

27 this Island (Dagar& Dagar, 1991). Among the different medicinal plants of the Island, *Cassia*
28 *alata* belonging to the family Ceaselpinaceae is one of the wild medicinal plant distributed
29 throughout the Islands. It is commonly known as ring worm shrub because of its antifungal
30 activity against fungal infection. *Cassia alata* L. is a small tree generally grown in the
31 gardens and not far away from human dwellings (Lee *et al.*, 2011). Traditionally, it is used to
32 treat superficial fungal infections apart from treating uterine disorders and snakebites. It is
33 also being used as anthelmintic, antibacterial, diuretic and laxative (Palanichamy *et al.*, 1990;
34 Berkland *et al.*, 2002; Somchit *et al.*, 2003). The water extract of the leaves had been found
35 to be effective against *Staphylococcus aureus* at concentration comparable to standard
36 antifungal agent, Tioconazole. The leaves of *C. alata* are used as an effective treatment
37 against ringworm and also against other skin diseases such as eczema and chronic skin
38 impurities (Hennebelle *et al.*, 2009).

39 Characterization of accessions of this medicinal plant *Cassia alata* will help in
40 conservation, domestication and its subsequent utilization in crop improvement programmes.
41 Molecular approaches represents a potential tool for effective characterization of germplasm
42 (Ferguson *et al.*, 2004; Figiulol and Perrino, 2004; Maestri *et al.*, 2002; Krishna Kumar *et al.*,
43 2010.), which circumvents the limitations associated with morphological and biochemical
44 characterization (Caetano-Anollecs, 1991). ISSR marker analysis was described as a
45 powerful technique to assess genetic diversity (Milbourne *et al.*, 1997; Huang *et al.*, 2008;
46 Israr Ahmad *et al.*, 2012) and to detect similarities between and within species levels
47 (Mattioni *et al.*, 2002; Manimekalai *et al.*, 2006). The present study has been undertaken with
48 the objective to assess the level of genetic diversity among different accessions of *Cassia*
49 *alata* Linn from different parts of South Andaman using ISSR marker.

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51 **Materials and Methods**

52 Twenty-nine different accessions of *Cassia alata* were collected from different places of
53 South Andaman. For molecular analysis, total DNA was isolated from freeze-dried powdered
54 young leaf tissue of *Cassia alata* samples according to the CTAB method (Saghai-Marooft *et*
55 *al.*, 1984; Doyle and Doyle, 1990) with minor modifications. After that, DNA was quantified
56 and adjusted to 25-50 ng/μl for PCR amplification, and DNA quality and purity were
57 evaluated by electrophoresis on 0.8% agarose gel and the ratio of A260/A280. A total number
58 of seven ISSR primers, were screened using a few DNA samples. PCR amplification was
59 performed in S1000 thermo cycler (BIO-RAD). The PCR was performed by initial
60 denaturation at 94°C for 5 minutes followed by 45 cycle of denaturation at 94°C for one
61 minutes, annealing at 52°C for one minute, and extension at 72°C for two minutes and final
62 elongation at 72°C for 7 minutes. Reaction was carried out in a total volume of 20 μl
63 containing about 100 ng of template DNA, 200 mM dNTP's and 3.5 mM MgCl₂, 60 pg of
64 primers, 2.0 μl 10 × Taq DNA polymerase buffer and 1.5 U Taq DNA polymerase. The
65 amplification products was analyzed by electrophoresis on 1.4% agarose gel in 1 × TAE
66 buffer (pH 8.3) and detected by ethidium bromide staining (Yang *et al.*, 1996), 100 bp DNA
67 Ladder was used to determine the size of the ISSR fragments. From the preliminary screening
68 of twelve primers, five primers that could amplify visible bands and they were selected for
69 further examination. The gel pictures were taken under UV light using gel documentation
70 system. Amplified products were scored across the lane with respect to their molecular size.
71 All the genotypes were scored for presence and absence of the ISSR bands. The 0/1 matrix
72 was used to calculate similarity as Jaccard's coefficient using SIMQUAL subroutine in
73 similarity routine. The resultant similarity matrix was employed to construct dendrogram
74 using SAHN based UPGMA to deduce genetic relationship (Rohlf FJ. 1998).

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77 **Results and Discussion**

78 The five ISSR markers amplified a total of 194 alleles, of which seven (3.6%) were poly-
79 morphic (Table 1). The number of alleles produced by each marker varied from 1 to 5, with
80 an average of 6.85 bands per primer (Figure 1). Markers UBC 842 and UBC 840 produced
81 the largest number of clear bands, while marker UBC 827 produced the smallest number of
82 bands. The number of alleles and polymorphic nature of these ISSR markers indicated their
83 robustness in fingerprinting of species. However, the number of alleles amplified by any
84 ISSR marker was not directly related to its ability to distinguish the cultivars. The presence
85 and absence of a particular allele was scored for each of the 29 accessions, and presence was
86 represented by 1 and absence by 0. The resulting genotyping files of all 29 varieties were
87 aligned. From these data, a homology tree was constructed by the Multiple Sequence
88 Alignment Program that showed 61 to 100% similarities among the 29 accessions (Figure 2).
89 The maximum homology of 100% was observed between Ca8, 9, 11 and Ca 3, 4. The 29
90 accessions clustered into two major groups. Group I consisted of 28 accessions and could be
91 further divided into three subgroups, group IA, group IB and group IC. Group IA comprised
92 fourteen accessions, and all these cultivars shared 80% homology. A maximum homology of
93 100% was observed between ca8, 9, 11 and ca3, 4. Group IB consisted of six accessions and
94 these cultivars showed 65% homology Group IC consisted of eight accessions showing 63%
95 homology. It has been shown that ISSR molecular markers have sufficient discriminatory
96 power to distinguish different cultivars (Powell *et al.*, 1996; Joshi *et al.*, 2000; Israr Ahmad *et*
97 *al* 2010). The dendrogram (Figure 2) showed that there was a high degree of similarity
98 among the cultivars analyzed.

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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.

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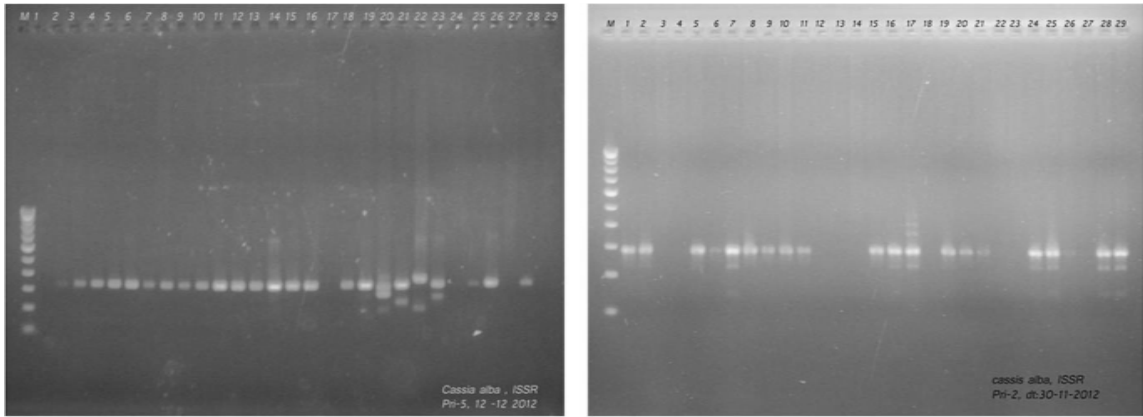
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174 Table: 1.

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) ₈ G	3	2	24	8.00	58.62	0.2-0.6	0.398	66.66
2.	ISSR-11	(CT) ₈ RC	3	3	35	11.66	82.75	0.5-0.8	0.420	100.00
3.	UBC842	(GA) ₈ G	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) ₈ T	3	3	36	12.00	68.96	0.5-0.8	0.427	100.00

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183 **Fig.1.** PCR profile of *Cassia alata* using ISSR primers.
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188 **Fig.2.**Dendrogram showing genetic diversity amongst *Cassia alata* genotypes by ISSR
189 primers.

