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REVIEW ARTICLE

A STUDY OF GENETIC DIVERSITY IN DIFFERENT ACCESSIONS OF URGINEA SPECIES HYACINTHACEAE, AS REVEALED BY ISSR

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ABSTRACT

Urginea is a very important and rare medicinal plant, found growing in India, Africa and Mediterranean regions. Due to its medicinal properties the bulb of *Urginea* has found its place in British and European pharmacopeia. In the present study, genetic fingerprints of seven accessions of *Urginea* from different parts of Karnataka, South India were developed using PCR-based markers-ISSR. 3 ISSR primers were used to elucidate genetic diversity important for management and conservation. A total of 935 clear, distinct and reproducible bands were observed corresponding to an average of 311 bands per primer of which 899 bands were polymorphic in nature and percentage of polymorphism obtained was 96%. The binary matrix was used to calculate the distance matrix by Neighbour joining using free tree software. The matrix has a genetic distance of 0.52715 to 0.68282 with a mean of 0.5823. The smallest distance value of 0.52715 was observed between Ranganathittu and Bukkapatna indicating that these ecotypes are similar. The maximum distance value 0.68282 suggesting high divergence detected between Gulbarga and Channamallipura accessions. Dendrogram was constructed according to the data recorded. The accession B. R. Hills falls to cluster I and Ranganathittu, Magadi, C M Pura, Karighatta, Gulbarga and Bukkapatna falls to cluster II with three subclusters. High genetic diversity documented in the present study provides a baseline data for optimization of conservation of the *Urginea* species.

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INTRODUCTION

Urginea species is an important medicinal plant found growing in India, Africa and Mediterranean regions. The genus *Urginea* derived from an Arabian tribe, Ben Urgin was coined by a German botanist Adolphe Steinhill (1834) along with the identification of seven species with about hundred species. A taxonomic revision of the genus has been made by Deb & Dasgupta (1987) & has recognized five species. It was later placed under Hyacinthaceae family. There are about seven species of *Urginea* among which two are been taken for our present work – *Urginea indica* and *Urginea wightii. U. indica* has long inflorescence and the flowers are night blooming while *U. wightii* has short inflorescence and have day blooming flowers. The genetic variability and genomic studies are hot topic in research. Thus, it has become necessary to precisely characterize the genetic diversity that exists in the accessions, advanced selections and native population.

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This is one step towards providing accurate genetic information for future breeding and germplasm collection efforts. Unlike morphological markers, cytological (chromosome number, karyotype, nuclear DNA content) and molecular markers (ISSR) are not prone to environmental influences and accurately characterize the plants portraying the extent of genetic diversity among taxa (Bennett and Smith, 1991; Rodriguez *et al.,* 1999). Molecular markers are fragments of DNA that are associated with a certain location within the genome. There are many types of genetic markers, each with particular limitations and strengths. These markers allow for the amplification of particular sequence within the genome for comparison and analysis. Molecular markers are effective because they identify an abundance of genetic linkage between identifiable locations within a chromosome and are able to be repeated for verification. They can identify small changes within the mapping population enabling distinction between a mapping species, allowing for segregation of traits and identity. They identify particular locations on a chromosome, allowing for creation of physical maps. Lastly they can identify how many alleles an organism has for a particular trait. ISSR molecular markers were chosen for the present study of diversity analysis in *Urginea* species.

The Inter-simple sequence repeat (ISSR) is semi arbitrary markers amplified by polymerase chain reaction (PCR) in the presence of one primer complementary to a target microsatellite. Each band corresponds to a DNA sequence delimited by two inverted microsatellites (Zietkiewicz *et al.,* 1994; Tsumara *et al.,* 1995; Nagaoka & Ogihara, 1997). It does not require genome sequence information; it leads to multilocus, highly polymorphous patterns and produces dominant markers (Mishra *et al.,* 2003). ISSR PCR is a fast, inexpensive genotyping technique based on variation in the regions between microsatellites. This method has a wide range of uses, including the characterization of genetic relatedness among populations, genetic fingerprinting, gene tagging, detection of clonal variation, cultivar identification, phylogenetic analysis, detection of genomic instability and assessment of hybridization.

Fig. 1. Vegetative bulbs of *Urginea*

ISSRs have been used in genetic diversity studies in different crop plants (Nagaraju *et al.,*2002; Reddy *et al.,* 2002; Obeed *et al.*, 2008). ISSR markers are also suitable for the identification and DNA fingerprinting (Gupta *et al.,* 2002; Gupta & Varshney, 2000). This method has several benefits over other techniques: it is known to be able to discriminate between closely related genotypes (Fang & Roose, 1997; Hodkinson *et al.,* 2002) and it can detect polymorphisms without any previous knowledge of the crop's DNA sequence. ISSRs are like RAPDs markers in that they are quick and easy to handle, but they seem to have the reproducibility of SSR markers because of the longer length of their primers. However, ISSR is more informative than RAPD in wheat, fruit plants and the common bean for the evaluation of genetic diversity (Korbin *et al.,* 2002; Rakoczy-Trojanowska *et al.,* 2004). It was proven to be reproducible, and quick for characterization many cultivars like poplar (Tang *et al.,* 2006).

MATERIALS AND METHODS

Bulbs of *Urginea indica* were collected from seven different parts of Karnataka, South India. Voucher specimens have been deposited in the herbarium of Botany Department. Plants are grown in the green house of Department of Botany, Bangalore University and maintained under respective growth conditions.

DNA Isolation: Frozen tissue of young leaves was ground using 750 µl of CTAB buffer in a mortar and pestle, until it forms a fine paste and the volume was made upto 1000 µl. 5µl of the RNase solution is added and gently mixed 5-6 times by inverting the vial. The sample was incubated at 65°C for 10 min with intermittent mixing. 1ml of Lysis Buffer was added and vortexed for a minute and incubated at 65°C for 15 min. The sample is centrifuged at 10,000 rpm for 2 min in a desktop centrifuge. The aqueous layer was recovered and mixed with equal amount of Isopropanol and centrifuged at 10,000 rpm for 15 min, to precipitate the DNA. The pellet was washed in1ml of 70% ethanol and centrifuged at 10,000 rpm for 15 min. The above process is repeated and the pellet obtained is dried at 37°C for 10 min and the DNA was suspended by placing the vials at 65°C for 15 min or at 4°C overnight. DNA was extracted separately from each individual plant. In all cases, extracted DNA was subjected to polymerase chain reaction (PCR) amplification.DNA quantity and quality were estimated using 1% agarose gel electrophoresis by comparing band intensity with λ DNA of known concentrations.

Primer Design: Primers and ISSR assay

A total of 12 primers were tested to amplify the isolated DNA, of which only 3 gave reproducible polymorphic bands, 5'$(CA)_8$AG 3', 5'$(GA)_8$CG3' and 5'$(CA)_8$CT3'. Amplification reactions were done in a 25µl volume containing:

Reagents	Quantity
gDNA	1.0 µl
Primer (100ng/µl)	2.0 µl
dNTPs (10mM)	1.0 µl
ChromTaq Assay buffer (10X)	2.5 µl
ChromTaq DNA Polymérase (3U/µl)	0.5 µl
Water	15.0 µl
Total	25 µl

PCR Amplification

PCR was performed using ISSR and amplification reactions were carried out in an Eppendorf Master Cycler Gradient. The apparatus is programmed to execute the following conditions were: 1 cycle – a denaturation step of 5 min at 94°C, followed by 40 cycles composed of 30 s at 94°C, 90 s at the annealing temperature 56^0 C, and 90 s at 72°C. A final extension of 72°C for 5 min was included. ISSR amplification products were analyzed by gel electrophoresis in 2% agarose in 1X TBE buffer, stained with Ethidium Bromide (0.5 µg/ml) and digitally photographed under ultraviolet light at 300 nm. Reproducibility of the patterns was checked by running the reactions in duplicates.

Data analysis

For Each DNA sample, ISSR bands were transformed into a binary matrix where the presence of reproducible polymorphic DNA band at particularly position on gels is scored manually as 1(present), while a 0(absent) denotes its absence of co-migrating fragments for all accessions. Only the clearest and strongest reproducible bands across two PCR amplification replicates were used for cluster analysis. Clearly detectable amplified ISSR ranged from 100 to 2500 bp in size. "The genetic similarity matrices and dendrograms were constructed using Neighbor- joining method using arithmetic average. In addition to cluster analysis, principal component was used to confirm the results of cluster analysis.

RESULTS

The present study showed that ISSR-PCR analysis is quick, reliable, produces sufficient polymorphisms for large-scale DNA fingerprinting purposes, and also showed that ISSR markers are able to reveal variability between *Urginea* accessions (Fig. 2). In ISSR, according to the reported results,

first twelve primers were used and after initial screening three out of them were eventually selected for the final analysis. A total of 935 clear, distinct and reproducible bands were observed corresponding to an average of 311 bands per primer of which 899 bands were polymorphic in nature and percentage of polymorphism obtained was 96%. The Fig. shows the results of amplification with primers on agarose 2% with 9lanes gel tray. From the results, good amplification products were obtained from primers based on AC, repeats ($(CA)_7AG$ and $(AC)_8T$). Since, primers based on CT, GT, CAG and CAA repeats produced few large separate bands which were eliminated for the final analysis.

Table 1. Distance matrix using Neighbour joining

	R1	R2	R3	R4	R5	R6	R7
R1		0.52733	0.64651	0.53345	0.55420	0.54030	0.53425
R2	0.52733		0.67773	0.52715	0.54277	0.57751	0.55153
R3	0.64651	0.67773		0.62626	0.66366	0.68282	0.62483
R4	0.53345	0.52715	0.62626		0.59934	0.56381	0.60236
R5	0.55420	0.54277	0.66366	0.59934		0.56874	0.54727
R6	0.54030	0.57751	0.68282	0.56381	0.56874		0.53525
R7	0.53425	0.55153	0.62483	0.60236	0.54727	0.53525	

The binary matrix was used to calculate the distance matrix by Neighbour joining using free tree software(table 1). The matrix has a genetic distance of 0.52715 to 0.68282 with a mean of 0.5823. Thus, it may be assumed that the accessions are characterized by a high degree of genetic diversity at the DNA level.

The smallest distance value of 0.52715 was observed between Ranganathittu and Bukkapatna indicating that these ecotypes are similar. The maximum distance value 0.68282 suggesting high divergence detected between Gulbarga and Channamallipura accessions. According to the data recorded, the accession B. R. Hills falls to cluster I and Ranganathittu, Magadi, C M Pura, Karighatta, Gulbarga and Bukkapatna falls to cluster II with three subclusters (Fig. 2).

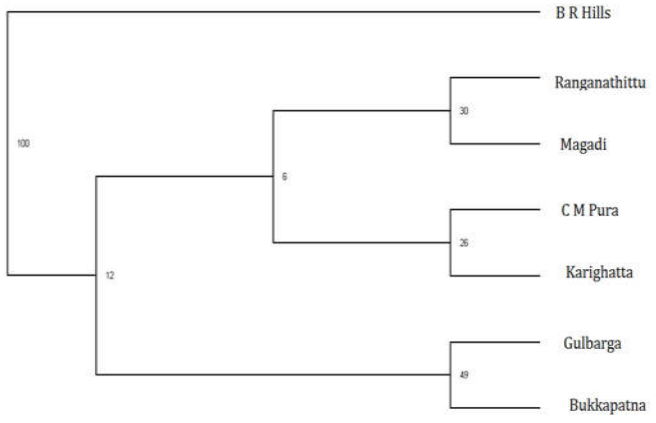
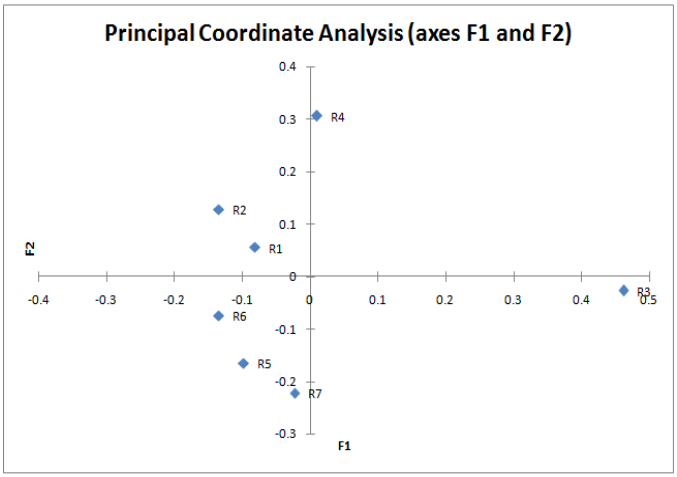


Fig. 2. Dendrogram of *Urginea* accessions as revealed by ISSR markers

The Principle coordinate analysis (PCA) based on genetic similarity matrices were used to visualize the genetic relationships among genotypes (Fig 3).



R1– B R Hills;
R2 – Ranganthittu;
R3 – Gulbarga;
R4 – Bukkapatna;
R5- Magadi;
R6 – Channamallipura;
R7 - Karighatta

Fig. 3. PCoA of different accessions of *Urginea*

DISCUSSION

Accurate identification and characterization of different germplasm resources is important for species identification, certification and conservation. With the advent of molecular biological techniques, DNA-based markers very efficiently augment the morphological, cytological and biochemical characterization of germplasm, varietal identification, assessment of genetic diversity, validation of genetic relationship, phylogenetic and evolutionary studies. Application of PCR-based molecular markers like RAPD, ISSR and AFLP had been proved by many works in molecular characterization of different species (Mukherjee *et al*, 2003; Dikshit *et al*. (2007); Fu *et al*. (2008); Lu *et al* (2009). Molecular markers are more reliable for investigation of genetic similarity/diversity due to their neutral behaviour to environment and coverage of the whole genome for amplification (Alluri *et al.,* 2015). The ISSR technique provided an efficient assessment of genetic variability in the sweet potato population, as was also found in other studies of this crop (Hu *et al.,* 2003; Qiang *et al.,* 2008). Qiang *et al.* (2008) used ISSR markers, found an association between genetic and geographic distances working with accessions from various Asian countries, different from the study of Veasey *et al.* (2008). The intraspecific changes were also noted in the genome size of *Urginea* species due to heterochromatin polymorphism, B-chromosomes, aneuploidy, polyploidy or hybridity (Ramya & Shivkameshwari, 2016).

The large difference in gene diversity as well as genome size among the studied population groups reveals the presence of strong genetic structure between them and thus significant differences exist in the genotypic diversity among themselves. Some variability could, however, be due to the different environmental conditions experienced by the population. Thus, ISSR data along with cytological data enlightens the wide the genetic diversity base present among these seven accessions of *Urginea*. However, limited sample size used in this study restricts the relevance of analysis and the reliability of results for more generalized conclusions on the diversity levels in

Urginea. Further studies should therefore be carried out using larger samples derived from more extended geographical regions to classify the general attitude of *Urginea* genetic variation and define valuable germplasm for the improvement and conservation programmes in this multi-potential medicinal plant.

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