451 |
| Imphal-East | 2.726 | 16372 | 14.80939 | 1.480939 |
| Imphal-West | 2.757 | 12715 | 12.51381 | 1.251381 |
| Senapati | 2.749 | 213 | 0.00043 | 0.00004 |
| Ukhrul | 2.721 | 945 | 0.002368 | 0.000237 |
| Churachandpur | 2.766 | 1095 | 1.08920265 | 0.10892 |

Those cultivars which grouped under the same cluster in dendrogram derived from RAPD and ISSR marker systems showed significant difference in the chemical analyses data. Adams (2000b) had reported that in the study of Juniperus section Juniperus, no comparable degree of correlation could be observed between the two sets of markers viz RAPD and chemical data. Hence we suggest that the genotypic differences may not be responsible for the differences in the chemical composition.

## CONCLUSION

The results obtained in the present study could be of practical application in classical breeding to produce better varieties and the cultivation of cultivars with known composition and ratio of individual essential oil constituents. In addition to this, the putative cultivar-specific RAPD markers could be converted to sequence characterized amplification regions (SCARs) after sequencing and designing primer pairs to develop robust cultivar specific markers.

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## References

1. Arnao MB, Cano A, Acosta M (2001) The hydrophilic and lipophilic contribution to total antioxidant activity. Food chem. 73: 239-244
2. Bestwick CS, Milne L, Duthie SJ (2007) Kaempferol induced inhibition of HL-60 cell growth results from a heterogeneous response, dominated by cell cycle alterations. Chem Biol Interact 170(2): 76-85
3. Das A, Kesari V, Satyanarayana VM, Parida A, Rangan L(2011) Genetic Relationship of Curcuma Species from Northeast India Using PCR-Based Markers. Mol Biotechnol 49: 65–76
4. Doyle JJ & Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19 :11–15
5. Haldimann P, Steinger T, Scharer HM (2003) Low genetic differentiation among seasonal cohorts in Senecio vulgaris as revealed by AFLP analysis. Mol Ecol 12 : 2541–2551
6. Hale AL, Farnham MW, Menz MA (2006) Effectiveness of PCR-based markers for differentiating elite broccoli inbreds. J Am Soc Hortic Sci 131: 418–423
7. Hangelbroek HH, Ouborg NJ, Santamaria L, Schwenk S (2002) Clonal diversity and structure within a population of the pondweed Potamogeton pectinatus foraged by Bewick's swans. Mol Ecol 11 : 2137–2150
8. Hu J, Quiros CF (1991) Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Rep 10 : 505–511
9. Islam MA, Meister A, Schubert V, Kloppstech K, Esch E (2007) Genetic diversity and cytogenetic analyses in *Curcuma zedoaria* (Christm.) Roscoe from Bangladesh. Genet Resour Crop Evol 54 : 149–156
10. Jatoi SA, Kikuchi A, Mimura M, Yi SS, Watanabe KN (2008) Relationships of Zingiber species and genetic variability assessment in ginger (*Zingiber officinale*) accessions from ex-situ genebank, on-farm and rural markets. Breed Sci 58: 261–270.
11. Kalpana D, Choi SH, Choi TK, Senthil K, Lee YS (2012) Assessment of genetic diversity among varieties of mulberry using RAPD and ISSR fingerprinting. Sci Hortic 134: 79–87
12. Kizhakkayil J, Sasikumar B (2010) Genetic diversity analysis of ginger (*Zingiber officinale* Rosc.) germplasm based on RAPD and ISSR markers. Sci Hort 125: 73–76
13. Kosuge T, Yokota M, Sugiyama K, Saito M, Iwata Y, Nakura M, Yamamoto T (1985) Studies on anticancer principles in Chinesemedicines II. Cytotoxic principles in Biota orientalis (L.) Endl. and *Kaempferia galanga* L. Chem Pharm Bull 33: 55-65
14. Liu G, Liu L, Wang Y, Yu F, Shen H, Gui W (2007) Seed genetic purity testing of F1 hybrid cabbage with molecular marker analysis. Seed Sci Technol 35: 47
15. Loarce Y, Gallego R, Ferrer E (1996) A comparative analysis of genetic relationships between rye cultivars using RFLP and RAPD markers. Euphytica 88: 107–115
16. Lopez-Lazaro M (2009) Distribution and biological activities of the flavonoid luteolin. Mini Rev Med Chem 9: 31-59
17. Louarn S, Torp AM, Holme IB, Andersen SB, Jensen BD (2007) Database derived microsatellite markers (SSRs) for cultivar differentiation in *Brassica oleracea*. Genet Resour Crop Evol 54: 1717–1725
18. Lu X, Lu L, Gong Y, Zhao L, Song X, Zhu X (2009) Cultivar identification and genetic diversity analysis of broccoli and its related species with RAPD and ISSR markers. Sci Hort 122: 645–648
19. Mohanty S, Parida R, Singh S, Joshi RK, Subudhi E, Nayak S (2011) Biochemical and molecular profiling of micropropagated and conventionally grown *Kaempferia galanga*. Plant Cell Tiss Organ Cult 106:39–46
20. Nayak S, Naik PK, Acharya L, Mukherjee AK, Panda PC, Das P (2005) Assessment of genetic diversity among 16 promising cultivars of ginger using cytological and molecular markers. Z Naturforsch 60c: 485–492
21. Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci U.S.A. 76: 5269–5273
22. Okada Y, Okada M (1998) Scaveenging effect of soluble protein in broad beans of free radicals and active oxygen species. J Agric Food Chem 46: 401-406
23. Papas AM (1999) Antioxidant status, diet nutrients and health CRC Press. Boca Raton. FL
24. Rahman MM, Amin MN, Ahamed T, Ali MR, Habib A (2004) Efficient plant regeneration through somatic embryogenesis from leaf base-derived callus of Kaempferia galanga L. Asian Journal of Plant Science 3(6): 675-678

25. Rohlf FJ (1998) NTSYS-PC Numerical taxonomy and multivariate analysis system, version 2.00. Exeter Software, Setauket, NY
26. Sajeev S, Roy AR, Iangrai B, Pattanayak A, Deka BC (2011) Genetic diversity analysis in the traditional and improved ginger (*Zingiber officinale* Rosc.) clones cultivated in North- East India. Sci Hortic 128: 182-188
27. Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic- Phosptungstic acid reagents. Am J Enol Viticult 16: 144-158
28. Sreekumar VB, Renuka C (2005) Assessment of genetic diversity in Calamus thwaitessii BECC (Arecaceae) using RAPD markers. Biochem Systemat Ecol 34: 397–405
29. Vijayan K, Tidaker A, Kar PK, Srivatava, Aswathi AK, Thangavelu K, Saratchandra (2006) Assessment of genetic relationships between wild and cultivated mulberry (Morus) species using PCR based markers. Genet Resour Crop Evol 53: 873–882
30. Wood TH, Whitten WM, Williams NH (2000) Phylogeny of Hedychium and related genera (Zingiberaceae) based on ITS sequence data. Edinburgh Journal of Botany 57: 261–270
31. Wu TL, Larsen K (2000) Zingiberaceae. In: Wu, Z.-Y. and P. H. Raven (eds.). Flora of China Sci Press, Beijing, China. 24: 333-346
32. Yonemoto Y, Chowdhury AK, Kato H, Macha MM (2006) Cultivars identification and their genetic relationships in Dimocarpus longan subspecies based on RAPD markers. Sci Hortic 109: 147–152


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