Existence of co-sexuality in palmyrah palm and study of relationship between monoecious and dioecious palms using molecular markers

Keywords: *Borassus flabellifer* L., dioecious, hermaphrodite, monoecious, RAPD

Palmyrah is a slow growing perennial dioecious palm. The first flowering takes place when the palm is 12-15 years of age; only then is the sex of each tree revealed. Though palmyrah is a dioecious palm, recently a high yielding monoecious palmyrah palm has been located in Undeswarapuram near to Rajamundry in Andhra Pradesh State (Venkataramana, personal communication).

The term cosexual (Lloyd, 1984) is used when individual plants have both sex functions, whether present within each flower (hermaphrodite), or in separate male and female flowers (monoecious). Minorities of plant species are sexually polymorphic including dioecious species, with separate males and females. Many dioecious species with hermaphrodite relatives have evident rudiments of opposite sex structures in flowers of plants of each sex, suggesting recent evolution of unisexual flowers (Darwin, 1877). The low frequency and scattered taxonomic distribution of dioecy and sex chromosomes suggest that cosexality is the ancestral angiosperm state (Charlesworth, 1985; Renner and Ricklefs, 1995).

The development of molecular markers linked to sex has been attempted in a number of dioecious species. Random amplified polymorphic DNA (RAPD) markers have been used for determining sex in hermaphrodite and male *Carica papaya* (Deputy *et al*., 2002), *Pistacia vera* (Hormaza *et al*., 1994; Kafkas *et al*., 2001), *Atriplex garrettii* (Claudete *et al*., 1998), *Trichosanthes dioica* (Singh *et al.,* 2002), *Salix viminalis* (Alstrom-Rapaport *et al*., 1998; Gunter *et al*., 2003), *Phoenix dactylifera* (Bekheet *et al*., 2008). Using this method, Mulchahy *et al*. (1992) identified markers specific to the Y chromosome in *Silene latifolia.* The presence of a male sex-linked RAPD marker has previously been reported in palmyrah palm through bulk segregant analysis (Jiji *et al*., 2007). The objectives of the present study were to examine the relationship between monoecious and dioecious palmyrah palm at DNA level and sex determination of seven seedlings generated from monoecious palm using the male sex-linked RAPD marker.

Leaf samples were collected for DNA extraction from the monoecious palm located at Undeswarapuram and its seven progenies planted in 2003 and maintained at Andhra Agricultural University, Pandirimamidi Immature leaf samples from pistillate and staminate palms were collected from Kasaragod, Kerala to serve as control. Immature leaflets of these palms were collected and placed in polybags and transported to the laboratory in icebox where they were kept at 4 ºC prior to DNA extraction. Genomic DNA was extracted from all the samples using the SDS method. The DNA concentration was determined for each sample spectrometrically at 260 nm. PCR amplification was performed as reported earlier (Jiji *et al*., 2007).

In monoecious palmyrah palm (Fig. 1 a), three types of spadices were noticed:

(1) An intermediate type of spadix in which both female and male flowers are present. The male flowers are fertile where as the female flowers are sterile and do not set fruits (Fig. 1 b).

(2) Female spadix possesses only 2-4 spikes, which are ensheathed by spathes. The upper half of the spike is imbricated by bracts while the lower end is a smooth peduncle. A barren bract ensheaths the spike, from where the flowers rise (Fig. 1 c).

(3) Male spadix has 5-10 branches and each branch is ensheathed by a spathe. Each branch has 2-3 spikes. Each spike is stout, cylindrical, 30-40 cm long and 2.5 -4.0 cm wide (Fig. 1 d).

The male-specific RAPD primer (OPA-06) (Jiji *et al*., 2007) was used to screen the monoecious palm and

Fig.1. Monoecious palmyrah palm and its three types of inflorescences
a. Monoecious palm located at Undeswarapuram (Andhra Pradesh); b. Intermediate type of spadix with male and female flowers
c. Female spadix; d. Male spadix

its progenies. It was observed that the male-specific band of size 600 bp was present in monoecious palm as well as in six out of the seven progenies tested (Fig. 2). From the results, it was revealed that one progeny is possibly female (palm No. 7 at Pandirimamidi Station) and the rest of the progenies (palm No. 4, 5, 8, 9, 11 and 12) may

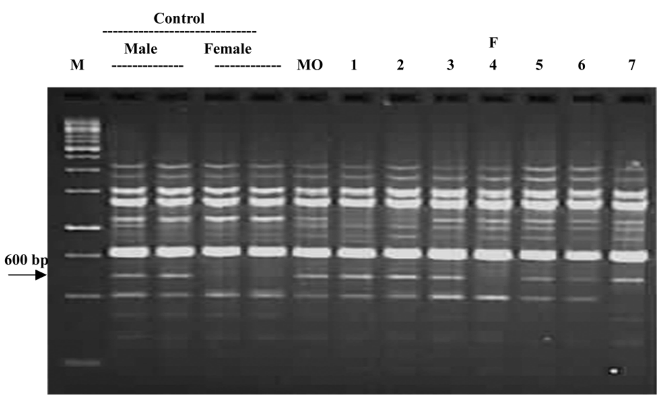

Fig. 2. RAPD banding profile of monoecious palm and its seedlings using male-specific primer OPA-06

MO	**:**	**Monoecious palm**	**1 to 3 & 5 to 7**	**:**	**Monoecious or males**
F	**:**	**Female**	**M**	**:**	**1 kb ladder**

be either monoecious or males. No marker has been identified as yet to differentiate monoecious and males. Perhaps there is only one monoecious palm and so identification of a marker specific to monoecious palm is a tedious task.

Claudete *et al.* (1998) reported that the male sex-specific RAPD marker of size 2075 bp fragment was present in male and hermaphrodite *Atriplex garrettii*. In papaya, three sex types are present male, female and hermaphrodite. The SCAR marker developed by Deputy *et al.* (2002) could detect 800 bp maker in hermaphrodite and male but not in female. Our study also revealed that the male specific marker was also present in monoecious palm.

A genetic model of the evolutionary transition from cosexuality to dioecy suggests, however, that linkage may often be necessary from the outset (Charlesworth and Charlesworth, 1978a). Starting from cosexuality, the evolution of two sexes must generally require at least two genetic changes, one (male-sterility) creating females and the other (female-sterilty) producing males. The process may sometimes have been more gradual, with partial sterility mutations (Charlesworth and Charlesworth, 1978b). A more reasonable hypothesis is that gynodioecy or androdioecy resulting from a single mutation may have provided likely intermediary step between hermaphroditism and dioecy (Ainsworth, 2000).

In monoecious palmyrah the intermediate type of inflorescence is a female-sterile (male) mutant, which is similar to female-sterile mutant in androdioecy. Charlesworth and Chalesworth (1978b) reported that early stages in the evolution of dimorphism will be difficult to distinguish from the range of variation found in cosexual populations, and at the first stages (nearing strict dioecy) inconsistencies can be expected on both sexes. But only in this case the intermediate type of spadix showed androdioecious nature where in the palm has both male and female spadix as in monoecious individual.

Allen (1940) reported most mutations in the sex genes of hermaphroditic species would tend towards unisexuality, whereas most mutations in doecious species will tend towards hermaphroditism (or monoecism). One approach for studying the direction of evolution is to look for reappearance of ancestral traits and to analyse these mutant carrying such ancestral traits. Such mutants have been termed atavistic as they give phenotypes resembling the presumed ancestral condition (Manuela *et al.*, 2005). Atavism occurs because genes for previously existing phenotypical features are often preserved in DNA, even though the genes are not expressed in some or most of the organisms possessing them.

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