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GENETIC VARIATION IN *ARENGA WIGHTII* GRIFF. (ARECACEAE) POPULATIONS USING INTER SIMPLE SEQUENCE REPEAT (ISSR) MARKERS

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Abstract

The genetic variability was determined for thirty-two natural populations of *Arenga wightii* Griff. (Arecaceae)-an unbranched monecious palm most commonly grows in wild or semi-wild condition and endemic to Western Ghats regions of Southern India. Inter simple sequence repeat (ISSR) markers were used to estimate genetic variability among the populations which are collected from different geographical regions within Karnataka, India. Ten ISSR primers were used to study the genetic diversity within populations of *Arenga wightii*. Among them, 5 primers successfully generated the amplicons of enviable DNA band sizes. An overall band amplified from those primers is 994 with an average of 198.8 bands per primer. The UPGMA-based clustering analysis showed the genetic similarity index ranged from 0.556 to 0.999, indicates considerable variability within the species and close similarity among individuals of a particular population. The similarity coefficients were utilized as input data source for cluster analysis constructed by a dendrogram similarity matrix was measured based on Jaccard index. Among the five analyzed populations, the maximum genetic diversity was observed in Kodagu population followed by Shringeri, Charmudi. This shows that the plants are more similar to one another; the similarity might be due to the same geographical area. These results suggest that ISSR markers are efficient for determining genetic variability in *Arenga wightii*. The obtained results demonstrate that the ISSR markers may be used for further several other molecular marker systems that will help in germplasm characterization of *Arenga wightii* for further conservation and sustainable utilization.

Key words : Wild-palms, Peninsular India, threatened species, conservation, polymorphism, NTSys-pc, dendrogram.

Introduction

Arecaceae/Palmae is a large plant group consists of around 212 genera and 3000 species (Takhtajan, 1987). Globally, palms are most commonly distributed in tropical and subtropical regions. This family is one of the extensively utilized plant families to the man, which stands after grasses in tropics region and it is also equal to legumes. Other than the well-known crops, coconut (*Cocos nucifera* L.), oil palm (*Elaeis oleifera* [Kunth] Cortes ex Prain), and date palm (*Phoenix dactylifera* L.), there are many other species of palm which facilitate various useful products such as foodstuffs, fibers and for various ailments (Balick and Beck, 1990). However, many species of palms prosper well in extremely assorted habitats. This is very well exemplified by the species inhabit in deserts, semi-arid regions, and tropical rain forests. Whereas, India comprises of 22 genera and 105 species are extensively distributed in three major geographical regions such as Peninsular India, North-Eastern India, and Andaman and Nicobar Islands (Hooker, 1872-1897). A small group of palm species also occur elsewhere in India, mainly in Gangetic plains and in the lower hill valleys of Northern India. Peninsular India is symbolized of 11 genera and about 32 species (Renuka, 1999). The genus *Arenga* Labill. is one among the South East Asian group consists of 25 species, which comprises only three species in India namely, *A. wightii* Griff., *A. westerhoutii* Griff. and *A. pinnata* (Wurmb) Merr., in which *A. wightii* is considered as the threatened and endemic species of India (IUCN, 2017; Lawand *et al.,*

2018). *A. wightii* commonly called as Wight's Sago Palm. Usually present on steep slopes in low and medium elevation wet evergreen forests, up to 1500 m. Various parts of *A. wightii* proven to possess several medicinal properties. Starch obtained from pith was orally consumed for painful urination and leucorrhoea. Sap attained from inflorescence is used as a cooling antidiarrheal agent. Fresh toddy collected from young inflorescence is utilized to treat jaundice (Manithottam and Francis, 2007).

Conservation of threatened species depends on the genetic diversity information of both within and among populations. The particulars resting on the genetic assortment of a plant are very much essential because the genetic divergence influences its subsistence in the expected population. The plants with high genetic diversity will easily adapt to environmental changes. Kang and Chung (1997) reported that information of genetic diversity and gene flow systems are supposed to be evaluated to conclude the efficiency of *in-situ* and *ex-situ* conservation program. Genetic markers play a vital role for identification of species and evaluation of relatedness (Bassam *et al.,* 1991; Mahmood *et al.,* 2010; Rabbani *et al.,* 2010). There are several information on utilization of DNA markers for analysis of heritable assortment of Arecaceae member such as; Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR), Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLPs) and Simple Sequence Repeats (SSR) (Zehdi *et al.,* 2002; Adawy *et al.,* 2005; Younis *et al.,* 2008; Shinwari *et al.,* 2011; Zeb *et al.,* 2011; Hamza *et al.,* 2012). Inter Simple Sequence Repeats (ISSR) is one among the PCR-dependent methods which engrosses the intensification of inter-tandem replicates of short DNA sequences. Inter simple sequence reiterates are extremely polymorphic, potent technique, fast, trouble-free, reproducible and cost effective for analysis of inherent relationship/assortment or to categorize intimately correlated crop plants which also includes fruit crops (Blair *et al.,* 1999; Moghaieb *et al.,* 2010; Nookaraju and Agrawal, 2012; Turi *et al.,* 2012). It is applied in differentiating polymorphism devoid of preceding acquaintance of DNA succession in the region flanked by two microsatellites (Khanam *et al.,* 2012). Single primer is utilized in this technique to mark compound genomic loci (Hussein *et al.,* 2005). It requires a simple procedure with good reproducibility, low-cost price, permitting its uses for genetic diversity analysis of population (Chen *et al.,* 2005; Li and Xia, 2005; Karim *et al.,* 2010). In this study, we employed ISSRs to estimate the degree of genetic variation among 32 populations *Arenga wightii* collected from Western Ghats of Karnataka.

Materials and Methods

Plant

The plant samples were collected in wild condition in and across Western Ghats of Karnataka in their natural habitats. The plant, being a threatened species has a limited distribution range. The samples were collected from the Dakshina Kannada, Shringeri, Agumbe, particularly Madikeri consists of certain other major divisions namely Bagamandala, Karike and Sampaje of Western Ghats of Karnataka. The list of the region of plant collection and their accession name is given in table 1. Young leaves were collected from the wild habitat and stored at -80°C prior to DNA extraction.

DNA Extraction

Total genomic DNA was extracted by fresh leaves using cetyltrimethylammonium bromide method (Sambrook and Russell 2001) the quality of DNA was obtained from 0.8% (w/v) agarose gel electrophoresis. Overall the genomic DNA of fresh young leaves was obtained by means of cetyltrimethylammonium bromide method (CTAB) described by Sambrook *et al.* (2001), and the quality confirmed by 0.8% (w/v) agarose gel electrophoresis in 1X TAE buffer. DNA qualitative and quantitative was determined using Quawell Q3000 UV Spectrophotometer.

ISSR-PCR Amplification

The investigation of genetic assortment of *A. wightii* was done using ISSR primers. The ISSR primers were chosen selectively based on those which gave amplification for palm species. Ten primers with good and clear amplified bands were selected for genetic diversity analysis. ISSR amplification was carried out using The Master Cycler Gradient (Eppendorf AG 22331, Hamburg, Germany) in each 10 ìl PCR reaction consists of Taq buffer, $MgCl_2$, primers, dNTPs, Milli Q water and Taq polymerase. PCR intensification was done in a 10 µl reaction mixture comprise of 1µl genomic DNA, Taq Buffer 1.5 µl, $MgCl_2$ 1µl, primer 1 µl, dNTPs 1µl, Milli Q water 3.75 µl, and Taq Polymerase 0.25 µl. Amplification was performed with primarily with denaturation step at 95°C for 3 min, pursued by 32 cycles denaturation 95°C for 30 sec. Primer annealing temperature was considered based on the annealing temperature of each primer for 45 sec, primer extension step was done at 72°C for 2 min and ultimately the extension step was performed at 72° C for 10 min (table 2). The PCR products were visualized on a 1.5% agarose gel with a standard 1kb

ladder. The ISSR-PCR was repeated three times.

Data Analyses

The amplified DNA fragments were scored as either 1 or 0, which represents the presence or absence of the band, respectively. Data analysis was performed using numerical taxonomy multivariate analysis system (NTSys-pc) version 2.0. A dendrogram (Sokal and Michener, 1958; Rohlf, 2000) was obtained later rely upon the genetic similarity matrix which is then followed by unweighted pair group method with arithmetic average (UPGMA) (Sneath and Sokal, 1973).

Results and Discussion

The present research was performed to scrutinize the genetic variation among 32 accessions of *A. wightii*. They are collected from varied geographical regions of Western Ghats of Karnataka. It is very important to understand the genetic variation and relatedness of *A. wightii*, a threatened species is mainly concerned with developing a conservation and utilization strategy. Genetic variability helps in forming new types, alteration and a species without adequate genetic diversity is thought to be unable to handle with changing climate (Nei, 1973; Schaal *et al.,* 1991).

A total of ten ISSR primers that were reported earlier to be specific for palm species were selected and tested for their ability to generate expected polymorphic ISSR bands in collected palm species (Madar and Devarajan, 2017). Among them, 5 primers successfully generated the amplicons of enviable DNA band sizes. The primers UBC 834, UBC 835, UBC 841, UBC 868 and UBC 880 were capable of producing bands in 32 populations of *A. wightii*. An overall band amplified from those primers is 994 with an average of 198.8 bands for every primer. The maximum amount of amplicons was produced with the primer UBC 834 (211 amplicons) which was followed by UBC 868 (208 amplicons) and UBC 841 (202 amplicons). The lowest number of amplicon produced with primer UBC 880 (177 amplicons). The five ISSR primers generated 10.86% polymorphism and 89.14% monomorphic bands. The primers UBC 834, UBC 868 capable of forming DNA bands with the molecular weight range of 600 to 1800 bp, the primer UBC 841 and UBC 835 creates DNA bands with molecular weight with an average of 500 to 2000 bp respectively. The primer UBC 880 capable of generating DNA bands with molecular weight of 600-1500 bp (fig. 2). Based on the polymorphic banding pattern, a genetic similarity matrix was constructed. The similarity matrix is given in table 3. The similarity coefficients were used as input data for cluster analysis performed by NTSYSpc (version 2.0) program. The resulting dendrogram is shown in the fig. 1. Genetic similarity coefficient among 32 accessions of *A. wightii* ranged from 0.556 to 0.999. The dendrogram divides the collected 32 plants samples into 2 major groups A and B. Out of 32 samples two are included in the group A, In group A, the plants collected from the Agu Z and Agu Z1 shows more similarity of 0.556. The group B consists of thirty samples in which the plants were grown at Shringeri (Shru), Bagamandala-Karike (Bagk), Charmudi Ghat (Char) and Sampaje (Samp) are more closely related compare to all others. In which group B is again divided into cluster B1, B2, and B3. In cluster B1, the plants collected from Shru2, BagkY, CharV, Shru1 shows more similarity of 0.999, Cluster B2 comprises of CharU, BagkT, BagkS, BagkR, SampQ, SampP, BagkO, BagkN, BagkM, ShruL, CharK, CharI, CharH, CharG, ShruF, BagkE, BagkD, CharA, BagkB, Shru3, Shru4 are more similar to a range of 0.999. The plants collected at the Charmudi Ghat (CharW and CharX) are more similar to a range of 0.857 and are placed in a separate cluster B3. This shows that the plants

Table 1 : List 32 populations of *Arenga wightii* Griff. Location, Accession name and GPS coordinates.

S. No.	Location	Accession name	No. of Samples	Latitude (°N)	Longitude (°L)
1.	Charmadi ghat, Dakshina Kannada district	CHAR	14	12°.91'	74°.85'
2.	Bagamandala karike	BAGK	11	12°.41'	75°.73'
3.	Agumbe Pond	AGUP	2	13°.86'	75°.50'
4.	Shingeri Road	SHRU	6	13°.42'	75°.26'
5.	Sampaje	SAMP	2	12°.43'	75°.59'

Table 2 : List of ISSR primers sequences and annealing temperature used for the present study.

S. No.	ISSR Primers	Sequence (5'-3')	Annealing temp. (°C)
1.	UBC834	AGAGAGAGAGAGAGAGYT	51.9°C
2.	UBC835	AGAGAGAGAGAGAGAGYC	44.7°C
3.	UBC841	GAGAGAGAGAGAGAGAYC	46.7°C
4.	UBC855	ACACACACACACACACYT	60.2°C
5.	UBC868	GAAGAAGAAGAAGAAGAA	50.8°C
6.	UBC880	GGAGAGGAGGAGAGGAGA	52.1°C
7.	UBC890	VHVGTGTGTGTGTGTGT	89.3°C
8.	UBC826	ACACACACACACACACC	53.3°C
9.	UBC827	ACACACACACACACACG	54.9°C
10.	UBC810	GAGAGAGAGAGAGAGAT	42.9°C

Table 3: Genetic similarity matrix

	Char A	Bagk B	Bagk C	Bagk D	Bagk E	Shru F	Char G	Char H	Char I	Char J	Char K	Shru L	Bagk M	Bagk N	Bagk O	Samp P	Samp Q	Bagk R	Bagk S	Bagk T	Char U	Char V	Char W	Char X	Bag Y	Agu Z	Shru 1	Shru 2	Shru 3	Agu Z1	Shru 3	Shru 4
Char A	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Bagk B	1.000	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Bagk C	1.000	1.000	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Bagk D	1.000	1.000	1.000	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Bagk E	1.000	1.000	1.000	1.000	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Shru F	1.000	1.000	1.000	1.000	1.000	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Char G	1.000	1.000	1.000	1.000	1.000	1.000	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Char H	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Char I	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Char J	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Char K	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Shru L	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Bagk M	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Bagk N	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Bagk O	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Samp P	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Samp Q	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1	1.000	1.000	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Bagk R	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1	1.000	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Bagk S	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1	1.000	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Bagk T	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1	1.000	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Char U	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1	1.000	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Char V	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1	0.667	0.778	0.889	0.444	0.889	0.889	0.889	0.556	0.900	0.900
Char W	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.667	1	0.857	0.750	0.429	0.750	0.750	0.750	0.571	0.600	0.600
Char X	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.857	1	0.875	0.571	0.875	0.875	0.875	0.714	0.700	0.700
Bag Y	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.750	0.875	1	0.500	1.000	1.000	1.000	0.625	0.800	0.800
Agu Z	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.429	0.571	0.500	1	0.500	0.500	0.500	0.800	0.400	0.400
Shru 1	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.750	0.875	1.000	0.500	1	1.000	1.000	0.625	0.800	0.800
Shru 2	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.750	0.875	1.000	0.500	1.000	1	1.000	0.625	0.800	0.800
Shru 3	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.750	0.875	1.000	0.500	1.000	1.000	1	0.625	0.800	0.800
Agu Z1	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.571	0.714	0.625	0.800	0.625	0.625	0.625	1	0.500	0.500
Shru 3	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.600	0.700	0.800	0.400	0.800	0.800	0.800	0.500	1	1.000
Shru 4	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.600	0.700	0.800	0.400	0.800	0.800	0.800	0.500	1.000	1

are more similar to one another; the similarity may be of the same geographical area.

Genetic variation analysis is a prominent tool for conservation of plant and breeding programmes. Usually, genetic richness is performed using certain genetic diversity constraints such as, to know the proportion of polymorphic loci and genetic diversity manifestation (Yeh, 2000). The proportion of polymorphic loci augmented in *A. wightii* was 10.8%. In respect to these studies, moderately lower percentage of polymorphic loci was obtained in *Gliricidia sepium* (Chalmers *et al.,* 1992) and *Theobroma cocao* (Russell *et al.,* 1993). The high percentage of polymorphism has been reported in several tropical and subtropical species such as *Caesalpinia echinata* Lam. (Cardoso *et al.,* 1998), *Euterpe edulis* (Cardoso *et al.,* 2000) and in *Calamus metzianus*. The maximum fraction of polymorphism possesses a maximum level of genetic dissimilarity within the species. It is reported that a high percentage of polymorphism shows the high level of outcrossing within and between the populations (Loveless and Hamrick, 1984; Karron, 1991). The present study possesses very low difference among populations due to the same geographical diversity, but few differences were also observed in Agumbe population due to the variation observed in the climatic features and altitude. With reference to this, low variation was also observed in certain wind pollinated, woody and long-lived tree species possess comparatively least variation within the population (Hamrick and Godt, 1990) as in *Populus tremuloides* (Yeh *et al.,* 1995) and *Quercus petrea* (De Greef *et al.,* 1998). It is also compared with herbaceous and insect-pollinated genotypes, which shows more or less similarity in *Lilium martagon* (Persson *et al.,* 1998) and *Argyroxiphium*

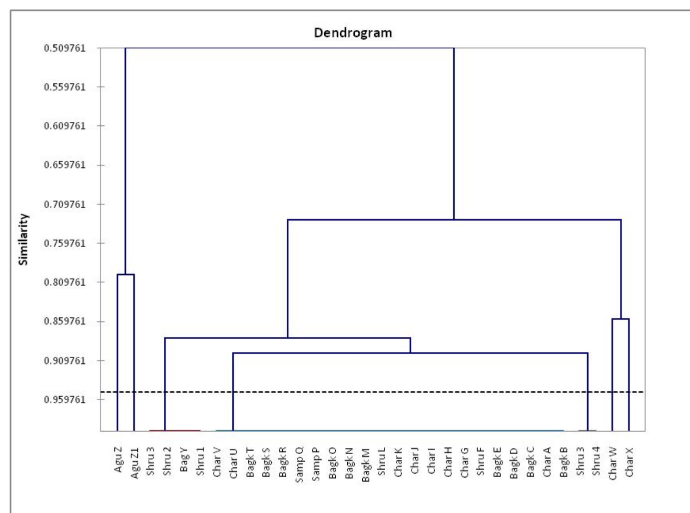

Fig. 1: UPGMA dendogram of 32 *Arenga wightii* Griff. provenances constructed from genetic distance.

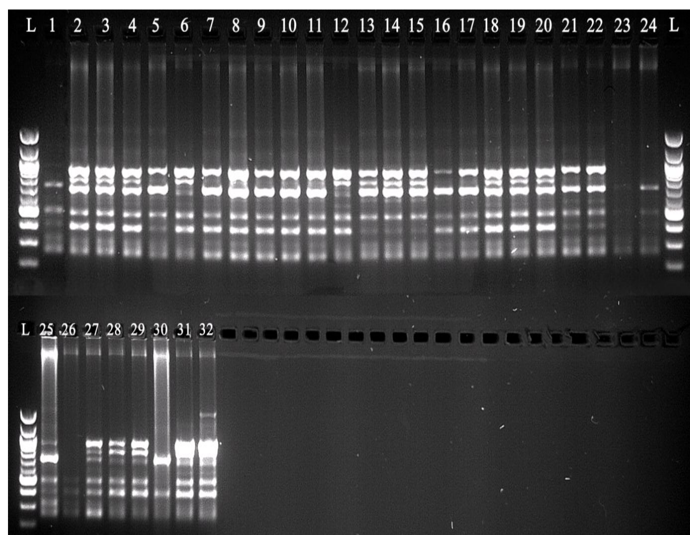

Fig. 2 : ISSR marker profile of 32 Arenga genotypes produced by primer UBC-834.

sandwicense (Frier *et al.,* 1996). Among the five analyzed populations, the highest genetic diversity measures were observed in Kodagu population followed by Shringeri, Charmudi. The eminent gene assortment was observed in Kodagu population when compared to that of other populations, it possesses high population density compared to those of other populations. It is also one of the important reservoirs of potentially useful genes required to provide high priority for management strategies and conservation. The key factors affecting genetic diversity within populations are the geographic distribution and mode of seed dispersal (Hamrick *et al.,* 1991). This study revealed the presence of minimum genetic variation among the populations used for analysis, suggesting that ISSR markers are an important tool to assess the genetic variation and relatedness in populations.

Conclusion

The obtained results indicated that the Arenga genotypes investigated in this study have minimum genetic diversity. This shows that the plants are more similar to one another; the similarity may be of the same geographical area. Cluster analysis using the UPGMA method grouped into two main clusters, mainly due to its geographical origin. For the better understanding of the genetic diversity of *Arenga wightii*, future studies should focus on a larger number of populations and accessions collected from more geographical regions.

References

Adawy, S.S., E.H.A. Hussein, S.E.M.E. Ismail and H.A. ElItriby (2005). Genomic diversity in date palm (*Phoenix dactylifera* L.) as revealed by AFLPs in comparison to RAPDs and ISSRs. *Arab J. Biotech*., **8(1)** : 99-114.

Balick. M.J. and H.T. Beck (I990). Useful palms of the world: a synoptic bibliography. Columbia University Press, New York.

Bassam, B.J., G. Caetano-Anolles and P.M. Gresshoff (1991). Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal. Biochem*., **196** : 80-83.

Blair, M.W., O. Panaud and S.R. McCouch (1999). Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). *Theor. Appl. Genet*., **98** : 780-792.

Cardoso, M.A., J. Proven., W. Powell., C.G. Ferreira and D.E. De Oliviera (1998). High genetic differentiation among remnant populations of the endangered *Caesalpinia echinata* Lam. (Leguminosae - Caesalpinioideae). *Molecular Ecology*, **7**: 601-608.

Cardoso, S.R. S., N.B. Eloy., J. Provan., M.A. Cardoso and P.C. G. Ferreira (2000). Genetic differentiation of *Euterpe edulis* Mart. populations estimated by AFLP analysis. *Molecular Ecology*, **9**: 1753-1760.

Chalmers, K.J., R. Waugh., J.I. Sprent., M.J. Simons and W. Powel (1992). Detection of genetic variation between and within populations of *Gliricidia sepium* and *G. maculata* using RAPD markers. *Heredity*, **69**: 465-472.

Chen, J.M., X. Liu, W.R. Gituru, J.Y. Wang and Q.F. Wang (2005). Genetic variation within the endangered quillwort *Isotoma petraea* (Lobeliaceae). *Mol. Ecol*., **8** : 775-789.

De Greef, B., L. Triest, B. De Cuyper and J. Van Slycken (1998). Assess-ment of intraspecific variation in half-sibs of *Quercus petraea* (Matt.) Liebl. «plus» trees. *Heredity*, **81** : 284-290.

Frier, E.A., R.H. Robichaux and D.W. Mount (1996) Molecular genetic variation following a population crash in the

endangered Mauna Kea silverswood, *Argyroxiphum sandwicense* ssp. sanwicense (Asteraceae). *Molecular Ecology*, **5** : 678-691.

Hamrick, J.L. and M.J.W. Godt (1990). Allozyme diversity in plant species. In: Plant Population Genetics, Breeding, and Genetic Resources, Brown, A.H.D., M.T. Clegg, A.L. Kahler and B.S. Weir (eds), Sinauer, Sunderland, 43-63.

Hamrick, J.L., M.J.W. Godt and S.L. Sherman-Broyles (1991). Factors affecting levels of genetic diversity in woody plant species. *New Forests*, **6**: 95-124.

Hamza, H., M.A. BenAbderrahim, M. Elbekkay, G. Ferdaous, T. Triki and A. Ferchichi (2012). Investigation of genetic variation in Tunisian date palm (*Phoenix dactylifera* L.) cultivars using ISSR marker systems and their relation with fruit characteristics. *Turk. J. Biol*., **36**: 449-458.

Hooker, J.D (1872-1897). Flora of British India vol 1 to 7. Reeve and Company Ltd. Ashford, Kent, England.

Hussein, E.H.A., S.S. Adawy, S.E.M.E. Ismail and H.A. ElItriby (2005). Molecular characterization of some Egyptian date palm germplasm using RAPD and ISSR markers. *Arab. J. Biotech*., **8(1)**: 83-98.

IUCN (2017). The IUCN Red List of Threatened Species. Version 2017-3. <http://www.iucnredlist.org>.

Kang, S.S. and M.G. Chung (1997). Genetic variation and population structure in Korean endemic species. IV *Hemerocalis hakuunensis* (Liliaceae). *J. Plant Res*., **110**: 209-217.

Karim, K., B. Chokri, S. Amel, H. Wafa, H. Richid and D. Nouredine (2010). Genetic diversity of Tunisian date palm using ISSR markers. *Int. J. Bot*., **6(2)**: 182-186.

Karron, J.D. (1991). Patterns of genetic variation and breeding systems in rare plant species. In: Genetics and Conservation of Rare Plants, Falk, D.A. and K.E. Holsinger (eds), Oxford University Press, Oxford.

Khanam, S., A. Sham, J.L. Bennetzen and M.A.M. Aly (2012). Analysis of molecular marker based characterization and genetic variation in date palm (*Phoenix dactylifera* L.). *AJCS*, **6(8)**: 1236-1244.

Lawand, P.R., S.S. Kambale, V.B. Shimpale and S.R. Yadav (2018). Lectotypification of *Hyphaene dichotoma* and *Arenga wightii* (Arecaceae). *Rheedea*, **28(1)**: 51-53.

Li, F. and N. Xia (2005). Population structure and genetic diversity of an endangered species, *Glyptostrobus pensilis* (Cupressaceae). *Bot. Bull. Sinica*., **46**: 155-162.

Loveless, M.D. and J.L. Hamrick (1984). Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics*, **15**: 65-95.

Madar, U. and T. Devarajan (2017). Unmasking genetic relationships among *Corypha umbraculifera* populations from Karnataka in India using simple sequence repeat markers. *Scientific Papers. Series A. Agronomy*, **60**: 307-313.

Mahmood, T., S. Muhammad and Z.K. Shinwari (2010). Molecular and morphological characterization of *Caralluma* species. *Pak. J. Bot*., **42(2)**: 1163-1171.

Manithottam, J. and M.S. Francis (2007). *Arenga wightii* griff, a unique source of starch and beverage for Muthuvan tribe of Idukki district, Kerala. *Ind. J. Trad. Know*., **6**: 195-198.

Moghaieb, R.E.A., A.A. Abdel-Hadi, M.R.A. Ahmed and A.G. M. Hassan (2010). Genetic diversity and sex determination in date palms (*Phoenix dactylifera* L.) based on DNA markers. *Arab. J. Biotech*., **13(2)**: 143-156.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proc. Nat.l Acad. Sci*., **70** : 3321-3323.

Nookaraju, A. and D.C. Agrawal (2012). Genetic homogeneity of *in vitro* raised plants of grapevine cv. crimson seedless revealed by ISSR and microsatellite markers. *S. Afr. J. Bot*., **78**: 302-306.

Persson, H.A., K. Lundquist and H. Nybom (1998). RAPD analysis of genetic variation within and among populations of Turk's cap lily (*Lilium martagon* L.). *Heriditas*, **128:** 213-220.

Rabbani, M.A., M.S. Masood, Z.K. Shinwari and K.Y. Shinozaki (2010). Genetic analysis of basmati and non basmati Pakistani rice (*Oryza sativa* L.) cultivars using microsatellite markers. *Pak. J. Bot*., **42(4)**: 2551-2564.

Renuka, C. (1999). Indian rattan distribution - an update. *Indian Forester*, **125**: 591-598.

Rohlf, F.J. (2000). NTSYS-PC: numerical taxonomy and multivariate analysis system, version 2.1. New York, USA.

Sambrook, J. and D.W. Russell (2001). Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 2100.

Schaal, B.A., W.J. Leverich and S.H. Rogstad (1991) Comparison of methods for assessing genetic variation in plant conservation biology. In : Genetics and Conservation of Rare Plants, Falk, D.A and K.E. Holsinger (eds), Oxford University Press, New York, USA, 123–134.

Shannon, C.E. and W. Weaver (1949). The mathematical theory of communication. University of Illinois Press, Urbana, 1-117.

Shinwari, Z.K., S. Sultan and T. Mehmood (2011). Molecular and morphological characterization of selected mentha species. *Pak. J. Bot*., **43(3)**: 1433-1436.

Sneath, P.H. and R.R. Sokal (1973). Numerical taxonomy: the principal and practice of numerical classification. W.H. Freeman and Company, San Francisco, 573.

Sokal, R.R. and C.D. Michener (1958). A statistical method for evaluating systematic relationships. *Univ. Kansas Sci. Bull*., **28**: 1409-1438.

Takhtajan, A. (1987). Systema Magnoliophytorum (in Russian). Nauka Publication, Moscow. 1-439.

Turi, N.A., M.A. Farhatullah, Rabbani and Z.K. Shinwari (2012). Genetic diversity in the locally collected *Brassica* species

of Pakistan based on microsatellite markers. *Pak. J. Bot*., **44(3)**: 1029-1035.

Yeh, F.C. (2000). Population genetics. In: Conservation Genetics Principles and practice, Young, A., D. Boshier and T. Boyle (eds), Forest, CSIRO publishing, United Kingdom.

Yeh, F.C., D.K. Xo and R.C. Yang (1995). RAPD variation within and among natural populations of trembling asper (*Populus tremuloides* Michx.) from Alberta. *Journal of Heredity*, **86**: 454-460.

Younis, R.A.A., O.M. Ismail and S.S. Soliman (2008). Identification of sex-specific DNA markers for date palm (*Phoenix dactylifera* L.) using RAPD and ISSR techniques. *Res. J. Agric. Biol. Sci*., **4(4)**: 278-284.

Zeb, A., Z.K. Shinwari and T. Mahmood (2011). Molecular markers assisted genetic characterization of some selected wild Poaceae species. *Pak. J. Bot*., **43(5)**: 2285-2288.