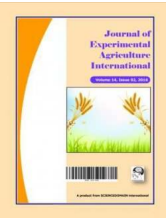

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Genetic Diversity Analysis of Medicinal Plant *Cassia alata* in Andaman Island through ISSR Markers

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Authors' contributions

This work was carried out in collaboration between all authors. Author KA designed the experiment and wrote the first draft of the manuscript. Author DRS reviewed the data, participated in the data analysis and writing the first draft of the manuscript. Author PS managed the laboratory experiments. Author VB participated in literature review and writing the first draft of the manuscript. All authors read and approved the final draft of the manuscript.

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ABSTRACT

Cassia alata is one of the important medicinal plants which are traditionally used to treat skin infections. It is also being used as antihelmintic, antibacterial, diuretic and laxative. The leaves of *C. alata* are used as an effective treatment against ringworm and also against other skin diseases such as eczema and chronic skin impurities. The local people of the Andaman and Nicobar Island use this medicinal plant *Cassia alata* L. against centipede bites and instant relief from joint and muscle pains. Wide variability was observed in this species and is distributed throughout the Islands. In the present study, genetic diversity among 29 different accessions of *Cassia alata* collected from different parts of South Andaman were studied using ISSR marker. A set of seven ISSR primers were taken for DNA fingerprinting, among them 5 ISSR primers produced194 clear and prominent bands with 61% homology. The maximum discriminating bands obtained from primer ISSR 7 and ISSR 9. Cluster analysis of ISSR divided into two clusters. An assessment of genetic diversity among species would assist in planning for future germplasm collection, conservation and domestication programmes.

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Keywords: Cassia alata; genetic diversity; ISSR; Andaman and Nicobar Islands.

1. INTRODUCTION

Andaman and Nicobar Islands is hot spot of biodiversity of medicinal plants representing a great emporium of ethnobotanical wealth. The island is an abode six indigenous tribes namely the Nicobarese, Onges, Sentinalese, Jarawas and the Great Andamanese. The strong enthnomedicinal uses of the tribes are the strength of the Island and are documented by different workers [1]. There exists wide biodiversity in the distribution of medicinal plants in the Island and there are about 71 medicinal plants which are endemic to this Island [2]. Among the different medicinal plants of the Island, *Cassia alata* belonging to the family Ceaselpinaceae is one of the wild medicinal plant distributed throughout the Islands. It is commonly known as ring worm shrub because of its antifungal activity against fungal infection. *Cassia alata* L. is a small tree generally grown in the gardens and not far away from human dwellings [3]. Traditionally, it is used to treat superficial fungal infections apart from treating uterine disorders and snakebites. It is also being used as anthelmintic, antibacterial, diuretic and laxative [4,5,6]. The water extract of the leaves had been found to be effective against *Staphylococcus aureus* at concentration comparable to standard antifungal agent, Tioconazole. The leaves of *C. alata* are used as an effective treatment against ringworm and also against other skin diseases such as eczema and chronic skin impurities [7].

Characterization of accessions of this medicinal plant *Cassia alata* will help in conservation, domestication and its subsequent utilization in crop improvement programmes. Molecular approaches represents a potential tool for effective characterization of germplasm [8,9,10,11], which circumvents the limitations associated with morphological and biochemical characterization [12]. ISSR marker analysis was described as a powerful technique to assess genetic diversity [13,14,15] and to detect similarities between and within species levels [16,17]. The present study has been undertaken with the objective to assess the level of genetic diversity among different accessions of *Cassia alata Linn* from different parts of South Andaman using ISSR marker.

2. MATERIALS AND METHODS

Twenty-nine different accessions of *Cassia alata* were collected from different places of South Andaman. For molecular analysis, total DNA was isolated from freeze-dried powdered young leaf tissue of *Cassia alata* samples according to the CTAB method [18,19] with minor modifications. After that, DNA was quantified and adjusted to 25-50 ng/µl for PCR amplification, and DNA quality and purity were evaluated by electrophoresis on 0.8% agarose gel and the ratio of A260/A280. A total number of seven ISSR primers, were screened using a few DNA samples. PCR amplification was performed in S1000 thermo cycler (BIO-RAD). The PCR was performed by initial denaturation at 94℃ for 5 minutes followed by 45 cycle of denaturation at 94℃ for one minutes, annealing at 52℃ for one minute, and extension at 72℃ for two minutes and final elongation at 72℃ for 7 minutes. Reaction was carried out in a total volume of 20 µl containing about 100 ng of template DNA, 200 mM dNTP's and 3.5 mM MgCl2, 60 pg of primers, 2.0 µl 10 × Taq DNA polymerase buffer and 1.5 U Taq DNA polymerase. The amplification products was analyzed by electrophoresis on 1.4% agarose gel in 1 × TAE buffer (pH 8.3) and detected by ethidium bromide staining [20], 100 bp DNA Ladder was used to determine the size of the ISSR fragments. From the preliminary screening of twelve primers, five primers that could amplify visible bands and they were selected for further examination. The gel pictures were taken under UV light using gel documentation system. Amplified products were scored across the lane with respect to their molecular size. All the genotypes were scored for presence and absence of the ISSR bands. The 0/1 matrix was used to calculate similarity as Jaccard's coefficient using SIMQUAL subroutine in similarity routine. The resultant similarity matrix was employed to construct dendrogram using SAHN based UPGMA to deduce genetic relationship [21].

3. RESULTS AND DISCUSSION

The five ISSR markers amplified a total of 194 alleles, of which seven (3.6%) were polymorphic (Table 1). The number of alleles produced by each marker varied from 1 to 5, with an average of 6.85 bands per primer (Fig. 1). Markers UBC 842 and UBC 840 produced the largest number of clear bands, while marker UBC 827 produced

the smallest number of bands. The number of alleles and polymorphic nature of these ISSR markers indicated their robustness in fingerprinting of species. However, the number of alleles amplified by any ISSR marker was not directly related to its ability to distinguish the cultivars. The presence and absence of a particular allele was scored for each of the 29 accessions, and presence was represented by 1 and absence by 0. The resulting genotyping files of all 29 varieties were aligned. From these data, a homology tree was constructed by the Multiple Sequence Alignment Program that showed 61 to 100% similarities among the 29 accessions (Fig. 2). The maximum homology of 100% was observed between Ca8, 9, 11 and Ca 3, 4. The 29 accessions clustered into two major groups. Group I consisted of 28 accessions and could be further divided into three subgroups, group IA, group IB and group IC. Group IA comprised fourteen accessions, and all these cultivars shared 80% homology. A maximum homology of 100% was observed between ca8, 9, 11 and ca3, 4. Group IB consisted of six accessions and these cultivars showed 65% homology Group IC consisted of eight accessions showing 63% homology. It has been shown that ISSR molecular markers have sufficient discriminatory power to distinguish different cultivars [22,23,24]. The dendrogram (Fig. 2) showed that there was a high degree of similarity among the cultivars analyzed.

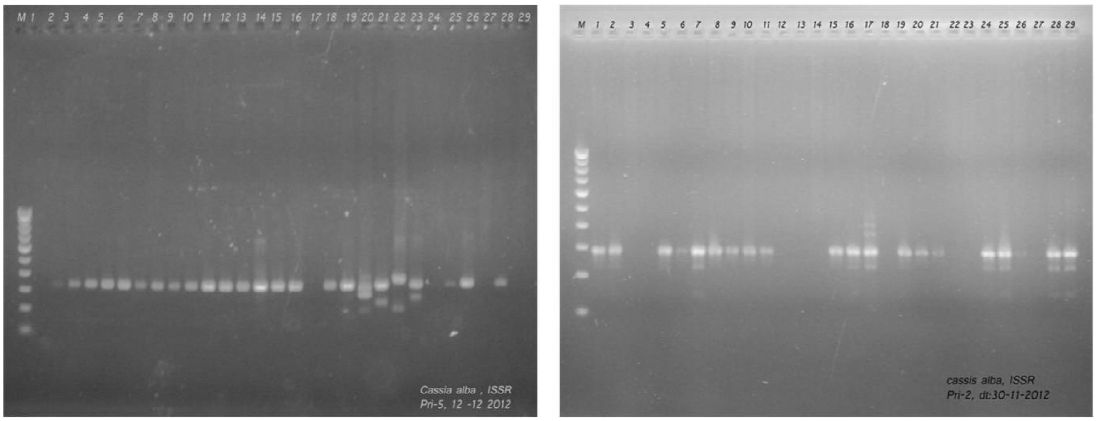

Fig. 1. PCR profile of *Cassia alata* using ISSR primers

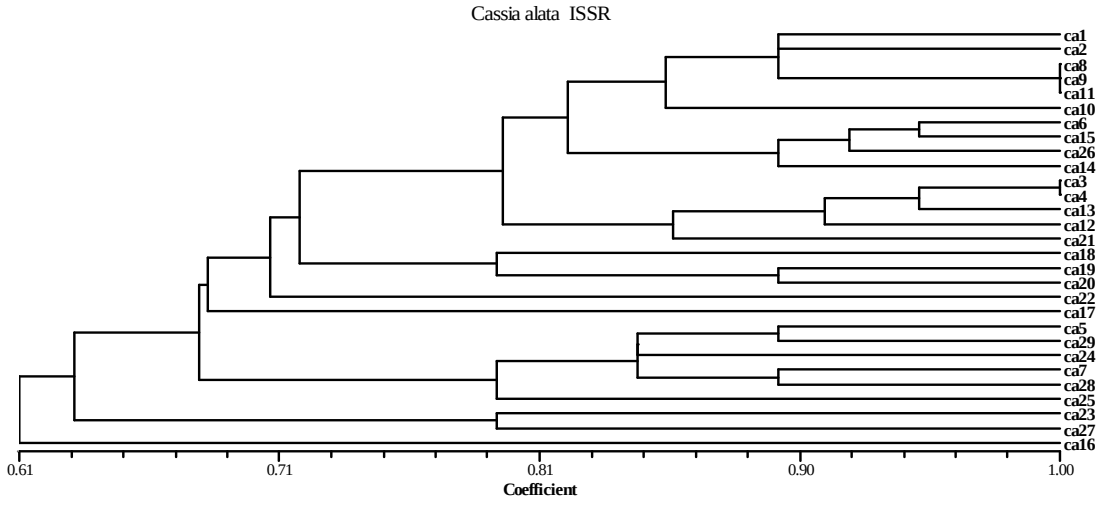

Fig. 2. Dendrogram showing genetic diversity amongst *Cassia alata* genotypes by ISSR primers

Table 1. Total number of polymorphic amplicons and polymorphic loci (%) as revealed by ISSR markers

S.no.	primer name	Primer sequence (5' – 3')	Total No. of bands	Total No. of polymorphic bands	Total no. of scorable bands	Average No. of bands across genotypes	Percentage of expressivity	Marker range (kb)	PIC value	% of polymorphism
1.	UBC827	$(GC)_8G$	3	2	24	8.00	58.62	0.2-0.6	0.398	66.66
2.	ISSR-11	$(CT)_8RC$	3	3	35	11.66	82.75	0.5-0.8	0.420	100.00
3.	UBC842	$(GA)_8G$	4	3	54	13.50	82.75	0.5-0.8	0.497	75.00
4.	UBC840	$(GA)_6$ YT	3	3	40	13.33	75.86	0.2-0.8	0.451	100.00
5.	UBC807	$(AG)_8T$	3	3	36	12.00	68.96	0.5-0.8	0.427	100.00

4. CONCLUSION

The assignment of 28 of 29 (96%) genotypes in one cluster is indicative of the narrow genetic base of these plant species, which means that most of the accessions of *Cassia alata* may share the same genetic background, based on common ancestors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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