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Start codon targeted (SCoT) polymorphism for evaluation of genetic diversity of wild population of *Maytenus emarginata*

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ABSTRACT

Maytenus emarginata (Willd.) Ding Hou (family Celastraceae), an evergreen and multipurpose tree species of Indian Thar Desert, is a promising source of anticancer agents i.e. sesquiterpene pyridine alkaloid Emarginatine B and Emarginatine F. The knowledge of the genetic diversity of *M. emarginata* is important for the selection of appropriate genotype with medicinal interest. However, the genetic analysis or breeding system of *M. emarginata* remains unrevealed, therefore, it is difficult to draw any conclusion regarding its natural genetic diversity, gene flow or genetic differentiation. In the present investigation, start codon targeted polymorphism technique was used first time to examine the genetic diversity of *M. emarginata*. A total of 51 individuals comprising seven populations, collected from the larger geographical area of arid, semiarid and sub humid regions of western Rajasthan, India, were tested for existing natural genetic diversity in *M. emarginata*. Of 36 primers screened, 12 SCoT markers produced a total 156 amplicons ranging from 8 to 17 per primer, of which 114 (73%) were polymorphic. Average polymorphic information content, Nei's gene diversity (h), Shannon index (I) and Percentage of polymorphic loci (Pp) were 0.517, 0.171, 0.257 and 49.54, respectively. Results revealed high level of genetic differentiation (Gst − 0.223) and the gene flow v (Nm − 1.74) in *M. emarginata* populations. Analysis of molecular variance showed that percent molecular variance was higher within population (84%) than among population (16%). The cluster analysis carried out by unweighted pair-group with arithmetic mean and the principal coordinate analysis showed high genetic variation among the genotypes and clustered all 51 genotypes into four major clusters while two genotypes in separate groups. High degree of genetic diversity in genotypes/populations may be useful in conservation program and selection of appropriate genotypes for exploitation of this important tree of Indian Thar desert.

1. Introduction

Maytenus emarginata (Willd.) Ding Hou (family Celastraceae), popularly known as "Kankero" in Hindi and "Thorny Staff Tree" in English, is an evergreen tree species of Indian Thar Desert and neighbouring Aravallies (Bhandari, 1990; Purohit and Shekhawat, 2012). *M. emarginata* is an ecologically and economically important tree species that produces biomass for fuel, fodder, yields timber of good durability and stabilizes sandy soil (Rathore et al., 1992). Traditionally, different part of this plant is used in the treatment of gastrointestinal disorders, mouth ulcers, jaundice and in purifying blood (Bhandari, 1990; Spivey et al., 2002; Agrawal and Nag, 2009). The plant is reported to contain a number of alkaloids namely Emarginatinine, Emarginatine B, Emarginatine-C, Emarginatine-D, Emarginatine-E, Emarginatine-F and Emarginatine-G. Antitumour activity of *M. emarginata* is mainly due to presence of a sesquiterpene pyridine alkaloid Emarginatine B and Emarginatine F which showed potent cytotoxicity against human KB cells, ileocecal adenocarcinoma, melanoma and medulloblastoma tumour cells (Kuo et al., 1990, 1994). Because of economical and medicinal importance, *M. emarginata* is being overexploited (Rathore et al., 1992), therefore, there is a need for germplasm conservation and genetic improvement of this plant.

In the era of global warming and climatic change, molecular characterization of plant resources of the arid ecosystems particularly heat and drought resistant trees would help in identification of adaptive genetic variations which leads to an increase in our knowledge of

Abbreviations: AMOVA, analysis of molecular variance; CTAB, cetyl trimethyl ammonium bromide; DAMD, directly amplified minisatellite DNA; ISSR, inter simple sequence repeats; PCoA, the principal coordinate analysis; PIC, polymorphic information content; QTL, quantitative trait loci; RAPD, random amplified polymorphic DNA; SCAR, sequence characterized amplified region; SCoT, Start codon targeted; TBE, tris borate EDTA; UPGMA, unweighted pair-group with arithmetic mean

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molecular mechanisms involved in drought, heat and other abiotic stress tolerance (Shekhawat et al., 2012; Rai et al., 2017). Assessing genetic variations in genotypes and among species are major concerns in conservation genetics and breeding programmes (Kalia et al., 2011). DNA based molecular markers are valuable tools for assessing genetic variation of genotypes. In recent years, many new gene targeted markers have been applied for assessment of genetic variation. Start codon targeted (SCoT) polymorphism, developed by Collard and Mackill (2009), is a gene targeted promising marker that targets the conserved region flanking the translation initiation start codon (ATG) of plant genes. Owing to a number of valuable characteristics like simple, reproducible, novel and gene targeted marker, SCoT marker has been successfully utilized in a variety of applications like analysis of genetic diversity (Guo et al., 2012; Bhattacharyya et al., 2013; Sorkheh et al., 2016; Chhajer et al., 2017; Singh et al., 2017), population structure (Alikhani et al., 2014; Satya et al., 2015; Zhang et al., 2016; Bhawna et al., 2017), genetic relationship between individuals of a species or in different species (Xiong et al., 2011; Luo et al., 2012; Amirmoradi et al., 2012; Rajesh et al., 2015), identification of QTL (Gorji et al., 2011; Yan et al., 2016), differential gene expression and screening of stress-related genes (Luo et al., 2014), evaluation of fingerprinting of offspring with their parents (Cabo et al., 2014), analysis of male and female genotypes (Heikrujam et al., 2015), development of co-dominant SCAR marker (Mulpuri et al., 2013) and genetic fidelity and variability of tissue culture raised plants (Rathore et al., 2014; Agarwal et al., 2015; Bhattacharyya et al., 2015; Rahmani et al., 2015; Vasudevan et al., 2017).

No previous reports on genetic analysis or breeding system of *M. emarginata* are available, therefore it is difficult to draw any conclusion regarding its natural genetic diversity, gene flow or genetic differentiation. However, little information on genetic diversity, relationship or population genetics of other *Maytenus* species using molecular markers has been reported. RAPD markers have been utilized in analysis of intra and inter populational genetic variability in *M. ilicifolia* (Mossi et al., 2007) as well as genetic diversity in population of *M. dasyclada* (Reichmann et al., 2017). In another study, Simplicio et al. (2015) estimated genetic diversity and population structure of *M. truncata* based on ISSR molecular marker.

The present investigation is the first attempt to use of SCoT marker to analyze the extent of genetic diversity among 51 genotypes representing seven populations of *M. emarginata*. All 51 genotypes were collected from the larger geographical area of arid, semiarid and sub humid regions of western Rajasthan, India where this tree grows extensively in wild habitat. The main objective of present study was to estimate the population genetic diversity and genetic relationship of *M. emarginata* genotypes using SCoT markers.

2. Materials and methods

2.1. Plant material and genomic DNA isolation

The young and juvenile leaves from 51 trees were collected from seven districts of Rajasthan i.e. Jodhpur, Bikaner, Jalore, Sikar, Churu, Nagaur and Pali for genetic diversity analysis of *M. emarginata*. The details of collection sites, population name and their acronyms and sample size of each population are presented in Table 1. A representative tree of *M. emarginata* and leaves along with flowers and fruits are shown in Fig. 1. The leaves collected from different geographic locations were stored at −20 °C for genomic DNA isolation. Total genomic DNA was isolated from frozen young leaves of *M. emarginata* by crushing in liquid nitrogen using autoclaved prechilled pestle and mortar using CTAB method described by Doyle and Doyle (1990) with minor modifications adopted by Gupta et al. (2011). The quality of the isolated DNA was assessed by determination of A_{260}/A_{280} absorbance ratio by spectrophotometer (UV–vis Elico spectrophotometer). The Quality and quantity of the DNA was also analysed by gel electrophoresis with 0.8% (w/v) agarose (Sigma, India) in 1X TBE buffer and estimated by 1 Kb DNA ladder (Hi-Media, India).

Table 1
Details of collection of samples and location of populations of *M. emarginata*.

Collection site	Population name (District)	Site Acronyms	No. of sample size	Latitude elevation	Longitude elevation
Guda Road	Jodhpur	JOD	8	26.1°N	73.1°E
Nokha	Bikaner	BIK	7	27.6°N	73.4°E
Bhadrajun	Jalore	JAL	8	25.6°N	72.8°E
Sewdara	Sikar	SIK	8	27.5°N	74.8°E
Salasar	Churu	CHR	8	27.7°N	74.7°E
Didwana	Nagaur	NAG	7	27.4°N	74.5°E
Vayad	Pali	PAL	5	25.7°N	72.9°E

2.2. SCoT primers and PCR amplification

A total of 36 SCoT primers designed by Collard and Mackill (2009) were used for DNA amplification in *M. emarginata*. All the primers were initially screened and primers generating clear and unambiguous amplicons were selected. All PCR amplification reactions were carried out in 15 µl volume reaction mixture containing 1.5 µl of 10 x PCR buffer [100 mM Tris (pH 9.0), 500 mM KCl, and 1% Triton X-100 with 15 mM $MgCl_2$]; 0.4 µl of dNTPs (10 mM; Sigma); 1.5 µl of $MgCl_2$ (2.5 mM); 1.5 µl of primer (10 mM; custom synthesized by Integrated DNA Technologies Inc., India); 0.5 µl of 3U Taq polymerase (Banglore Genei, India); and 50–70 ng of template DNA. Amplifications were carried out in a thermal cycler (Eppendorf 5331) with PCR program as an initial denaturation at 94 °C for 4 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at annealing temperature (Ta) for 1 min and extension at 72 °C for 2 min with a final extension at 72 °C for 10 min. The Ta was kept 2 °C below the Tm (melting temperature) of that particular primer sequence. PCR products were resolved on 1.4% agarose gel using 1X TBE buffer stained with ethidium bromide. DNA fragments were visualized and photographed under ultraviolet (UV) light in the gel documentation system (Syngene Gel Doc, Syngene, Synoptics Ltd., UK). A 50 bp DNA ladder (Hi-media) was used to estimate the size of fragments. To ensure the reproducibility of the results, the amplification reaction of each primer was performed twice.

2.3. Data analysis

The DNA fragments amplified by each SCoT primer were scored as a binary code (1, 0) for presence and absence. Only well-resolved and easy to score DNA fragments were considered as band present and total number of bands and polymorphic bands scored manually. The PIC was calculated as described by Botstein et al. (1980). In order to estimate population genetic diversity, different genetic parameters including effective no. of alleles (Ne), Shannon's information index (I), percentage of polymorphic loci (Pp), diversity among populations (G_{ST}) and fixation index (F_{ST}) were estimated using GenAlEx (Ver. 6.5). Gene flow (Nm) was calculated using following formula; $Nm = (0.5\ (1 - G_{ST})/G_{ST})$, whereas G_{ST} is diversity among populations. The PCoA and AMOVA were carried out using the GenAlEx (ver. 6.5) program. A pairwise matrix of distances between genotypes of *M. emarginata* was determined using Jaccard coefficient by the DARwin program (ver. 6.0). The relationship of populations was estimated from SCoT data using UPGMA clustering method. The UPGMA dendrogram was viewed using the same program.

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