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Molecular characterization of *Asplenium nidus* L. – A potential ornamental fern species from Andaman

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ABSTRACT

Asplenium nidus L. is one of the most important terrestrial fern in Andaman and Nicobar Islands with great ornamental value. Inter simple sequence repeat (ISSR) analysis was used to study the genetic diversity of the species *A. nidus* L. collected from different geographical locations of the Island. Among 15 ISSR primers screened, 9 primers produced amplification and produced a total of 222 reproducible amplified bands. The number of amplified bands varied from 10 in ISSR 11 to 44 in UBC 842 with an average of 24.7 bands per primer. The range of polymorphic bands and percent polymorphism observed were 1 to 5 and 33.3 to 83.3, respectively. The polymorphic information content value of ISSR marker ranged from 0.365 to 0.485. Cluster analysis grouped the twelve accessions into three clusters (Cluster I, II and III) at Jaccard's similarity coefficient value of 0.69 on basis of geographical locations of the accessions. The cluster I was further divided into two sub-clusters Ia and Ib at the coefficient value of 0.77 and it includes the accessions belonging to South Andaman, one each accession collected from two different locations, Little Andaman and Rangat (Middle Andaman) were grouped separately as clusters II and III, respectively. The genetic variability studies with ISSR marker grouped the accessions of *A. nidus* L.on basis of their geographical locations and it would help in rapid identification of polymorphism and assist in germplasm collection, conservation and domestication programmes.

Key words: Andaman, *Asplenium nidus*, Cluster analysis, Fern, Genetic diversity, ISSR marker.

INTRODUCTION

Ferns are very ancient family of plants that are older than land animals. Ferns have worldwide distribution growing in all continents except Antartica and most Islands favouring moist temperature and tropical regions (Burkhill, 1935). The fern species are characterized by two independent generations namely the sporophyte producing spores and an independent gametophyte producing gametes. There are more than 12000 species of ferns distributed all over the world with a variety of utilization like ornamental value, medicinal valueand habitat conservation, etc. The Andaman and Nicobar Islands is a hot spot of biodiversity with rich fern flora. The ferns are found to grow luxuriantly in the Islands due to the prevailing favourable moist climate. About 126 diverse species of fern flora have been reported from Andaman and Nicobar Islands and have been considered to be a very significant feature of the Island flora. *Asplenium nidus* L. commonly known as Bird's Nest fernbelonging to family, Ploypodiaceae is a potential underexploited ornamental fern of the Island. In India, this fern is distributed only in Andaman and Nicobar Islands and North Eastern region (Nath *et al.*,2013).It is a spectacular, epiphytic fern with apple green frondsvisually similar to banana leaves, with the fronds growing to 50–150 cm long and 10–20 cm broad. They are often crinkled, with a black midrib, and exhibit circinate venation. Spores develop in sori on the underside of the fronds. These sori form long rows extending out from the midrib on the back of the outer part of the lamina (frond). The fronds roll back and they become brown and create a massive leaf nest in the branches and trunks of trees. There is a wide distribution of this fern throughout the Andaman group of Islands and is highly fascinated as an indoor plant. There is enormous potential for exploitation of this particular fern as a commercial ornamental species. *Asplenium* is one of the groups of epiphytic ferns with simple leaves and lacks good qualitative taxonomic characters for species recognition (Yatabe *et al.*, 2001). The morphological descriptors are not highly dependable for identification of fern species as they are of natural origin and often quantitative characters which are used as descriptors are not highly reliable. Most morphological traits are highly influenced by environment or vary with the development of the plant and isozymes are limiting due to low levels of polymorphisms (Asha *et al.*, 2006).

Consequently DNA based techniques such as RAPD, ISSR and micro-satellites or simple sequence repeats (SSRs) are effective in assessing genetic diversity of plant species because they provide unlimited potential markers to

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reveal differences at molecular level (Ghafoor *et al*., 2007). When compared to other classes of markers, DNA based markers, often carry high numbers of alleles at very low frequencies (Vinod *et al*., 2007). Inter-simple sequence repeat (ISSR) is the molecular marker which was first employed by Zietkiewicz *et al.*, (1994) and Gupta *et al.*, (1994) has been proved to be a highly useful tool for estimating genetic diversity and assessing genetic relationships because it is simple, fast, cost-effective, reliable and highly discriminating. The assessment of genetic diversity within a species will form as basis for species conservation, genetic improvement and promotion of domestication of a population with desirable traits (Ahmad *et al*., 2010).There are reports that *A. nidus* lacks the good taxonomic characters available for species recognition and multiple cryptic species exists within *A. nidus* (Yatabe *et al.*, 2001).The extent of genetic diversity of *A. nidus* L. in the Islandis very much essential for maintenance and utilization of this fern in further breeding programs. There are very limited reports on genetic diversity studies on this potential fern *A. nidus*. Hence the present study has been undertaken with the objective to assess the level of genetic diversity among twelve different accessions of the species *A. nidus* L. collected from different parts of Andaman group of Islands.

MATERIALS AND METHODS

Twelve different accessions of *Asplenium nidus* were collected from different parts of the Andaman group of Islands (Table 1) and were maintained in pots in shade house at the experimental farm of ICAR-Central Island Agricultural Research Institute (CIARI), Port Blair. After three years of maintenance of the collections in pots, the morphological characters of the different accessions were recorded. The leaf samples were collected from the 12 different accessions and were stored at -80^{o}C until DNA extraction. Genomic DNA was isolated from young leaves using Cetyl Trimethyl - Ammonium Bromide (CTAB) method (Aitchitt *et al*., 1993). Purity of DNA was checked by UV spectrophotometer and running in 0.8 % agarose gel. The quantification of DNA was done using UV spectrophotometer. PCR reaction was performed in final volume of 20ul containing 10x assay buffer 2.5 mM dNTPs, 0.5 unit of *Taq* DNA polymerase obtained from Bangalore Genei,10 pmols/reaction of ISSR primer and 100 ng of template DNA. PCR reaction was performed using 15 ISSR primers (Clonitec) and nine primers amplified well. The primer list of ISSR markers used is presented in Table 2. The PCR was performed by initial denaturation at 94°C for 5 min. followed by 45 cycle of denaturationat 94°C for one minute, annealing at 52°C for one minute, and extension at 72°C for two minutes and final elongation at 72°C for 7 min. The PCR products were resolved on 1.2%Agarose gel (Bangalore Genei) prepared in 1x TAE buffer containing 0.5 ug/ml of the ethidium bromideat 80 V for 2.5 h. All the genotypes were scored for presence and absence of the ISSR bands and the data were entered into a binary matrix as discrete variables, 1 for presence and 0 for absence of character and this data matrix was subjected to further analysis. The 0/1 matrix was used to calculate similarity index using Jaccard's coefficient. The resultant similarity matrix was employed to construct dendrogram using SAHN based UPGMA to infer genetic relationship (Rohlf, 1998;Sneath *et al*., 1973).

RESULTS AND DISCUSSION

In the present study, the 12 different collections from different parts of Andaman group of Islands were studied for their morphological traits such as number of frond per plant, frond length and frond width as these ferns are highly valued for their cut foliage. The number of fronds per plant ranged from 19.0 to 29.0 with maximum frond length (149.2 cm) and frond width (18.5 cm) in accession 4, AnJT1. This particular collection is from marshy and high vegetative area near perennial water source. This might have led to the maximum growth of the fronds in the fern which is essential for using this plant in interior plantscaping as potted plant. For molecular characterization, 15 ISSR primers were

Table 1: Passport information for collection sites of *Asplenium nidus* in Andaman Islands

Code	Collection site	Location		Morphological traits		
		Latitude	Longitude	Number of fronds/ plant	Frond length (cm)	Frond width (cm)
An.CT1	Chidiyatapu	N 11^{o}30'11.2"	E 92^{o} 42'2.0"	23.0	120.3	16.5
An.CD	Chouldari	N 11^{o}38'16.7"	E 92^{o} 39'57.5"	19.0	117.8	14.1
An.MH1	Mount Harriet	N 11^{o}43'14.8"	E 92^{o}43'59.8"	29.0	90.3	12.6
An.JT1	Jirkatang	N 11^{o}35'54.9"	E 92^{o}39'58.4"	25.0	149.2	18.5
An.JT2	Jirkatang	N 11^{o}35'33.7"	E 92^{o}39'48.3"	27.0	136.4	17.2
An. MH2	Mount Harriet	N 11^{o}35'14.6"	E 92^{o}42'49.8"	25.0	100.7	15.9
An.HB	Hut Bay	N 11^{o}34' 33.7"	E 92^{o}40'44.1"	23.0	123.2	16.3
An.JT3	Jirkatang	N 11^{o}35'17.4"	E 92^{o}29'38.3"	24.0	139.1	16.4
An.RG	Rangat	N 12^{o}30' 44.2"	E 92^{o}57'52.9"	21.0	99.6	14.1
An.JT4	Jirkatang	N 11^{o}35'17.4"	E 92^{o}29'38.3"	25.0	115.4	16.4
An. MH3	Mount Harriet	N 11^{o}41'14.6"	E 92^{o}36'49.8"	27.0	131.1	16.1
An.CT2	Chidiyatapu	N 11^{o}30' 29.1"	E 92^{o} 42'13.9"	23.0	128.3	17.3

Table 2: The ISSR primer details along with number of polymorphic bands, % polymorphism and PIC value of the primer used for genetic diversity study of species *Asplenium nidus* L.

Primer	Sequence	TB*	PB	SB	AB	PE	MR	BS	PIC	% PM
ISSR 24	$(GA)_6CC$	3	2	18	6.0	84.6	0.2-0.6	300-500	0.375	66.7
UBC 809	$(AG)_8G$	3	2	37	12.3	92.3	0.2-0.5	100-400	0.421	66.7
ISSR 23	$(GA)_6GG$	3	1	20	6.7	84.6	0.2-0.6	400-600	0.365	33.3
ISSR 22	$(CA)_6AC$	3	2	18	6.0	76.9	0.2-0.5	400-500	0.420	66.7
ISSR 25	$(GAG)_3GC$	6	4	38	6.3	61.5	0.2-0.7	150-600	0.457	66.7
UBC 827	$(AC)_8G$	3	2	23	7.7	84.6	0.1-0.5	200-400	0.421	66.7
ISSR 11	$(CT)_8RC$	4	2	10	2.5	53.8	0.2-0.5	200-500	0.451	50.0
UBC 807	$(AG)_8T$	6	5	14	2.3	69.2	0.3-0.6	100-600	0.485	83.3
UBC 842	$(GA)_8G$	5	3	44	8.8	92.3	0.2-0.5	200-600	0.450	60.0
	Total	**36**	**23**	**222**	-	-	-	-	-	-
	Minimum	**3**	**1**	**10**	**2.3**	**53.8**	-	**100**	**0.365**	**33.3**
	Maximum	**6**	**5**	**44**	**12.3**	**92.3**	-	**600**	**0.485**	**83.3**

***TB**- Total number of bands; **PB**- Number of polymorphic bands; **SB**- Total number of scorable bands; **AB**- Average number of bands across genotypes; **PE**- Percentage of expressivity; **MR**- Marker range; **BS**- Size of bands; **PIC**- Polymorphic information content; **%PM**- Percent polymorphism

Table 3: Jaccard's similarity index of 12 *Asplenium nidus*accessions of Andaman based on ISSR marker.

1*	2	3	4	5	6	7	8	9	10	11	12
1.000											
0.571	1.000										
0.714	0.857	1.000									
0.857	0.714	0.857	1.000								
0.857	0.714	0.857	1.000	1.000							
0.714	0.857	1.000	0.857	0.857	1.000						
0.714	0.857	0.714	0.571	0.571	0.714	1.000					
0.857	0.714	0.857	1.000	1.000	0.857	0.571	1.000				
0.571	0.428	0.571	0.714	0.714	0.571	0.285	0.714	1.000			
0.857	0.714	0.857	1.000	1.000	0.857	0.571	1.000	0.714	1.000		
0.714	0.857	1.000	0.857	0.857	1.000	0.714	0.857	0.571	0.857	1.000	
1.000	0.571	0.714	0.857	0.857	0.714	0.714	0.857	0.571	0.857	0.714	1.000

***1** -An.CT1; **2** -An.CD; **3** -An.MH1; **4** -An.JT1; **5** -An.JT2; **6** -An. MH2; **7** -An.HB; **8** -An.JT3; **9** -An.RG; **10** -An.JT4; **11** -An. MH3; **12** -An.CT2

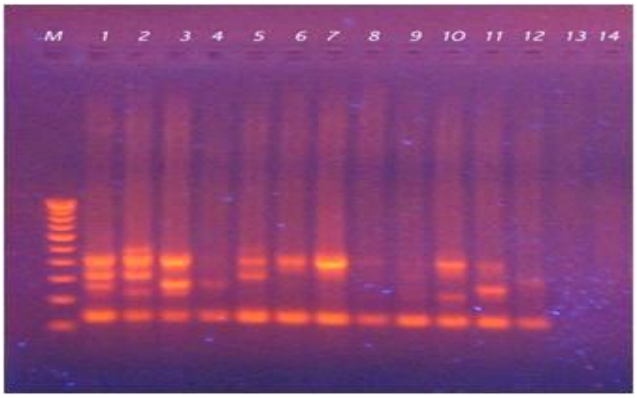


Fig 1: ISSR marker (ISSR 25) profile for twelve *Asplenium nidus* accessions.

used to study the relatedness within a species based on their geographical distribution in the Island. Among 15 ISSR primers used, 9 primers showed amplification and the ISSR profiles of primer ISSR 25 for twelve accessions of *A. nidus* were shown in Fig. 2. The total numbers of reproducible amplified bands are 222 and it varied from 10 in ISSR 11 to 44 in UBC 842 with an average of 24.7 bands per primer (Table 2). Polymorphism information content (PIC) values were calculated for ISSR markers to characterize the capacity of each primer to reveal or detect polymorphic loci among the genotypes. The PIC value of ISSR marker used in the study ranged from 0.365 (ISSR 23) to 0.485 (UBC 807). The level of polymorphism in *A. nidus* as number of polymorphic allele per primer and percent polymorphism ranged from1to 5 alleles and 33.3 to 83.3 %, respectively among twelve accessions. This vast range in percent polymorphism obtained in the present study proved the discriminatory power of ISSR molecular markers for distinguishing different cultivars reported in earlier literatures (Powell *et al*., 1996; Joshi *et al*., 2000; Ahmad *et al* 2010; Abirami *et al*., 2016).The present study revealed the ISSR marker, UBC 807 with higher number of polymorphic alleles and percent polymorphism; whereas, the marker, ISSR 23 with the lowest number of polymorphic alleles and percent polymorphism(Table 2). The number of alleles and polymorphic nature of these ISSR markers indicated their robustness in fingerprinting of species.

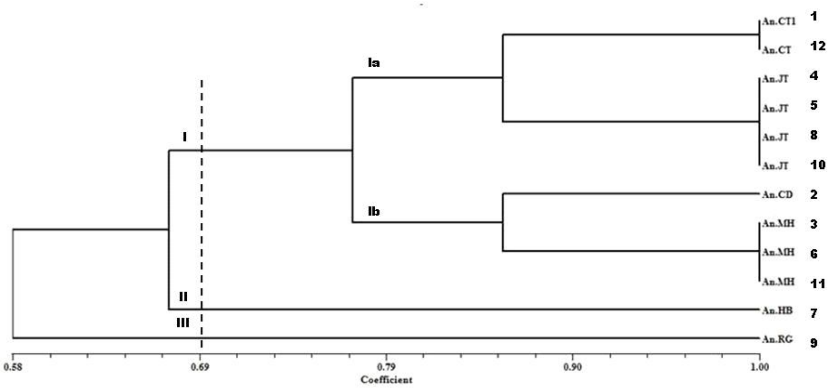


Fig 2: Dendrogram of 12 *Asplenium nidus* genotypes by ISSR marker using UPGMA method of cluster analysis based on Jaccard's similarity coefficient.

Dendrogram was generated by using UPGMA method of cluster analysis on basis of Jaccard's similarity coefficient that differentiated all the 12 *A. nidus* genotypes at 58 % similarity (Table 3). Dendrogram could be divided into three clusters namely Cluster I, II and III at Jaccard's similarity coefficient value of 0.69. Cluster'I'includes 10 accessions mainly collected from South Andaman viz., Chidiyatappu, Chouldari, Mount Harriet and Jirkatang, which had 77 % similarity. Cluster 'II' had only one genotype (An.HB) collected from Little Andaman and it has 67% similarity with all accessions, except An.RG (58% similarity). Cluster 'III' had genotype (An.RG) collected from Middle Andaman (Rangat) showed 58% similarity among accessions. Cluster 'I' was divided into two sub-clusters namely sub-cluster 'Ia'with six accessions and 'Ib' with four accessions at coefficient value of 0.77. Though, the accession An.CD from Chouldari showed 86% similarity with accessions from Mount Harriet, it showed only 77% similarity with accessions from Chidiyatappu and Jirkatang. The present study revealed the existence of influence of geographical origin of accessions with respect to genetic variation through grouping them together. Further, it revealed that the presence of variation between geographical locations only and the accessions from same place showed no variation. Generally, the relationships among accessions in the cluster could be attributed to their diversity, geographical locations and also due to exchange of plant genetic resources among farmers within and between the different regions of the Island. It has been shown that ISSR molecular markers have sufficient discriminatory power to distinguish different cultivars (Powell *et al*., 1996; Joshi *et al*., 2000; Ahmad *et al* 2010; Abirami *et al*. 2016) and reliably used for quantification of genetic diversity and relationships, species identification and evaluation of conservation status (Abirami *et al*., 2018). The variability within a species is low due to less degree of gene flow in population through random mating without barrier (Ahmad *et al*., 2010). As the morphology of *A. nidus* does not provide good qualitative taxonomic characters for species recognition (Yatabe *et al*, 2001), the present molecular characterization study using ISSR markers would be utilized as an effective tool for genetic diversity analysis and its relationships within species along with the conservation of this potential fern species in the Andaman and Nicobar Islands.

REFERENCES

Abirami, K., Singh, D R., Simhachalam, P. and Baskaran, V. (2016). Genetic diversity analysis of medicinal plant *Casssia alata* in Andaman Island through ISSR markers. *Indian J. Experimental Agri. Inter*., **14(4):** 1-6.

Abirami, K., Baskaran,V. and Simhachalam, P. (2018). ISSR marker based diversity assessment of *Piper spp*. in Bay Islands, India.*Indian J. Agrl. Res.*,**52(4):** 434-438.

Ahmad, I., Bhagat, S., Sharma, T. V. R. S., Kumar, K. and Simachalam, P. (2010). ISSR and RAPD marker based DNA fingerprinting and diversity assessment of *Annona spp*. in South Andaman. *Indian J. Hort.*,**67(2)**: 147-151.

Aitchitt, M., Ainsworth, C. C. and Thangavelu, M.(1993). A rapid and efficient method for the extraction of total DNA from mature leaves of the date palm (*Phoenix dactylifera* L.). *Plant Mol. Biol. Rep.,* **11(4)**:317-319.

Asha, K. L., Nair, G. M. and Nair, M. C. (2006). Inter-relationship among the species of *Dioscorea* revealed by morphological trait and RAPD marker. *Indian J. Plant Genet. Resources*.**19**: 40-46.

Burkhill, I. H.(1935).*A dictionary of the Economic Products of Malay Peninsular.* Ministry of Agriculture, Malaysia, 1542.

Ghafoor, S., Shah, M. M. and Ahmad, H. (2007). Molecular characterization of *Ephedra* species found in Pakistan. *Genet. Mol. Res.* **6**: 1123-1130.

Gupta, M., Chyi, Y. S., Romero-Severson. and Jowen, J. L. (1994). Amplification of DNA markers from evolutionary diverse genomes using simple sequence repeats. *Theor.Appl. Genet*.,**89**:998-1006.

Joshi, S. P., Gupta, V.S., Aggarwal, R.K. and Ranjekar, P. K. (2000). Genetic diversity and phylogenetic relationship as revealed by inter simple sequence repeat (ISSR) polymorphism in the genus Oryza. *Theor. Appl. Genet.,***100**: 1311-1320.

Nath, K., Bhattacharya,M. K., Sen, A. and Kar, S. (2013) Antibacterial activity of frond extract of *Asplenium nidus* L., a threatened ethnomedicinal fern of North East India. *International J. Pharmacog. and Phytochem.*, **28(2)**: 1169-1172.

Powell, W., Margenta, M., Andrew C., Hanfrey, M., Vogel, J., Tingey, S. and Rafalsky, A. (1996). The utility of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breed.*,**2**: 225-238.

Rohlf, F.J. (1998). NTSYS-PC Numerical Taxonomyand Multivariate Analysis System Version 2.02,Exeter Publications Setauket, New York.

Sneath, P.H.A. and Sokal, R.R. (1973). NumericalTaxonomy - The Principles and Practice of Numerical Classification. Freeman and Co. SanFrancisco. P. 573.

Vinod, K, Singh, G., Sharma, R. and Sharma, S. N. (2007) RAPD and protein profiles of cotton varieties. *Indian J. Plant Physiol.*,**12**: 115-119.

Yatabe Y, S., Masuyama, D., Darnaedi. and Murakami, N. (2001). Molecular systematic of the *Asplenium nidus* complex from Mt. Halimun National park, Indonesia: Evidence for reproductive isolation among three sympatric *RBC*L sequence types. *American J. Bot.*, **88(8)**: 1517-1522.

Zietkiewicz E., Rafalski, A. and Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. *Genomics*,**20**: 176-183.