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Short Communication

Ascertaining the genetic basis of variants in *Justicia adhatoda* L. collected from Tamil Nadu using ISSR- PCR Markers

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Abstract

In the present investigation genetic variability of Justicia adhatoda L. belonging to the family Acanthaceae was carried out. The healthy fresh young leaves were collected from four different regions of Tamil Nadu. The DNA was isolated with standard protocol and the ISSR analysis was studied with five primers. The primers produced distinct bands in each population. The polymorphic loci number was 12 and percentage was 36 respectively. In between the population the genetic distance ranged from 0.3610 to 0.0625 and 0.9394 to 0.6970 respectively. 1.3636 was the mean value of overall observed number of alleles and 1.2788 was the effective number of alleles. The overall value for the genetic diversity was 0.1553 and value for Shannon information index was 0.2243. The UPGMA dendrogram represents the phylogenetic relationship between different accessions of Justicia adhatoda. The present study helps to find out the accession which has more genetic variability for the conservation in near future.

Keywords: *Justicia adhatoda,* ISSR- PCR, genetic variability, medicinal plant.

Introduction

Justicia adhatoda L. is one among the medicinally important plants of the family Acanthaceae. The plant has been utilized by many village people to cure the number diseases. It is one of the important plants of the home garden of all the people. It is also cultivated in and around the home or garden for fencing. It is an evergreen, highly medicinal valuable shrub with lengthy opposite branches. The leaves are arranged in opposite manner without stipules and showing lance-shape. The white colour dense flowers are present in axillary spicate cyme inflorescence, with short peduncles, broadly ovate bracts, foliaceous[1,2]. The parts of the plant used to treat asthma, rheumatism, lumber pain, joint pain, eczema, chronic bronchitis, sprains, malaria, whooping cough, cold, swelling and venereal diseases in Traditional Medicinal Systems in India[3,4].

The study on population genetics and genetic diversity of medicinal plants helps to establish the effective and efficient conservation practices. The variety of molecular methods is used to find out genetic variability of medicinal plants, among them ISSR, is the powerful molecular marker, since they need no prior knowledge of the DNA sequence and are universally applicable[5]. Molecular markers used in various fields like genetic mapping, cultivars identification, paternal tests, population history, epidemiology, detect mutant genes and population studies[6]. The environmental factors are not influenced in any molecular markers and the molecular tools accurately testify the genetic relationship between and among plant groups. It is less time consuming and easy to handle in comparison with morphological and biochemical markers. It helps to display the closeness of species and hybrids accurately[7]. The following molecular markers ISSR, RAPD, SSR and AFLP are currently used in genetic diversity study of medicinal plants, depending upon the nature of the study the suitable markers will be selected [8-10].

ISSR markers are producing stable and reproducible bands than RAPD in genetic diversity studies of different plants[11,12]. ISSR is active, easy, stable and radioactivity assay is not necessary for usage. High levels of polymorphisms observed in ISSR markers studies[13]. Among the different molecular markers used for genome analysis, ISSR markers give significant information of plant species[14]. ISSR is a PCR based marker amplify the genomic DNA with arbitrary primer, repeatedly corrupt bases extended into oblique sequences[15]. ISSR markers differentiating closely accompanying cultivars with huge potential and produce very repeatable and reproducible bands[16]. The ISSR analysis was carried out in many medicinal plants like *Hedychium coronarium*[17] from Eastern India, *Andrographis paniculata*[18] from India, *Trifolium hybridum*[19]. The present research work evaluate the genetic component in *Justicia adhatoda* for conservation and management of genetic diversity included in the RET listed categories of India over a decade[20].

Materials and methods

Justicia adhatoda L. belongs to the family Acanthaceae was selected for the present study. The fresh and young leaves of

Justicia adhatoda were collected from Salem, Tirunelveli, Thoothukudi and Coimbatore regions of Tamil Nadu and stored in deep freezer (-70°C) for DNA isolation and ISSR analysis.

Extraction of plant Genomic DNA: *Justicia adhatoda* DNA was extracted using the standard protocol of Doyle and Doyle[21] with slight modification. The DNA was qualitatively check with 1% agarose gel electrophoresis and quantitatively with UV-Visible spectrophotometer optical density respectively and the isolated genomic DNA stored in deep freezer for studies.

ISSR PCR Analysis: The amplification of PCR mixture was done with 20µl reaction volume have 2x DyNAzyme II PCR Master Mix 10µl. Among ten primers, there are five primers UBC-814, 822, 827, 840 and 876 were selected for final experiment based on the ability of producing reproducible bands (Table-1). The PCR reaction mixture was mixed gently in spinwin and 35 cycles of reaction was performed in a PCR (Applied Bio systems) with the amplification profile; of 95^0C - 5.00 min for initial denaturation, 94^0C-0.45min for denaturation, 42^0C - 1.00min for annealing, 72^0C-1.30min for extension, 72^0C - 10.00min for final extension and 4^0C -∞ followed by cooling at 4^0C. The final product of the PCR was checked in more than one percent agarose gels. The resulting gel was visualized and documented in Bio-Rad Gel documentation system.

Table-1: Primers selected for the Genetic variability studies of Justicia adhatoda.

Primer Name	Sequence (5' → 3')
UBC-814	CTC TCT CTC TCT CTC TA
UBC-822	TCT CTC TCT CTC TCT CA
UBC-827	ACA CAC ACA CAC ACA CG
UBC-840	GAG AGA GAG AGA GAG AYT
UBC-876	GAT AGA TAG ACA GAC A

Interpretation of Data: The presence and absence of clear, dense and visible bands in the gels of different populations were noted[22]. The data was analysed with the help of Pop gene package version 1.31and the dendrogram was constructed with MEGA software. Nei and Li[23] method was used for the calculation of individual population similarity index.

Results and discussion

Genetic diversity analysis of different populations of *Justicia adhatoda* L. was carried out using ISSR-PCR markers in the present investigation. Four different accessions were collected from Salem, Coimbatore, Thoothukudi and Tirunelveli of Tamil Nadu. Out of ten primers, there are five primers selected based on the ability of frequent production of bands (Figure-1).

The PCR final products produce bands in varied range of molecular weight. The result indicated that similar bands developed in all the populations at various frequencies. Five primers yielded 12 polymorphic loci with polymorphic loci of 36.36%. The genetic distance and the identity between the populations ranged from 0.3610 to 0.0625 and from 0.9394 to 0.6970 respectively. The mean values of overall number of alleles observed was 1.3636 and effective numbers of alleles was 1.2788 (Table-2). The overall genetic diversity of Nei was 0.1553 and information index of Shannon was 0.2243. The summary statistics of genetic diversity of all primers and populations were displayed in Table-3. The UPGMA dendrogram constructed using MEGA software displayed genetic distance among the four accessions with three clusters in Figure-2**.** The first cluster represented highest similarity index between population 1 (Salem) and population 2 (Tirunelveli) with the genetic distance of 3.126. The second cluster showed more similarity between Population 3 (Thoothukudi) and Population 4(Coimbatore) with the genetic distance of 8.215. The distance between clusters one and three is 11.818 and the distance between clusters two and three is 6.729.

The molecular marker ISSR used to investigate the polymorphism of *Justicia adhatoda* o from different genomic regions in the present investigation. This method owing different evolutionary histories and genome coverage[24]. Due to the rapid usage of medicinal plants from wild leads to the loss of diversity, so that the study of genetic diversity and selection of superior genotype is necessary for the conservation of biodiversity. The genetic characters of the plants may be affected by the reproductive modes of the plants, seed dispersal mechanism and geographic distance of plant species. The conservation of genetic diversity contributed by widespread species is more compare to the small geographical range species[25]. Genetic variations caused by the modifications in the environmental gradients caused by altitudinal gradients because topographical heterogeneity of plants habitat[26,27]. The genetic diversity of plants are high in hybrid varieties compared to the species formed by selfing and clone[28]. Similar results reported in *Caldesia grandis*[29], *Dalbergia sissoo*[30]. Singh *et al.*[31] also studied DNA polymorphism in elite mung bean genotypes. Das *et al.*[32] explored similar results of ISSR markers based on the genotypes and the density of microsatellites, that contribute to development with species and the SSR patterns which targeted[33]. These features suggested that ISSR markers are suitable for the genetic assay of numerous plants[34-36].

Conclusion

The present study of *Justicia adhatoda* explored the significant genetic variations among the four different populations. For the development of sustainable management strategies of population structure of *Justicia adhatoda* provides the crucial and basic information of the plant. The result of the present investigation could be useful for the conservation of selected species or useful in breeding programmes.

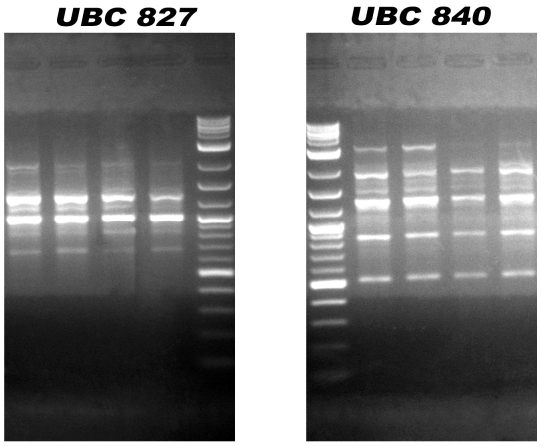

Figure-1: ISSR-PCR Profiles of *Justicia adhatoda.*

Table-2: Nei's genetic identity mentioned in above diagonal and Genetic distance in below diagonal.

Accessio ID	1	2	3	4
1	****	0.9394	0.6970	0.7273
2	0.0625	****	0.7576	0.7879
3	0.3610	0.2776	****	0.8485
4	0.3185	0.2384	0.1643	****

Table-3: All loci summary statistics of genetic diversity.

Statistic parameters	Mean value	Standard deviation
Alleles - Observed number (a)	1.3636	0.4885
Alleles - Effective number (ne)	1.2788	0.3935
Gene diversity (h) [23]	0.1553	0.2120
Shannon's information index (I)	0.2243	0.3039

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