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Research article

GENETIC DIVERSITY AND ANTIOXIDANT POTENTIAL OF ZINGIBER MONTANUM (KOENIG) LINK EX A. DIETR

K. Sanatombi* and Rajkumari Sanatombi

Department of Biotechnology, Manipur University-795003, India

ABSTRACT: The present study reports for the first time the assessment of genetic diversity among ten accessions of *Zingiber montanum* (Koenig) Link ex A. Dietr., collected from different parts of Manipur using random amplified polymorphic DNA (RAPD) markers and screening of their antioxidant activity by 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. The ten accessions exhibited genetic variability with 91.83% polymorphism among them and similarity coefficients ranged from 0.35 to 0.68 with an average of 0.44. UPGMA clustering based on Jaccard's similarity coefficient divided the accessions into three distinct clusters with respect to their location of collection. The antioxidant activity of the accessions also showed variations with value ranging from 59.03% to 74.30% inhibition.
Key words: Antioxidant activity, Genetic diversity, *Zingiber montanum*, RAPD, DPPH

*Corresponding author: K. Sanatombi, Department of Biotechnology, Manipur University-795003, India, E-mail: ksanatombi@rediffmail.com

INTRODUCTION

Zingiber montanum (J. Koenig) Link ex A. Dietr., commonly known as Cassumunar ginger, is a medicinally important aromatic perennial herb of the genus *Zingiber* belonging to family Zingiberaceae. It is found abundantly in Manipur and locally known as "Tekhao yaikhu". The rhizomes of Cassumunar ginger are used by traditional medicinal practitioners of Manipur for treatment of various ailments and several other disorders [1]. It has also been shown to have high antioxidant [2, 3, 4], anti-inflammatory [5, 6, 7, 8], anti-allergic [9], antibacterial and antifungal activity [10, 11].

Since the plant is used both as a dietary supplement and also as a medicinal herb by local practitioners, it is heavily exploited and efforts are needed for its conservation. However, there has been no report on the study of genetic variability among the Cassumunar ginger populations growing in different parts of Manipur. Therefore, the present investigation was undertaken to assess the genetic diversity among accessions of Cassumunar ginger collected from different parts of Manipur by using RAPD markers and also to analyze the antioxidant activity of the collected samples.

METHODOLOGY

RAPD analysis

Good quality genomic DNA was extracted from the rhizomes of ten accessions of Cassumunar ginger (Table 1) following the modified CTAB method of Ashraf *et al*., 2014[12] and PCR amplification reaction was performed in a total volume of 25µl containing 10X PCR buffer, 2mM $MgCl_2$, 0.4mM dNTPs (0.025mM each dNTP), 0.2µM decamer primer (Operon), 0.08U of Taq DNA (New England Biolab) and 25 ng of genomic DNA. The PCR cycle consisted of an initial denaturation at 94°C for 2 min followed by 45 cycles, each consisting of denaturation at 94°C for 1 min, annealing at 36°C for 1 min and extension at 72°C for 2 min. It was followed by a final extension at 72°C for 10 min. Each RAPD PCR reaction was repeated and observed three times for similar banding pattern. The PCR products were separated alongside a molecular weight marker (100 bp ladder, Himedia) by 1.5% agarose gel electrophoresis in 1X TBE buffer and gels were photographed in a Gel-documentation system (Vilber Lourmat). The amplified DNA fragments were scored as present (1) or absent (0) and used for statistical analyses. Genetic similarities were calculated by Jaccard's Similarity Coefficient and a dendrogram was constructed by the UPGMA (Unweighted Pair Group Method with Arithmetic mean) method using MEGA6.

DPPH radical scavenging activity

1g of dried rhizome powder was homogenized with 20 ml of 90% methanol for 2/3 hours and then centrifuged at 5000 rpm for 15 min. The supernatant obtained was collected and used for determination of antioxidant activity following the method of Shimada *et al.*, 1992 [13] using 1, 1-Diphenyl-2-picrylhydrazyl (DPPH). The scavenging activity of the samples corresponded to the intensity of quenching DPPH and results were expressed as percentage of inhibition,

$$\% \text{ inhibition} = \frac{(Ac - Ae)}{Ac} \times 100\ \%$$

Where Ac – Absorbance of the control, Ae – Absorbance of the plant extract after 30 min incubation

RESULTS AND DISCUSSIONS

Random amplified polymorphic DNA (RAPD) markers are rapid, easy and cost effective PCR-based DNA fingerprinting method [14]. It has been used for studying genetic variability of closely related species [15, 16, 17] including *Zingiber montanum*, *Zingiber officinale* Rosc, *Zingiber zerumbet* (L.) Smith and *Zingiber moran* [12, 18, 19, 20, 21, 22, 23, 24, 25]. In the present study, eleven decamer primers (Table 2) which gave consistent amplification products were used to assess the genetic diversity among the collected samples and significant polymorphism could be detected using the technique. Earlier, Singh *et al.* (2013) [22] used 15 RAPD and 8 ISSR primers to study the genetic diversity of *Zingiber officinale* cultivars collected from nine districts of Manipur. However, unlike the present study, no polymorphism could be detected among the cultivars of *Zingiber officinale* revealing their genetic stability. A total of 147 reproducible and scorable amplification products were generated using the primers, out of which 135 were polymorphic accounting for average polymorphism of 92.21% among the ten accessions of Cassumunar ginger. The genetic similarity coefficients (Jaccard's) obtained by RAPD varied considerably (0.35 to 0.68) among the accessions and the highest genetic similarity was observed between the accessions ZM1 and ZM3 (similarity index of 0.68) followed by similarity index of 0.64 between ZM5 and ZM9 and similarity index of 0.60 between ZM1 and ZM5 while the least genetic similarity (0.35) was obtained between ZM2 and ZM7 (Table 3). The dendrogram constructed by the UPGMA method grouped the accessions into three major clusters (I-III) which split at a Jaccard's Similarity Index (JSI) of 0.01 (Figure 1).

Table 1. Collection sources, details and antioxidant potential of *Zingiber montanum* accessions collected from different parts of Manipur used in the present study.

S.No	Accession number	Latitude, Longitude & Elevation(m)	Origin of collection	% DPPH inhibition (Mean ± S.E)
1	ZM1	24° 43' 5.086" N 93° 56' 3.926" E ,778 m	Lilong Chajing, Imphal West district	71.05±1.24
2	ZM2	24° 51' 29.415" N 93° 36' 59.037" E, 461 m	Noney,Tamenglong district	74.30±0.63
3	ZM3	24° 42' 59.193" N 93° 51' 44.828" E, 771 m	Nambol langpok, Bishnupur district	71.30±3.12
4	ZM4	24° 15' 25.412" N 93° 40' 10.443" E, 834 m	Geljang Hills, Churachandpur district,	69.56±2.79
5	ZM5	24° 44' 2.229" N 93° 7' 48.850" E , 46 m	Jiribam, Imphal East district	61.14±0.62
6	ZM6	24° 15' 24.080" N 94° 17' 47.639" E , 204 m	Moreh, Chandel district	66.31±4.80
7	ZM7	24° 48' 53.686" N 94° 21' 41.009" E , 1397 m	Phungyar, Ukhrul district	65.43±0.37
8	ZM8	24° 29' 48.384" N 93° 59' 13.554" E , 785 m	Kakching, Thoubal district	59.03±1.24
9	ZM9	25° 16' 32.650" N 94° 1' 58.470" E , 1051 m	Senapati, Senapati district	61.58±0.57
10	ZM10	24° 48' 34.180" N 93° 55' 25.405" E, 782 m	Uripok, Imphal West district	59.12±0.47

S. E – Standard Error

Cluster I was further divided into two subgroups: Cluster I (A) consisting of ZM1, ZM2, ZM3 and ZM4 and Cluster 1(B) consisting of ZM5 and ZM9 where the two nodes showed 0.18 JSI between them, which is found to be nearest to ZM1 and ZM3. The accessions ZM6 and ZM7 were grouped in cluster II while cluster III was represented by ZM8 and ZM10. Similar cases of the presence of a high level of genetic variation in Cassumunar ginger accessions collected from different regions have also been reported earlier [18, 25].

Since DPPH method offers a rapid, stable, easy way and acts as free radical- scavengers or hydrogen donors for studying the antioxidant activity [26, 27], it was used to screen the total antioxidant potential of the ten collections of *Zingiber montanum* and the result is shown in Table 1. The DPPH inhibition ratio varied from 59.03% exhibited by *Zingiber montanum* collected from Thoubal district to 74.30% exhibited by *Zingiber montanum* collected from Tamenglong district. A similar earlier study by Bua-in and Paisooksantivatana (2009) [28] reported significant difference in the antioxidant activity of Z. *cassumunar* collected from different locations of Thailand where the % inhibition activity were in the range of 57.68% to 80.88%.

Table 2. List of RAPD primers, number of amplified products and polymorphism percentage for Zingiber montanum accessions collected from different parts of Manipur.

Primers	Sequence (5' - 3')	Total no. of bands	No.of polymorphic bands	Polymorphism (%)	Size range of amplicons (kb)
OPC-02	GTGAGGCGTC	11	10	90.9	> 0.2-3.0
OPC-04	CCGCATCTAC	17	17	100	> 0.2-3.0
OPC-05	GATGACCGCC	16	15	93.75	0.1-3.0
OPC-09	CTCACCGTCC	10	9	90	0.5-3.0
OPC-14	TGCGTGCTTG	11	10	90.9	> 0.2-3.0
IDT 3	TGAGCACGAG	16	15	93.75	0.1 - < 3.0
IDT 4	CCATTCCCCA	14	13	92.85	0.2-3.0
IDT5	AGGACTGCCA	14	12	85.71	0.2-3.0
IDT 9	CTCTGCGCGT	11	11	100	0.1-1.5
IDT 11	CACAGCTGCC	10	10	100	0.5-3.0
IDT 15	GTCCCGACGA	17	13	76.47	0.3-3.0
Average		13.36	12.27	92.21	0.1-3.0

Table 3. Similarity matrix of *Zingiber montanum* accessions collected from different areas of Manipur generated using Jaccard's similarity coefficient.

Rows/ Columns	ZM1	ZM2	ZM3	ZM4	ZM5	ZM6	ZM7	ZM8	ZM9	ZM10
ZM1	1.00									
ZM2	0.49	1.00								
ZM3	0.68	0.55	1.00							
ZM4	0.49	0.49	0.52	1.00						
ZM5	0.60	0.50	0.57	0.58	1.00					
ZM6	0.42	0.39	0.44	0.43	0.47	1.00				
ZM7	0.39	0.35	0.43	0.38	0.46	0.44	1.00			
ZM8	0.47	0.37	0.45	0.46	0.46	0.44	0.39	1.00		
ZM9	0.43	0.44	0.40	0.40	0.64	0.36	0.37	0.37	1.00	
ZM10	0.38	0.36	0.39	0.37	0.40	0.39	0.42	0.48	0.44	1.00

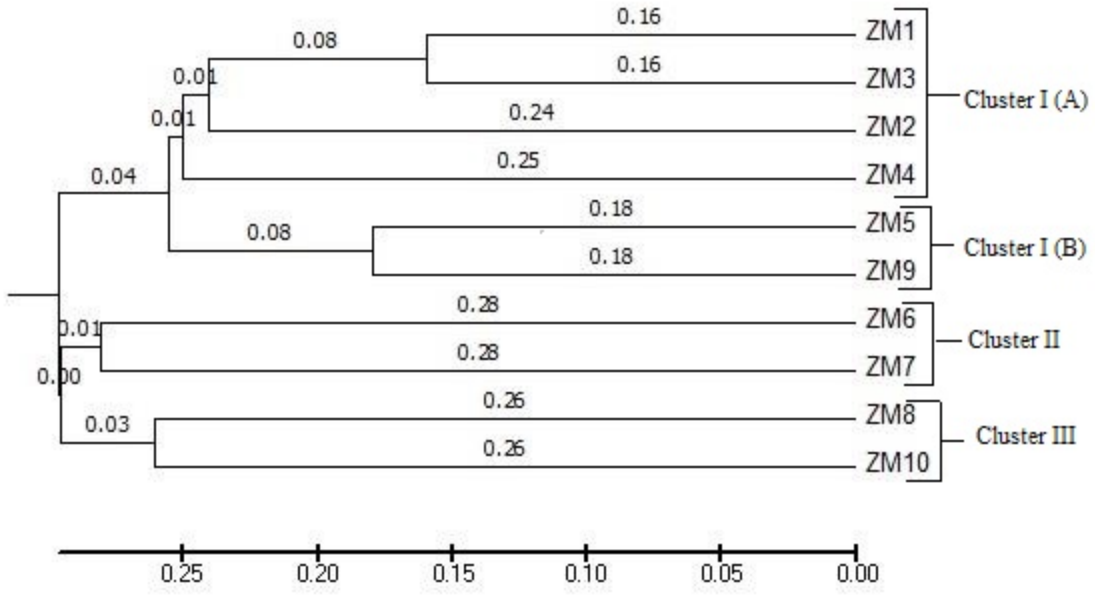

Figure 1. Dendrogram for the ten accessions of *Zingiber montanum* constructed by UPGMA cluster analysis from RAPD data generated using 11 primers.

CONCLUSIONS

The pair-wise genetic similarity indices, high level of polymorphism and the variations in antioxidant activity of *Zingiber montanum* accessions collected from different parts of Manipur indicates that there is considerable genetic diversity among them which could be attributed to different geographical and ecological conditions, wide range of distribution and type of molecular marker used.

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