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Microsatellite Markers Reveals Male-specific DNA Sequences in *Calamus thwaitesii* Becc., an Important Economic Rattan Palm

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ABSTRACT: *Calamus thwaitesii* Becc. is a perennial, dioecious rattan palm distributed in the Western Ghats, India. Currently, it arises as a valuable source of tribal economy owing to its high-quality flexible canes for furniture manufacturing and cottage industries. However, the unscientific harvesting can have manifold ecological consequences and affect the sustenance of the plant in its ecosystem. The sex of the plants can be identified only after flowering when it attains reproductive maturity by 10-15 years. Hence proper planning for effective breeding programme, sustainable forestry management and agroforestry systems cannot be accomplished. A study was carried out in this background and two male specific SSR markers for *C. thwaitesii* were identified. The diagnostic potential of microsatellite markers can be exploited to identify the sex of the plant at early seedling stage.

Keywords: microsatellites; SSR; *Calamus*; rattan palms; sex-marker; molecular marker

I. INTRODUCTION

Rattans represent high value non-wood forest produce primarily used for making furniture, handicraft items and an important raw material in the cottage industry. Rattans are the fourth economic palm significant both in international trade (*e.g.*, oil palm, date palm, coconut, rattan) and at the subsistence level in some of the poorest communities in the world. There are about 600 rattan species in 13 genera globally and 51 species in 4 genera in India, of which 75% are endemic and 40.5% threatened [1]. *Calamus* L. is the most widespread rattan genera, occurring in both the tropical and subtropical regions of Africa and south-east Asia [2]. A number of economically important species of rattans are extensively harvested, often leading to severe lack of regeneration [3,4]. Moreover, due to rapid degradation of natural habitats of these species and endemic nature of some species, these groups of plants are gradually heading towards endangerment and extinction.

Calamus thwaitesii Becc. is a robust, large diameter cane grows in evergreen, semi-evergreen and moist deciduous forest between 100 to 900 m elevation throughout the Western Ghats of India and Sri Lanka. They are perennial, dioecious, climbing and endemic rattan with least concern status but are more prone to destruction due to rapid urbanization.

Plant identification is mainly based on vegetative descriptors like morphology of stem, leaf, spines [5–7] and in later stages on the morphology of reproductive characters like flowers, fruits etc [5]. However, the distinction between male and female plants can be identified only after the first flowering by attaining reproductive maturity of 10 to 15 years in these robust canes [8]. Early sex identification in dioecious plants is important for breeders. Male plants are of greater value in *Asparagus officinalis* whose male individuals produce larger and thicker shoots [9]. Female plants are considered valuable in agriculture due to their ability to produce seeds include *Borassus flabellifer* [10] and *Phoenix dactylifera* [11]. In non-crop plants, early sex determination can be of environmental significance [12].

Besides furniture making and basketry, the stem sap of *C. thwaitesii* has been used as an antifertility drug by the tribal groups, Siddhis and Gowlis of Uttara Kannada district, Karnataka [13]. *C. thwaitesii* is used in ayurvedic drugs for curing various diseases like cough, urinary calculi, retention of urine, herpes, diarrhea, bleeding, gynecological disorders, prolapsed uterus, diabetes, fever, rabies, wounds, nervous disorders and diseases of blood [14].

Fruit production is influenced by the distribution and ratios of opposite sexes in any locality. The fruits are edible and new plants are propagated easily through seeds [15]. Indeed, the identification of sex-linked DNA markers will form the basis of a sustainable breeding programme for genetic improvement.

II. MATERIALS AND METHODS

The aim of the present study was to develop a genetic sex marker for *C. thwaitesii* to allow gender determination at any stage in its life cycle. Naturally distributed *C. thwaitesii* populations from Rosemala of Shendurney Wildlife Sanctuary in Southern Kerala, India were selected for the present study. Leaf materials were collected from six male and six female plants of *C. thwaitesii* in May 2016 at the peak seed-bearing stage. The leaf materials were stored at -80°C prior to use. The frozen young leaves were cleaned with distilled water and removed the midrib and spines carefully. DNeasy® Plant Mini Kit was used to extract the DNA by following the quick-start protocol (Qiagen®, USA). Quality of DNA was assessed by electrophoresis on 0.8% agarose gels and its quantity was evaluated in Biophotometer (Eppendorf India Ltd) to dilute DNA stock as 50 ng/µL in d_2H_2O to use in PCR amplification. Polymerase chain reaction (PCR) was carried out using 25 µl of reaction mixture consisting of 2.5µl of buffer with $MgCl_2$, 200 µM of deoxynucleotides, 15 pmol of forward and reverse primers, 50 ng DNA template and 1 unit of Taq polymerase. After 2 min at 94°C, 35 cycles were performed for 30 sec at 94°C, 1 min annealing at the optimized temperature, and 2 min extension at 72°C, and a final extension step of 7 min at 72°C. The PCR was carried out using SureCycler 8800 (Agilent Technologies, Malaysia). After the PCR, the amplified DNA products were electrophoresed on 3.5% agarose gels at 50 V for 5 h with ethidium bromide and product visualized under ultraviolet light using gel documentation (UVP Bio-Imaging, UK) system.

III. RESULTS AND DISCUSSION

The orthologous sequences that differentiated between individuals of male and female plant samples were considered as putative sex-linked markers. The 40 SSR primers examined in this study effectively generated clearly amplified SSR bands with different sizes ranging from 100 bp to 1500 bp. Two SSR primers, *viz.* RT 20 and RT 23 generated a 1200 bp and 590 bp band respectively in the male accessions (Fig. 1 and Fig. 2).

Morphological sexual differentiation in dioecious plants has constantly been a difficult effort until sexual maturity is reached [16]. Moreover, the karyological and cytogenetic analysis of the leaf tissues can be used for sex differentiation in only those groups of dioecious plants that have specific sex chromosomes [17]. Under these situations, a sex-linked molecular marker could be the utmost reliable genetic tool for gender determination in the vegetative growth stage of the plants.

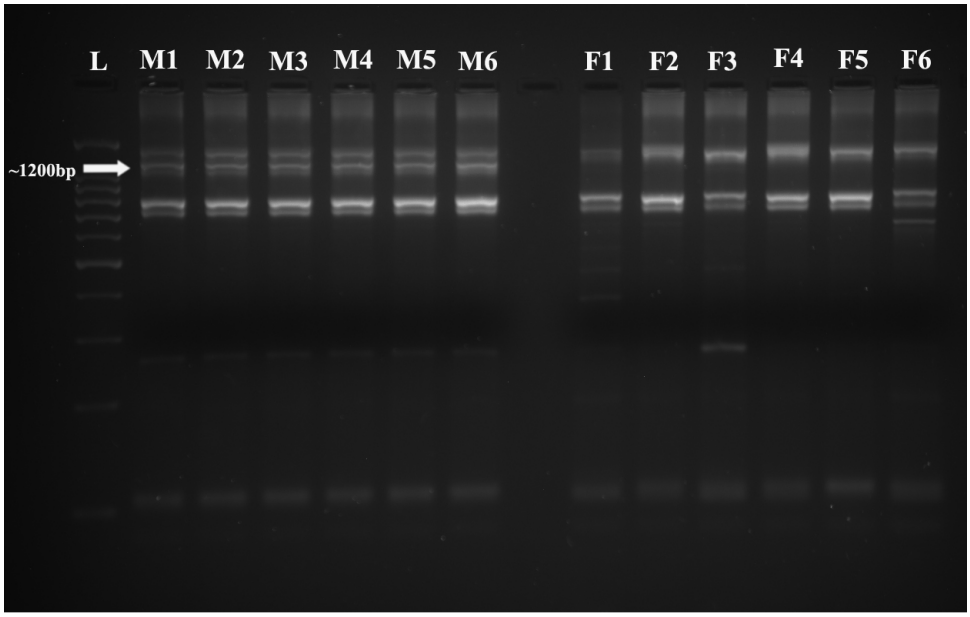

Fig. 1. SSR profiling of *Calamus thwaitesii.* The arrow indicates the unique band of ~1200 bp present in the male samples and absent in the female samples.

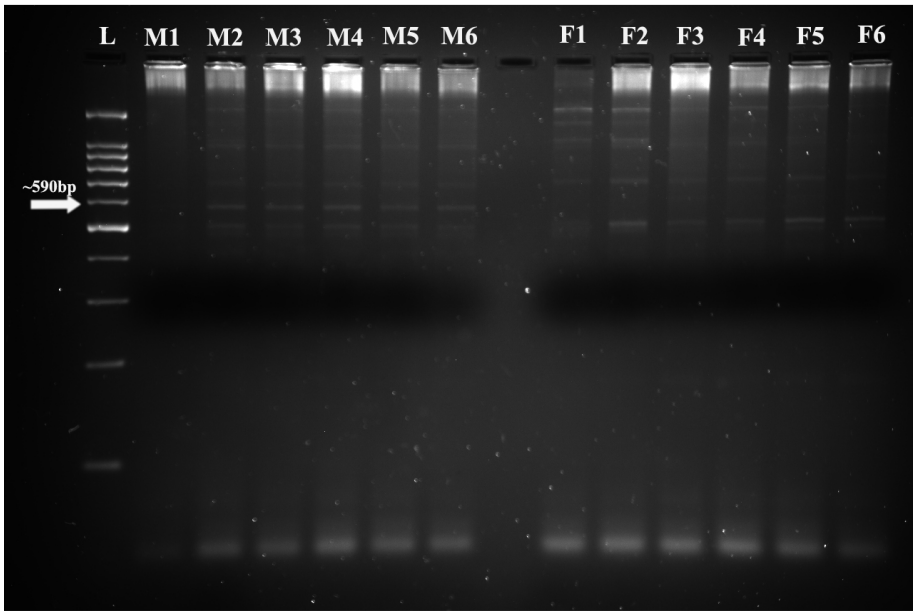

Fig. 2. SSR profiling of *Calamus thwaitesii.* The arrow indicates the unique band of ~590 bp present in the male samples and absent in the female samples.

Although having offbeat commercial values, *C. thwaitesii* and other related rattan species do not have an effective breeding programme due to dioecy and absence of a stable morphological or molecular marker for their segregation. Microsatellites [18] are explored for their potential to serve as sex-specific markers in *C. thwaitesii* owing to its relative abundance in coding and non-coding regions, hyper variable, locus specific, co-dominant and multiallelic nature [19]. Microsatellite primers used in sex identification of date palm at seedling stage were highly polymorphic and generated high number of alleles that confirmed the good transferability [20]. The higher the degree of genetic variability in the microsatellite DNA sequences, the larger those sequences are responsible for sex determination [21,22]. Additionally, the degree of genetic variability, the variation in allele length, insertions/deletions among sequences, transitions and transversions are also responsible for sex determination [23]. Regions adjacent to the microsatellite repeat are known to be hotspots for mutations. The male associated PCR markers are reported to be amplified in male plants and not in females [24]. In the case of the PCR products (could be SSR alleles or stutter bands - confirmed through repeated amplifications) linked to the male sex obtained in this study, non-amplified samples can be unambiguously considered as of female origin.

Microsatellite markers in other plants have been reported to be linked with male gender. The microsatellites $(GATA)_4$ and $(GAA)_6$ revealed male sex-specificity in *Carica papaya* [25]. A trinucleotide microsatellite repeat $(AGA)_7$ is tightly linked to male sex in *Humulus lupulus* [23]. Another microsatellite marker, mPdCIR048 revealed male sex-specificity in *Phoenix dactylifera* [26]. A microsatellite marker, RT17 revealed male sex-specific DNA sequences in *C. thwaitesii* [27]. A male-specific SCAR marker was developed in *C. simplicifolius* from the coding genome region [28]. The present study also affirms the utility of microsatellite markers as tightly linked loci on autosomes for the detection of sex-specific loci in *C. thwatesii*.

IV. CONCLUSION

Rattan palms are important components of many natural habitats and can have major ecological impact in the plant communities. They were benefitted for climate change mitigation, large scale carbon sequestration, reduce carbon footprints of products such as plastic, steel, concrete, etc by replacing them and can reduce pressure on use of forest timber resources. An adequate sex-ratio is to be maintained for these dioecious palms in natural habitat for producing offsprings having equal gene complements for continual evolution and sustainable utilization. Developing scientific management strategies and improved utilization of canes could help to generate employment locally and thus contribute to the socioeconomics sustainably. The study will significantly contribute to genetic improvement, sustainable forestry management and agroforestry systems by bulding green economies worldwide over millennia.

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REFERENCES

[1]. Renuka C. (1997). Distribution of rattan resources in India. In: Rattan Taxonomy and Ecology, Silviculture, Conservation, Genetic Improvement and Biotechnology. *IPGRI*;. p. 55–6.

[2]. Uhl NW, Dransfield J. (1987). Genera Palmarum: a classification of palms based on the work of H.E. Moore, Jr. Lawrence, KS: International Palm Society and L.H. Bailey *Hortorium*;.

[3]. Ravikanth G, Ganeshaiah KN, Shaanker RU. (2002). Identification of hot spots of species richness and genetic variability in rattans: an approach using geographical information systems (GIS) and molecular tools. *Plant Genet Resour Newsl.*; 17–21.

[4]. Narwade A V., Nageswara Rao M, Ravikanth G, Uma Shaankar R, Ganeshaiah KN. (2003). Genetic diversity and structure of endemic and non-endemic species of rattans in central western Ghats. In: Devar K V., editor. National symposium on conservation, management and utilization of bamboo and rattan resources. University of Agricultural Sciences, Dharwad, India.

[5]. Basu SK. (1992). Rattans (canes) in India: A monographic revision. Malaysia and Canada: FRIM and IDRC;.

[6]. Renuka C. (1992). Rattans of Western Ghats - A taxonomic manual. Peechi, Thrissur, India: Kerala Forest Research Institute.

[7]. Sarmah P, Barua PK, Sarma RN, Sen P, Deka PC. (2007). Genetic diversity among rattan genotypes from India based on RAPD-marker analysis. *Genet Resour Crop Evol.*; **54**(3): 593–600.

[8]. Shiva MP. (1992). Status of minor forest products with particular reference to cane, Rattan management and Utilization. In: Rattan (Cane) Seminar India. KFRI, India and IDRC, Canada; p. 48–65.

[9]. Gao WJ, Li RL, Li SF, Deng CL, Li SP. (2007). Identification of two markers linked to the sex locus in dioecious *Asparagus officinalis* plants. *Russ J Plant Physiol [Internet]*. **54**(6): 816–21. Available from: https://doi.org/10.1134/S1021443707060143

[10]. George J, Karun A, Manimekalai R, Rajesh MK, Remya P. (2007). Identification of RAPD markers linked to sex determination in palmyrah (*Borassus flabellifer* L.). *Curr Sci.*, **93**(8):1075–7.

[11]. Younis RAA, Ismail OM, Soliman SS. (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): 278–84.

[12]. Grewal A, Goyat S. (2012). (2015). Marker assisted sex differentiation in dioecious plants. *J Pharm Resaearch.;* **9**(8): 531–49.

[13]. Bhandary MJ, Chandrashekar KR, Kaveriappa KM. (1956). Medical ethnobotany of the Siddis of Uttara Kannada district, Karnataka, India. *J Ethnopharmacol* [Internet].; **47**(3):149–58.

[14]. Chopra RN. (1956). Glossary of Indian Medicinal Plants. In: Glossary of Indian Medicinal Plants. CSIR, New Delhi.

[15]. Mohamad A, Mohmod AL. (1993). Rattan research in Malaysia. *Ratt Manag Util.*; 1–8.

[16]. Irish EE, Nelson T. (1989). Sex determination in monoecious and dioecious plants. *Plant Cell.* **1**(8):737.

[17]. Rejón CR, Jamilena M, Ramos MG, Parker JS, Rejón MR. (1994). Cytogenetic and molecular analysis of the multiple sex-chromosome system of *Rumex acetosa*. *Heredity* (Edinb). **72**(2): 209–15.

[18]. Litt M, Luty JA. (1989). A hypervariable microsatellite revealed by *in vitro* amplification of a di nucleotide repeat within the cardiac muscle actin gene. *Am J Hum Genet*., **44**: 397–401.

[19]. Kar B, Nayak S, Joshi RK. (2014). Development and evaluation of STS diagnostic marker to track turmeric (*Curcuma longa* L.) resistance against rhizome rot caused by *Pythium aphanidermatum. Australas plant Pathol*.; **43**(2): 167–75.

[20]. Billotte N, Marseillac N, Brottier P, Noyer J, Jacquemoud-Collet J, Moreau C, et al. (2004). Nuclear microsatellite markers for the date palm (*Phoenix dactylifera* L.): characterization and utility across the genus Phoenix and in other palm genera. *Mol Ecol Resour.*; **4**(2): 256–8.

[21]. Ellegren H. (2000). Microsatellite mutations in the germline:: implications for evolutionary inference. *Trends Genet*.; **16**(12): 551–8.

[22]. Korpelainen H, Bisang I, Hedenäs L, Kolehmainen J. (2008). The first sex-508 specific molecular marker discovered in the moss *Pseudocalliergon trifarium. J Hered*. **99**: 581–587.

[23]. Jakse J, Stajner N, Kozjak P, Cerenak A, Javornik B. (2008). Trinucleotide microsatellite repeat is tightly linked to male sex in hop (*Humulus lupulus* L.). *Mol Breed*.; **21**(2): 139–48.

[24]. Polley A, Ganal MW, Seigner E. (1997). Identification of sex in hop (*Humulus lupulus*) using molecular markers. *Genome*. **40**(3): 357–61.

[25]. Parasnis AS, Ramakrishna W, Chowdari K V, Gupta VS, Ranjekar PK. (1999). Microsatellite (GATA)n reveals sex-specific differences in Papaya. *Theor Appl Genet*.; **99**(6): 1047–52.

[26]. Elmeer K, Mattat I. (2012). Marker-assisted sex differentiation in date palm using simple sequence repeats. *3 Biotech.,* **2**(3): 241–7.

[27]. Sarmah P, Barua VJ, Nath J, Sarma RN, Kurian B, Hemanthkumar AS, et al. (2016). ISSR and SSR markers reveal sex-specific DNA sequences in three *Calamus* species from India. *Agrofor Syst [Internet].*; 1–5. Available from: http://dx.doi.org/10.1007/s10457-016-9952-9.

[28]. Li M, Yang H, Li F, Yang F, Yin G, Gan S. (2010). A male-specific SCAR marker in *Calamus simplicifolius*, a dioecious rattan species endemic to China. *Mol Breed [Internet]* **25**(3): 549–51. Available from: http://link.springer.com/10.1007/s11032-009-9349-9.