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# Molecular Characterization of *Acmella paniculata* (Asteraceae) from Arunachal Himalayan Region through RAPD and ISSR Markers

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## Abstract

*Acmella paniculata* (Wall. ex DC.) R.K. Jansen (synonyms: *Spilanthes paniculata* DC and *Spilanthes calva* DC.) belonging to the family Compositae (Asteraceae) distributed across both tropical and subtropical nations have been reported to be useful in traditional medicine and dietary system among different tribal communities of the world. Worldwide utilization of *A. paniculata* (Wall. ex DC.) R.K. Jansen in modern medicine has made it necessary for authentication of this particular species at the molecular levels in order to avoid confusions regarding taxonomic positions prevalent throughout the globe. Here, a total of 60 individuals of *A. paniculata* (Wall. ex DC.) R.K. Jansen belonging to nine groups of populations from six districts of Arunachal Himalayan Region of India with elevation ranging from 200–3000 m from the mean sea level was taken in order to establish genetic diversity within and among its populations using RAPD and ISSR markers. A total of 25 RAPD and 15 ISSR primers of varying concentrations were used for the present study. In case of RAPD a total of 523 loci were obtained in which 247 loci were monomorphic and 276 loci were polymorphic thereby showing 52.77% polymorphism while in case of ISSR a total of 208 loci were obtained in which 75 loci were monomorphic and 133 loci were polymorphic thereby showing 63.94% polymorphism. The Polymorphic Information Content (PIC) value of the RAPD and ISSR marker system was 0.65 and 0.78 respectively. The Resolving Power (Rp) values of the RAPD primers ranged from 4.20 to 7.65 while the ISSR primers ranged from 5.34 to 7.47. The Percentage of Polymorphic loci (Pp) ranging from 31% to 76% in case of RAPD while 52% to 77% in case of ISSR. Nei's Gene Diversity (h) from 0.10 to 0.17 with mean Nei's Gene Diversity of 0.25, and Shannon's Information Index (I) values ranging from 0.14 to 0.26 with an average value of 0.47 were found in the study. Global Coefficient of Genetic Differentiation (GST), showed that relative genetic diversity of the populations was moderately high. However, the low value of gene flow was revealed by the RAPD and ISSR marker (Nm = 0.25). The average similarity matrix was used to generate a tree for cluster analysis by UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method which showed higher genetic diversity in some of the populations of *A. paniculata* (Wall. ex DC.) R.K Jansen. The results of Analysis of Molecular Variance (AMOVA) revealed that variation among the populations (71.36%) was significantly higher than within the populations (28.64%). Therefore, the present investigation validates the utility of RAPD and ISSR markers system to estimate the genetic diversity of *A. paniculata* (Wall. ex DC.) R.K. Jansen, and that it can be seen as a starting point for future molecular research on the population and evolutionary genetics of this particular species under the genus Acmella of high medicinal and nutraceutical values.

**Keywords:** Genetic Diversity; RAPD; ISSR; Nei's Gene Diversity; UPGMA; AMOVA

## Abbreviations

PCR: Polymerase Chain Reaction; DNA: Deoxyribonucleic Acid; RAPD: Random Amplified Polymorphic DNA; ISSR: Inter Simple Sequence Repeat; SCAR: Sequence Characterized Amplified Regions; RGU: Rajiv Gandhi University; CTAB: Cetyltriethylammonium Bromide; PIC: Polymorphic Information Content; AMOVA: Analysis of Molecular Variance; RP: Resolving Power; SCoT: Start Codon Targeted Polymorphism; TRAP: Target Region Amplification Polymorphism; DAMD: Directed Amplification of Minisatellites.

## Introduction

*Acmella paniculata* (Wall. Ex DC.) R.K. Jansen is a medicinally important species belonging to the family Asteraceae, commonly known as toothache plant, or Shormoni among the Bengali community, and Bud and Marsang among the Nyishi and Adi community of Arunachal Himalayan Region of India. The genus comprises of 30 species and nine additional infra specific taxa that are mainly distributed in the tropical and subtropical regions around the world [1]. It is also called Para cress or Eyeball plant, which is native to the tropics of Brazil, and is grown as both medicinal and ornamental species in various parts of the world [2]. The raw leaves are used as flavouring for salads, soups and meats in Brazil and India, and are grown widely as an ornamental because of the attractive colourful heads [3]. The plant is also used in the traditional medicine and dietary system for the treatment of various disease complications including toothache, infections of throat and gums, paralysis of tongue, a popular remedy for stammering in children and as diuretic [4]. Leaves are used externally in treatment of skin diseases [5]. Root decoction is used as purgative and diuretic [4]. The plant also plays a significant role in soil nutrient enrichment in poorly managed shifting cultivation systems [6]. *A. paniculata* (Wall. Ex DC.) R.K. Jansen is rich in essential oil 'spilanthol' and biologically active compounds especially belonging to the class alkylamides which this plant possesses in large amounts [7].

The worldwide significance of *Acmella paniculata* (Wall. Ex DC.) R.K. Jansen in traditional and modern medicine has made it a necessary for authentication of this particular species at the molecular levels to determine the genotypic variation within and among the population group in order to avoid confusions regarding taxonomic positions that is prevalent throughout the globe [8]. Lack of adequate molecular based literature on the genus *Acmella* [9,10] has necessitated the present authors for undertaking this molecular investigation to check the genetic diversity if exist at all within and among the wild populations naturally grown in a total geographical area of around 90,000 Sq. km and elevation ranging from 200–3000 m from the mean sea level covering tropical and temperate region of Eastern Himalaya. PCR based approaches have been in demand for a long time because of their simplicity and requirement for only small quantities of sample DNA. Among the techniques used for genetic evaluation, Randomly Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) markers are basic approaches that have been commonly used for molecular authentication and validation of plant since last few decades. Despite the development of newer techniques

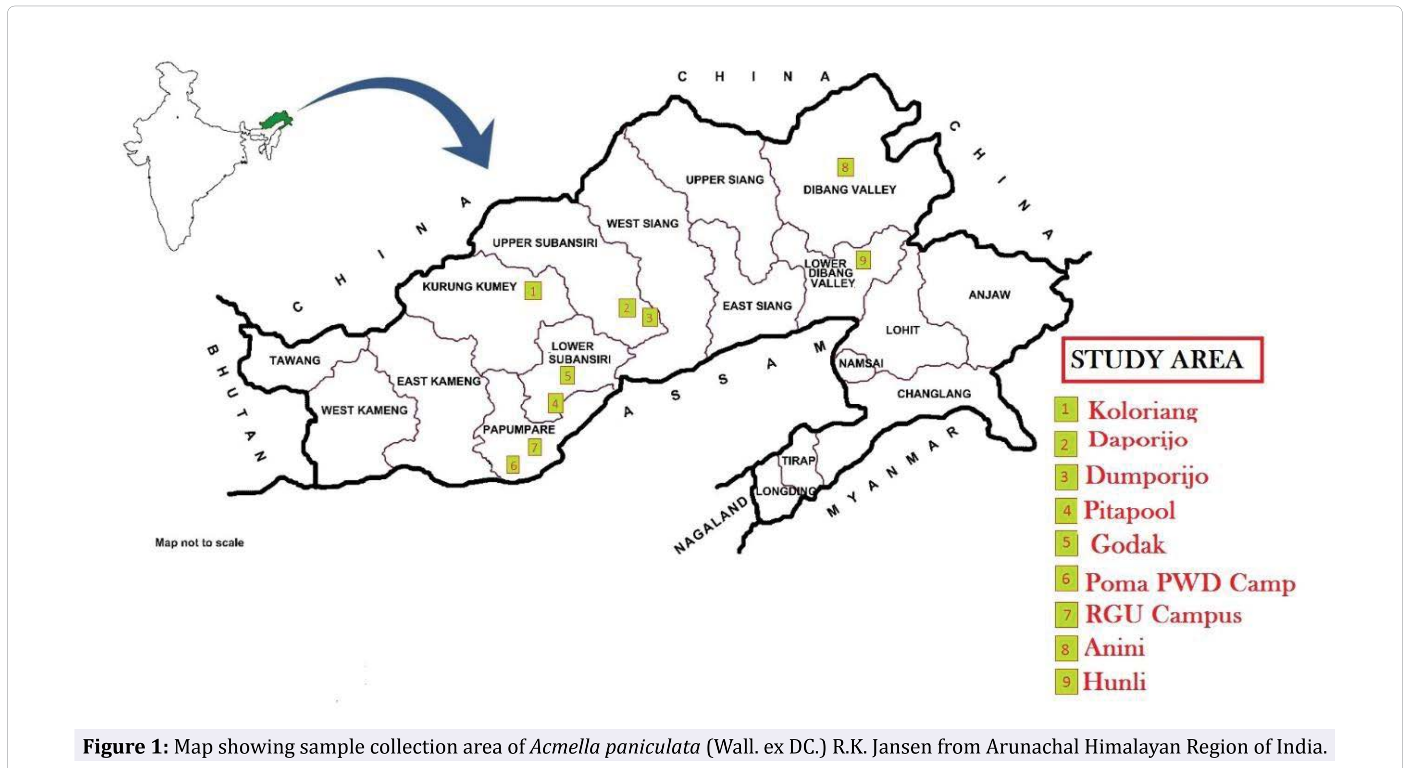


**Figure 1:** Map showing sample collection area of *Acmella paniculata* (Wall. ex DC.) R.K. Jansen from Arunachal Himalayan Region of India.

they are much faster, cheaper requiring no prior information of DNA sequence and have shown to give detail and robust estimates of intra-specific genetic diversity [11]. RAPD can scan the entire genome while ISSR markers can target multiple microsatellite loci that are distributed across the genome and discriminative ISSR markers could also be converted to Sequence Characterized Amplified Regions (SCAR) markers with improved specificity and reproducibility. Moreover combining these two markers can detect genome wide genetic variation among a large number of plants.

Therefore, in our present work, RAPD and ISSR markers have been used to establish the genetic diversity within and among populations of *A. Paniculata* (Wall. Ex DC.) R.K. Jansen collected from a total of six districts of Arunachal Pradesh (Arunachal Himalayan Region of India) covering different altitudinal gradient to know the genetic variation at the population level to develop an understanding on genotypic differences at molecular level that possibly are influenced by the physical barriers, geographical distance, habitat and climate type which could help in deducing phylogenetic relationship among the wild populations growing in different geographical ranges and habitat pattern. This study is as such the first study on population level of this particular species at molecular level from Arunachal Himalayan Region.

## Materials and Methods

### Study Sites and Sample Collection

Sixty individuals (type) of *Acmella paniculata* (Wall. Ex DC.) R.K. Jansen representing nine natural wild populations from six districts of Arunachal Himalayan Region of India was collected through extensive field survey covering a total geographical area of 90,000 sq. Km. The population was sampled across different elevation range to cross check the genetic diversity if exist based on altitudinal gradient. The spots of sampling distance between Central Arunachal Himalayan Region and Far East Arunachal Himalayan Region was maintained at 800 km. The specimen were authenticatedat Herbarium of Botanical Survey of India, Arunachal Pradesh Regional Circle, Itanagar, and Herbarium of Department of Botany, Rajiv Gandhi University, Rono Hills, Arunachal Pradesh. The taxonomic characters and distribution pattern was verified through standard flora such as Flora of British India [12] and Materials for the Flora of Arunachal Pradesh [13]. The accepted name and synonymswere verified through website of the Plant List Version 1.1 2013 (www.theplantlist.org) [14] hosted by Royal Botanical Garden, Kew UK and Missouri Botanical Garden USA. The voucher specimen was prepared, labelled and deposited at Herbarium of Department of Botany, Rajiv Gandhi University, Rono Hills, Doimukh, Arunachal Pradesh, India for future reference.

### Genomic DNA Extraction

Total genomic DNA was extracted from young leaves of *A. paniculata* (Wall. Ex DC.) R.K. Jansen following the 2X CTAB protocol [15] with some minor modifications. The quantity and purity of the isolated DNA were checked using a UV spectrophotometer (UV-2600, Schimadzu wavelength range 300–1100 nm). The ratio of absorbance at two wavelengths ($A_{260}$ and $A_{280}$) was compared with the standard ratio of pure DNA. The quantities of the DNA isolated were found to be optimum for further PCR amplification because of their low $A_{230}$ values suggesting lesser amount of polysaccharides and proteins.

### RAPD and ISSR-PCR Amplification

A total of 25 RAPD and 15 ISSR primers custom synthesized from Metabion Inc. Ltd., Germany was used for carrying out the RAPD reactions all the PCR reactions were carried out in 25 µl volumes containing 40 ng of template DNA, 2 µm of each of the four dntps, 1X PCR buffer (10 mm Tris, pH 9.0, 50 mm kcl), 1.5 mm $MgCl_2$, 1U Taq polymerase (Bangalore Genei, India) and 10 pMol of primer. The reaction program of RAPD-PCR was set following the protocol of Williams [16]. The temperature program was set at 94°C for 3 min followed by 45 cycles of at 94°C for 45 sec, 36°C for 30 sec and 72°C for 2 min. followed by final extension at 72°C for 7 min. Similarly ISSR-PCR was carried out following the method of Zietkiewicz [17]. The temperature profile for ISSR was done at 94°C for 3 min followed by 40 cycles of at 92°C for 1min, (42-58)°C for 1 min and 72°C for 2 min followed by final extension at 72°C for

7 min in a thermal cycler 2720 (Applied Biosystems, USA). After completion of the amplification, 3 µl of 10X blue dye was added to the samples, and the amplified DNA was analysed on 1.5% agarose gel in 1X TAE buffer at (70–80) V for 3–4 hrs. DNA fragments were photographed using Chemi Doc MP System (Biorad, USA). Due to the low reproducibility of RAPD and ISSR markers and also to check the consistency of the electrophoretic patterns and the polymorphism detected, every PCR reaction was repeated twice. Moreover all the PCR amplifications included a negative control (i.e. no DNA) to avoid erroneous interpretations regardless of the marker system used.

### Data Analysis

Banding profiles generated by RAPD and ISSR primers were compiled into a binary data matrix in an excel sheet based on the presence (1) or absence (0) of the selected band. We took into account only amplified bands which were clear, unambiguous and reproducible for the scoring and further statistical data analysis. Smeared and weak bands were excluded. The ability of the primers to distinguish between genotypes through RAPD and ISSR primers were obtained by calculating Resolving Power (Rp) of the primers used [18]. This function has been found to be strongly correlated with the ability to distinguish between genotypes, and the formula Rp = σib where Band In formativeness, [Ib = 1 − (2 × |0.5 − p|)] and p is the proportion of genotypes containing band I. Polymorphic Information Content (PIC) value was also calculated using the formula [PIC = 1 − Σpi2], where pi is the frequency of the $i^{th}$ allele [19]. Genetic diversity parameters including the Percentages of Polymorphic Loci (Pp), Nei's Gene Diversity (h), and Shannon Information Index (I) were calculated with POPGENE version 1.31 to estimate the genetic variation level [20]. Gene Flow (Nm) was estimated from the formula Nm = $[0.25 \times (1 - G_{ST})/G_{ST}]$. Analysis of Molecular Variance (AMOVA) and Fixation Index or F statistics ($F_{ST}$) was performed using Arlequin version 3.01 at two hierarchical levels to evaluate differences within and among populations [21]. The significance of this analog was evaluated by 1000 random permutations of sequences among populations [22]. Pair-wise similarity matrices were generated by Jaccard's coefficient of similarity, using the SIMQUAL format of ntsyspc [23]. Dendrogram was constructed by using the unweighted pair group method with arithmetic average (UPGMA) with the SAHN module of ntsyspc to show a phenetic representation of genetic relationships as revealed by the similarity coefficient [24].

## Results

A total of 25 RAPD primers used for genetic diversity studies of *Acmella paniculata* (Wall. Ex DC.) R.K. Jansen produced a total of 523 loci were obtained in which 247 loci were monomorphic and 276 loci were polymorphic thereby showing 52.77% polymorphism (Figure 2A, Table1). The average number of amplification products was approximately 21 per primer; the maximum was 32 with primer OPAA-14, whereas the minimum was 15 with primers OPAG-13 and OPBH-01 and OPE-18. The PIC value for the RAPD primers was 0.65 (Table 1). The Rp value of the primers ranged from 4.20 (OPAL-13) to 7.65 (OPAB-06). The genetic distance recorded using Jaccard's Coefficient of Similarity ranged from 0.13 to 0.92 (Table 1).

In case of ISSR marker, a total of 208 loci were obtained in which 75 loci were monomorphic and 133 loci were polymorphic thereby showing 63.94% polymorphism (Figure 2B, Table 2). The average number of amplification products was approximately 13 per primer; the maximum was 22 with primer ISSR-17, whereas the minimum was 10 with primer ISSR-02. The PIC value for the ISSR primers was 0.78 (Table 2). The Rp value of the primers ranged from 5.34 (ISSR-27) to 7.47 (ISSR-05). The genetic distance recorded using Jaccard's Coefficient of Similarity ranged from 0.15 to 0.85 (Table 2).

### Cluster Analysis

The dendrogram derived from UPGMA cluster analysis of the RAPD and ISSR data matrix indicated two major clusters groups of the 60 genotypes branching at a similarity value of 68 % (Figure 3). Cluster I, consisted of populations from Papum Pare [RGU (1–7), PWD (1–7)], and Lower Subansiri districts [PTP (1–7), GDK (1–7)] while Cluster II, comprised of populations from other five districts viz. Upper Subansiri [DMPJ (1–5), DPJ (1–6)], kurungkumey [KLR (1–5)], Lower Dibang Valley [HNL (1–8)] and Dibang Valley [ANN (1–8)] (Figure 3).

### Population Structure

The observed number of alleles (Na) ranged from 1.1757–1.8730 while the effective number of alleles (Ne) ranged between 1.1142–1.3827 respectively. Similarly, Nei's Gene Diversity (h) and Shannon's Information Index (I) ranged between 0.10–0.16 with an average value of 0.25 and 0.14–0.26 with average value of 0.47 respectively. The percentage of Polymorphic Loci (Pp) was estimated in the range of 17.59% (DPJ) to 40.81% (PWD). The Gene Flow value (Nm) and the Diversity among Populations ($G_{ST}$) were found to be 0.25 and 0.46 respectively (Table 3). The Fixation Index or F statistics ($F_{ST}$) was found to be 0.42. The Nei's unbiased measures of genetic identity and genetic distance among the population was also calculated (Table 4). AMOVA ($p < 0.001$) showed that genetic variation of 28.64% was observed within the populations, whereas the variance among populations was 71.36% (Table 5).

## Discussion

Genetic diversity and its distribution of plant populations is essential to develop an understanding on genotypic variation within and among the populations, that would help in breeding experiment, cultivation and conservation for economic utilization. It also helps in determining appropriate agro-climatic and altitudinal range for study of breeding behaviour, cultivation and conservation of such genotypic variant population that successfully thrive in particular elevation and agro-climatic zones [25]. It also helps in understanding the taxonomy and delimitation of taxa at species, subspecies and varietal level, deducing conclusion on origin and evolution of plant species of ecological and evolutionary significance grown in a different agro-climatic and geographical regions of the world. A combined knowledge of both these topics is essential for studying plant species especially with the one having taxonomic confusion at species, subspecies and varietal level.

Our studies have found a lower level of gene flow among the

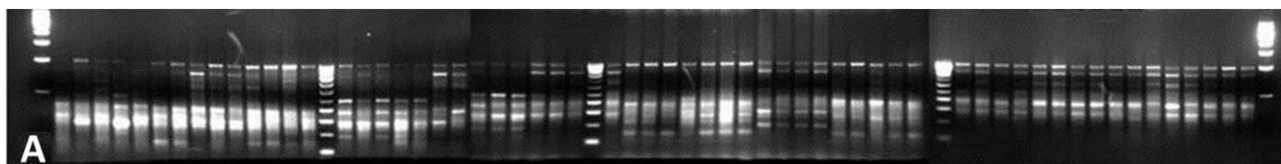


**Figure 2A:** Representative picture of RAPD of 60 individuals of Acmella paniculata (Wall. ex DC.) R.K. Jansen comprising nine populations group using primer OPBH 01.

| SL No | Primer Name | Primer sequence | Total no. of bands | No. of poly-morphic bands | No. of mono-morphic bands | % of Ply-mor-phisim (P) | Resolving Power (Rp) | PICa | Distance range (Jaccard's coefficient) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | OPAA-14 | 5'AACGGGCCAA3' | 32 | 20 | 12 | 62.5 | 6.23 | 0.65 | 0.1303 – 0.9231 |
| 2 | OPAA-16 | 5'GGAACCCACA3' | 27 | 10 | 17 | 37.03 | 5.27 | | |
| 3 | OPAB-06 | 5'GTGGCTTGGA3' | 23 | 16 | 7 | 69.56 | 7.65 | | |
| 4 | OPAC-10 | 5'AGCAGCGAGG3' | 23 | 12 | 11 | 52.17 | 5.80 | | |
| 5 | OPAG-13 | 5'GGCTTGGCGA3' | 15 | 7 | 8 | 46.66 | 4.50 | | |
| 6 | OPAH-12 | 5'TCCAACGGCT3' | 17 | 6 | 11 | 35.29 | 5.70 | | |
| 7 | OPAI-05 | 5'GTCGTAGCGG3' | 22 | 7 | 15 | 31.81 | 6.52 | | |
| 8 | OPAL-04 | 5'ACAACGGTCC3' | 18 | 9 | 9 | 50.00 | 6.75 | | |
| 9. | OPAL-13 | 5'GAATGGCACC3' | 24 | 9 | 15 | 37.50 | 4.20 | | |
| 10. | OPBC-13 | 5'CCTGGCACAG3' | 26 | 10 | 16 | 38.46 | 4.89 | | |
| 11. | OPBE-10 | 5'AAGCGGCCCT3' | 30 | 12 | 18 | 40.00 | 6.57 | | |
| 12. | OPBH-01 | 5'CCGACTCTGG3' | 15 | 6 | 9 | 40.00 | 4.73 | | |
| 13. | OPBH-06 | 5'TCGTGGCACA3' | 20 | 8 | 12 | 40.00 | 7.50 | | |
| 14. | OPE-18 | 5'GGACTGCAGA3' | 15 | 5 | 10 | 33.33 | 7.45 | | |
| 15. | OPF-06 | 5'GGGAATTCGG3' | 18 | 8 | 10 | 44.44 | 5.15 | | |
| 16. | OPA-07 | 5'GAAACGGGTG3' | 20 | 12 | 8 | 60.00 | 6.76 | | |
| 17. | OPA-08 | 5′-GTGACGTAGG | 24 | 15 | 9 | 62.50 | 7.02 | | |
| 18. | OPA-10 | 5′-GTGATCGCAG | 17 | 11 | 6 | 64.70 | 5.12 | | |
| 19. | OPA-11 | 5′-CAATCGCCGT | 21 | 16 | 5 | 76.19 | 6.14 | | |
| 20. | OPA-16 | 5′-AGCCAGCGAA | 16 | 10 | 6 | 62.5 | 7.24 | | |
| 21. | OPA-18 | 5'-GTGATCGCAG-3' | 19 | 11 | 8 | 57.89 | 4.89 | | |
| 22. | OPA-22 | 5'-TCGGCGATAG-3' | 22 | 17 | 5 | 72.27 | 5.90 | | |
| 23. | OPA-25 | 5'-CAGCACCCAC-3' | 17 | 9 | 8 | 52.94 | 6.45 | | |
| 24. | OPA-26 | 5'-TGAGCGGACA-3' | 20 | 13 | 7 | 65.00 | 5.92 | | |
| 25. | OPA-30 | 5'-TGAGCGGACA-3' | 22 | 17 | 5 | 72.27 | 7.11 | | |
| Total Number of Bands | | | 523 | 276 | 247 | | | | |
| Average | | | 20.92 | 11.04 | 9.88 | | | | |

**Table 1:** Data of RAPD primers used in the present study and the extent of polymorphism.

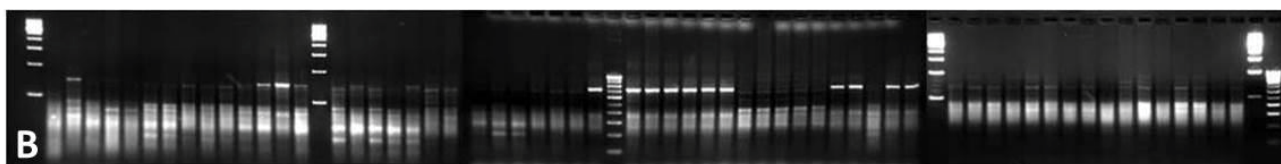


**Figure 2B:** Representative picture of ISSR of 60 individuals of *Acmella paniculata* (Wall. ex DC.) R.K. Jansen comprising nine populations group using primer ISSR 17.

| SL No | Primer Name | Primer sequence | Total no. of bands | No. of ply-mor-phic bands | No. of mono-mor-phic bands | % of Poly-mor-phisim (P) | Resolving Power (Rp) | PICa | Distance range (Jaccard's coefficient) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | ISSR02 | 5'CAGGCCCTTC3' | 10 | 7 | 3 | 70.00 | 6.23 | 0.78 | 0.1520 – 0.8505 |
| 2 | ISSR05 | 5'AGTCAGCCAC3' | 12 | 8 | 4 | 66.66 | 7.47 | | |
| 3 | ISSR06 | 5'AATCGGGCTG3' | 13 | 9 | 4 | 69.23 | 6.09 | | |
| 4 | ISSR10 | 5'CAGCACCCAC3' | 14 | 7 | 7 | 50.00 | 7.12 | | |
| 5 | ISSR12 | 5'GGTCGGAGAA3' | 16 | 13 | 3 | 81.25 | 6.15 | | |
| 6 | ISSR13 | 5'TCGGACGTGA3' | 14 | 12 | 2 | 85.71 | 5.70 | | |
| 7 | ISSR15 | 5'AGACGTCCAC3' | 11 | 8 | 3 | 72.72 | 7.14 | | |
| 8 | ISSR16 | 5'GACGCC ACAC3' | 13 | 8 | 5 | 61.53 | 6.70 | | |
| 9. | ISSR17 | 5'GTCGTAGCGG3' | 22 | 15 | 7 | 68.18 | 6.52 | | |
| 10. | ISSR19 | 5'ACAACGGTCC3' | 18 | 9 | 9 | 50.00 | 6.75 | | |
| 11. | ISSR22 | 5'$(GACA)_2$3' | 15 | 8 | 7 | 53.33 | 5.85 | | |
| 12. | ISSR25 | 5'C$(GTG)_3$3' | 14 | 9 | 5 | 64.28 | 6.09 | | |
| 13. | ISSR27 | 5' T$(CGA)_3$3' | 11 | 5 | 6 | 45.45 | 5.34 | | |
| 14. | ISSR29 | 5'A$(ACA)_3$3' | 13 | 8 | 5 | 61.53 | 6.34 | | |
| 15. | ISSR30 | 5'$(CGT)_3$G3' | 12 | 7 | 5 | 58.33 | 6.89 | | |
| Total Number of Bands | | | 208 | 133 | 75 | | | | |
| Average | | | 13.86 | 8.86 | 5 | | | | |

**Table 2:** Data of ISSR primers used in the present study and the extent of polymorphism.

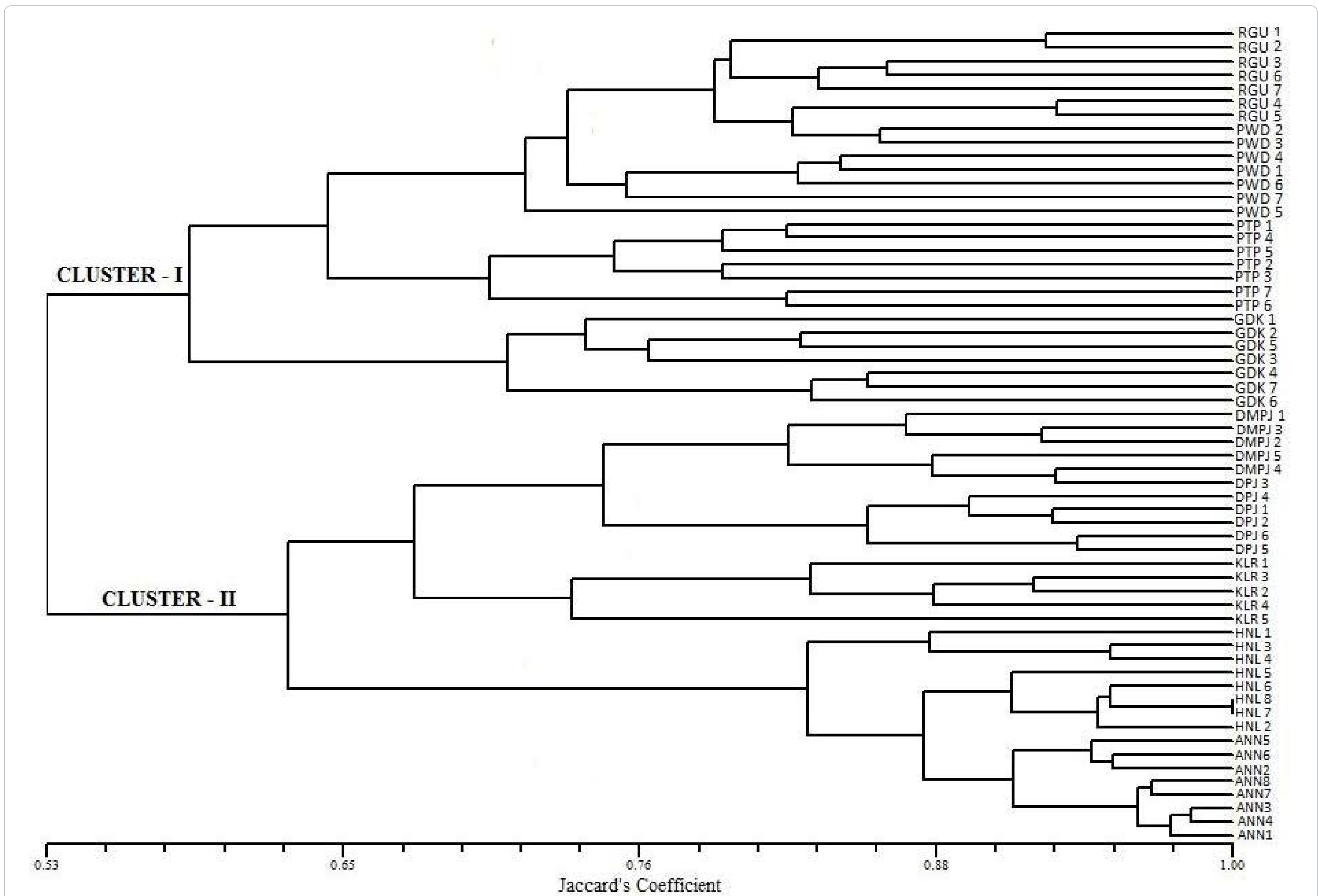


**Figure 3:** UPGMA clustering of 60 individuals belonging to nine populations group of *Acmella paniculata* (Wall. ex DC.) R.K. Jansen based on RAPD and ISSR markers.

| Populations | Ss | Na±SD | Ne±SD | h± SD | I ± SD | Pp | Hsp | Hpop | Gst | Nm | $F_{ST}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| RGU | 7 | 1.28 ± 0.42 | 1.14 ± 0.30 | 0.10 ± 0.19 | 0.17 ± 0.23 | 32.79 | | | | | |
| PWD | 7 | 1.38 ± 0.33 | 1.26 ± 0.34 | 0.16 ± 0.20 | 0.22 ± 0.27 | 40.81 | | | | | |
| PTP | 7 | 1.31 ± 0.46 | 1.22 ± 0.37 | 0.12 ± 0.19 | 0.17 ± 0.28 | 31.02 | | | | | |
| GDK | 7 | 1.25 ± 0.43 | 1.15 ± 0.31 | 0.11 ± 0.17 | 0.14 ± 0.24 | 25.00 | | | | | |
| DPJ | 6 | 1.17 ± 0.38 | 1.11 ± 0.27 | 0.14 ± 0.15 | 0.20 ± 0.21 | 17.59 | | | | | |
| DMPJ | 5 | 1.23 ± 0.42 | 1.16 ± 0.32 | 0.16 ± 0.17 | 0.18 ± 0.25 | 23.61 | | | | | |
| KLR | 5 | 1.19 ± 0.38 | 1.11 ± 0.27 | 0.11 ± 0.15 | 0.17 ± 0.21 | 18.38 | | | | | |
| HNL | 8 | 1.23 ± 0.38 | 1.12 ± 0.25 | 0.17 ± 0.19 | 0.23 ± 0.24 | 27.69 | | | | | |
| ANN | 8 | 1.21 ± 0.42 | 1.26 ± 0.35 | 0.16 ± 0.21 | 0.26 ± 0.26 | 29.30 | | | | | |
| Total | 60 | 1.87 ± 0.22 | 1.38 ± 0.25 | 0.25 ± 0.18 | 0.47 ± 0.18 | 87.88 | 0.17 | 0.083 | 0.46 | 0.25 | 0.42 |

**Table 3:** Analysis of divergence of genetic variation in populations of *A. paniculata* (Wall. ex DC.) R.K. Jansen. (H: Nei's gene diversity; I: Shannon's information index; Na: Observed number of alleles; Ne: Effective number of alleles; Np: Number of polymorphic loci; Pp: Percentage of polymorphic loci; SD: Standard deviation; $G_{ST}$: Diversity among populations; Nm: Gene flow 0.25 $(1 - G_{ST}) / G_{ST}$; Hpop: Variability within populations, Hsp: Total variability; Ss: Number of individuals; $F_{ST}$: Fixation index or F statistics).

populations grown in different latitudinal and altitudinal gradient. The population sampled from eastern region of Arunachal Himalaya with higher elevation range 1500–3000 m located at higher latitude region have shown a distinct clustering pattern while the populations sampled from middle and lower elevation range of Central Arunachal Himalayan Region 200–1500 m located at low latitude region have shown a distinct cluster. This clearly indicates the genotypic variations among the populations grown at different altitudinal gradient. The possible influence of agro-climatic factors such as rainfall, temperature, humidity, soil types, and UV-light intensity along the altitudinal gradient cannot be ruled out as per our present findings and this same theory have also been suggested by earlier workers [26,27].

Our studies have further established the fact that Asteraceae is relatively young family or chloroplast DNA is evolved at a slower rate in other families as observed in earlier phylogenetic studies which have shown ancient evolutionary split as suggested by the result of chloroplast DNA inversion studies [28]. The high frequency of occurrence of polyploidy as found through morphological studies in conjunction with observations of meiotic pairing in this genus

| Population ID | RGU | PWD | PTP | GDK | DPJ | DMPJ | KLR | HNL | ANN |
|---|---|---|---|---|---|---|---|---|---|
| RGU | **** | 0.7785 | 0.6235 | 0.9213 | 0.8738 | 0.5168 | 0.6433 | 0.7230 | 0.4825 |
| PWD | 0.2215 | **** | 0.1352 | 0.4425 | 0.7902 | 0.9256 | 0.8104 | 0.5205 | 0.3452 |
| PTP | 0.3765 | 0.9231 | **** | 0.5735 | 0.6648 | **** | 0.6832 | 0.6245 | 0.6678 |
| GDK | 0.0787 | 0.8204 | 0.7582 | **** | 0.5932 | 0.2456 | 0.3588 | 0.6786 | 0.1328 |
| DPJ | 0.1262 | 0.6135 | 0.5022 | 0.2684 | **** | 0.7783 | 0.6445 | 0.4265 | 0.2324 |
| DMPJ | 0.4832 | 0.7479 | 0.6434 | 0.3872 | 0.4201 | **** | 0.8876 | 0.3976 | 0.4659 |
| KLR | 0.3567 | 0.5668 | 0.2852 | 0.4824 | 0.3869 | 0.4986 | **** | 0.6435 | 0.7439 |
| HNL | 0.2770 | 0.2856 | 0.5390 | 0.5457 | 0.4680 | 0.7877 | 0.2214 | **** | 0.2772 |
| ANN | 0.5175 | 0.3884 | 0.4751 | 0.6123 | 0.7580 | 0.5915 | 0.5805 | 0.4472 | **** |

**Table 4:** Nei's unbiased measures of genetic identity and genetic distance for nine wild populations of Acmella paniculata (Wall. ex DC.) R.K. Jansen based on RAPD and ISSR marker data. (RGU: Rajiv Gandhi University; PWD: Poma PWD Camp; PTP: Pitapool; GDK: Godak; DPJ: Daporijo; DMPJ: Dumporijo; KLR: Koloriang; HNL: Hunli; ANN: Anini)

| Variation Source | df | Sum of Squares | Variance components | Percentage of variation |
|---|---|---|---|---|
| Among populations | 8 | 982.251 | 20.4822 | 71.36 |
| Within populations | 51 | 578.166 | 9.6075 | 28.64 |
| Total | 59 | 1560.417 | 30.0897 | |

**Table 5:** Analysis of molecular variance (AMOVA) results for nine wild populations of *Acmella paniculata* (Wall. ex DC.) R.K. Jansen from Arunachal Himalayan region through RAPD and ISSR marker. (df : Degrees of freedom).

emphasized the important role this process has had in speciation within Acmella might be an important aspect, and our studies where some populations have relatively higher genetic diversity might be moving towards a new species in long term evolutionary process [29]. RAPD and ISSR markers have been successful in determining genetic diversity of many plant populations [30–32] and our studies also shows the utility of such DNA based markers in determining genetic diversity of plant populations.

Moreover, a species with a broad distribution rarely has the same genetic makeup over its entire range. It could also be possible that these local populations are composed of clonal descendants of a small number of ancestral populations. Thus, our studies establishes the fact that the populations of *A. paniculata* (Wall. Ex DC.) R.K. Jansen might need further taxonomic studies at both morphological and DNA level by deriving more samples from wider geographical range and different altitudinal gradient for deducing better conclusion on different population of same species grown in different continents based on altitude and climate type. Such studies could prove to be useful in evolving overall phylogenetic clustering of the genus Acmella with rest of the Asteraceae members in holistic manner. An integrated research approach aims to develop clonal germplasm, medicinal and nutraceutical production through vegetative and seed propagation methods in same habitat and space by deriving seeds and vegetative samples from phenotypically variants population from different geographical locations could help in evolving new genotypic variants of commercial interests.

The information obtained through this study provides valuable baseline data of genetic variation at inter and intra population level in nine naturally distributed wild populations of *A. paniculata* (Wall. Ex DC.) R.K. Jansen. The use of RAPD and ISSR markers can further lead to the development of the Sequence Characterised Amplified Region (SCAR) markers [33–35] which could further help in the molecular authentication of this medicinally important plant species of the genus Acmella.

## Conclusion

Thus from the above study it is found that RAPD and ISSR markers are proven as an important molecular tools in determining genetic diversity within and among the wild population of *A. paniculata* (Wall. Ex DC.) R.K Jansen is naturally grown in the different altitudinal gradient of Eastern Himalayan Region of India. The study therefore confers further scope for use of subsequent gene based markers viz. Scot, TRAP, DAMD etc for further molecular authentication of the genetic distinctiveness of this medicinally important genus Acmella and few species found in Arunachal Himalayan region and other regions for deducing phylogenetic relationship of the inter specific and intra specific population, genotypic variants within and among the population, future conservation and sustainable utilization possibly through ex-situ agro-technology methods for large scale biomass production.

## Conflict of Interest

The authors declare that they have no conflict of interest financial or otherwise.

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