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Study of arbitrarily amplified (RAPD and ISSR) and gene targeted (SCoT and CBDP) markers for genetic diversity and population structure in Kalmegh [*Andrographis paniculata* (Burm. f.) Nees]

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ABSTRACT

Andrographis paniculata is a medicinal herb also known as 'King of Bitter'. This herb was studied using arbitrary amplified (RAPD and ISSR) and gene targeted (SCoT and CBDP) molecular markers. Thirty-nine accessions were collected from five agro-ecological regions of India. Eighteen RAPD and five ISSR primers generated a total of 124 and 32 amplicons with an average of 6.89 and 6.4 amplicons per primer respectively. Similarly, 22 SCoT and 19CBDP primers were used to characterize A. *paniculata* accessions. A total of 132 and 97 amplicons were generated, with an average of 6.0 and 5.11 amplicons per primer, respectively. Polymorphic information content (PIC) for RAPD and ISSR primers ranged from 0.32 to 0.45 and 0.25–0.42, with resolving power (Rp) ranging from 2.13 to 10.03 and 2.46–3.96, respectively. PIC values ranged from 0.09 to 0.48 and 0.30–0.46, with an average value of 0.34 and 0.41 per primer for SCoT and CBDP markers, respectively. The resolving power for SCoT and CBDP primers ranged from 2.36 to 10.54 and 1.39–13.15 per primer, respectively. Unweighted pair group method with arithmetic mean (UPGMA) based clustering grouped 39 accessions into three broad clusters based on all four marker systems studied, did not correlate with their agro-ecological regions. Population structure based analysis divided 39 accessions into seven populations, six populations and four populations using RAPD, ISSR and SCoT markers respectively. Whereas only three populations were observed using CBDP primers which corresponded to the grouping observed with the UPGMA. Analysis of molecular variance (AMOVA) based analysis showed that the maximum variation (10%) among agro-ecological regions was observed using CBDP primers; whereas, minimum variation (2%) was observed using SCoT markers. Taken together, our data suggest CBDP markers are better markers for genetic diversity study in *A. paniculata*.

1. Introduction

Kalmegh (*Andrographis paniculata* (Brum. F.) Nees), family Acanthaceae, is a medicinal herb, commonly known as 'King of Bitter' Maha-tita or Bhui-neem. *A. paniculata* is much smaller in size, but has a bitter taste similar to neem (*Azadirachta indica* A. Juss.) (Niranjan et al., 2010). Traditionally, this herb is used as anti-inflammatory, antibacterial, antioxidant, anti parasitic, antispasmodic, anti diabetic, anti carcinogenic, antipyretic, anti diarrheal, nematocidal, anti HIV and hepatoprotective drugs with wide geographic distribution from the peninsular of India, Sri Lanka, south-east Asia, China, America and West Indies to the Christmas Island in Indian ocean (Kumar et al., 2012; Lattoo et al., 2008). South India and Sri Lanka possibly represent the origin and diversity centers of *A. paniculata* because native populations of plants are found throughout these areas.

A. paniculata (Burm. F.) Nees, is a diploid (2n = 2x = 50) species usually found in wild but under cultivation in India. Plant is an erect herb, grows up to the height of 50–125 cm under favorable conditions. The stem is quadrangular with more branches. The leaves are simple, opposite, lanceolate and glabrous. The inflorescence is freely branched terminal racemose. The flower is zygomorphic, complete, pentamerous, hypogynous, pedicellate, and bisexual. Stigma is closely attached to the anthers till the flower open-

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ing and promotes the mechanism of self pollination (Sharma and Jain, 2015). The corolla is white with maroon streak on upper lip which attracts the insect pollinators like honey bees, butter flies etc. Around 4 per cent cross pollination takes place due to protoandry and insect pollinators. The fruit is capsule, linear, oblong and acute at both the ends. The seeds are small, sub quadrate and yellowish brown in colour and slightly smaller than mustard seed. The seeds per capsule are numerous and orthodox in nature. The plant is indeterminate and exhibits non synchronous maturity and seeds dispersed regularly through capsule dehiscence. The seeds of *A. paniculata* are dormant due to hard seed coat. The presence of seed dormancy is common survival strategy for an effective dispersal of wild and semi-domesticated species. In northern part of India, it is grown as rainy (Kharif) season (June–July) and harvested for its herbage in the month of October–November.

Evaluation and cataloguing of genetic variability is necessary for optimum genetic enrichment and effective conservation of the allelic and genotypic variability. Genetic diversity analysis based on morphological and biochemical traits is extremely useful. In the past few years, the application of molecular markers has accelerated the agglomeration of this information, which complements the characterizations based on morpho-chemical descriptors and geographic origin.

The limited knowledge about genetic diversity in *A. paniculata* provides the rationale for this study, which provides methods for determining intraspecific relatedness for the selection of different genotypes for breeding, effective conservation and management of its germplasm resources. DNA fingerprinting has emerged as an effective tool for genetic identification in crop plant and germplasm management (Jondle, 1992; Smith, 1998). Appropriate marker system and technique can be used to study genetic diversity in the best possible manner. Different marker system and techniques with set parameters are currently available for this kind of study. Markers like RAPD (Randomly Amplified Polymorphic DNA), ISSR (Inter Simple Sequence Repeats) and AFLP (Amplified Fragment Length Polymorphism) are collectively known as AAD markers (Arbitrarily Amplified Dominant) (Karp et al., 1996; Wolfe and Liston, 1998) which have been used in population genetic studies, QTL mapping and diagnostic genomic fingerprinting. Advancement in genomics have led to development of new alternative marker techniques such as Sequence Related Amplified Polymorphism (SRAP) (Li and Quiros, 2001), Target Region Amplification Polymorphism (TRAP) (Hu and Vick, 2003), Conserved Region Amplification Polymorphism (CRAP) (Wang et al., 2009), Start Codon Targeted Polymorphism (SCoT) (Collard and Mackill, 2009) and CAAT Box Derived Polymorphism (CBDP) (Singh et al., 2014) in which polymorphism is generated from genic regions in the genome. Among these SCoT has become very popular, because there are earlier reports of its application in genetic analysis and QTL mapping. This marker was developed from the short conserved region flanking the ATG start codon in plant genes. Another gene targeted marker, CAAT Box-Derived Polymorphism (CBDP) exploits CAAT box region of promoters in plant genes was studies on jute, cotton and linseed cultivars that have validated the significance of this marker (Singh et al., 2014).

A literature survey shows that for the molecular characterization of *A. paniculata*, only few works has been done using RAPD markers (Ghosh et al., 2014; Kumar and Shekhawat, 2009; Lattoo et al., 2008; Maison et al., 2005; Padmesh et al., 1999; Wijarat et al., 2011). Therefore, in the present study an effort was made to study the genetic diversity using both AAD markers (RAPD and ISSR) and gene targeted (SCoT and CBDP) markers and simultaneously to compare their efficiency in genetic diversity study of 39 accessions of *A. paniculata* collected from different agro-ecological regions of India.

2. Material and methods

2.1. Plant material

Thirty nine morphologically diverse accessions of *A. paniculata* were collected over the years from different agro-ecological regions of India were used in this study (Fig. 1). Explorations were under taken in the Himalayan Foot Hills and Gangatic Plains in the month of October-November, and in Eastern Ghats and Southern parts in the month of February-March to collect these diverse accessions. All the collections augmented from different sources were grown in the same year (2014) under identical conditions at Issapur experimental farm of NBPGR, New Delhi to collect samples for the study (Table 1).

2.2. DNA extraction

For DNA extraction, approximately 5gm fresh leaves of each accession collected from different plants were pooled and crushed into liquid nitrogen using mortar and pestle and total genomic DNA was isolated by using modified CTAB method (Saghai-Maroof et al., 1984). DNA quality was checked on 0.8% agarose gel and concentrations were estimated using nanodrop (Thermo Fisher, USA). After quantification, a working concentration of 10 ng/μl DNA stock was prepared and stored at 4 °C until further use.

2.3. Genotyping with RAPD markers

Initial screening was done with 120 RAPD primers to select polymorphic and reproducible primers. After initial screening, 18 primers were selected for further analysis (Table 2). The PCR amplification was performed in a total volume of 25 μl containing 50 ng of DNA Template, 2.5 μl of 10× PCR buffer, 2.5 μl of 10 mM deoxynucleotide tri-phosphates (dNTPs), 2.5 μl of 25 mM MgCl2, 2.0 μl of 5 pmol primer and 1U Taq DNA polymerase (Thermoscientific, USA). PCR reactions were amplified in a Thermal Cycler (G-Storm, UK) under following conditions: initial denaturation at 94 °C for 5 min followed by 42cycles of denaturation at 94 °C for 1 min, annealing at 37 °C for 1 min and extension at 72 °C for 2 min followed by final extension at 72 °C for 10 min. Reactions were stored at 4 °C until electrophoresis. Amplified products were separated on 1.4% agarose gel at 120 V for 1.5–2 h and stained with 0.5 μg/ml ethidium bromide solution. PCR products were visualized using gel documentation system (Alpha Innotech Corporation, USA). 1 Kb DNA ladder (Fermentas, USA) was used as standard.

2.4. Genotyping with ISSR markers

Out of 25 ISSR primers tested only five were found suitable for further analysis (Table 3). PCR reactions mix was prepared as described in RAPD, except ISSR primer volume (0.2 μl) and template DNA concentration (30–40 ng). The thermocycler program for PCR amplification was set same as mentioned in case of RAPD except annealing temperature which ranged from 48° C to 64° C. PCR amplification products were separated and visualized similar to RAPD.

2.5. Genotyping with SCoT markers

A total of 32 SCoT primers (Oligos, Macrogen, Seoul) were used for initial screening out of which, 22 were finally selected for genetic diversity analysis. PCR reactions were carried out in a total volume of 10 μl containing 25 ng of template DNA, 1.0 μl of 10× PCR buffer, 0.8 μM SCoT primers, 2.5 mM of each dNTPs and 1U of Taq DNA polymerase (Thermoscientific, USA). SCoT-PCR amplification was done under following conditions: Initial denaturation