Journal of Agriculture and Environment
Vol. 20 No. 1, 2024: 201-210

DNA fingerprinting of Date palm (*Phoenix dactilifera L.*) gender using random amplified polymorphic DNA – PCR technique

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ABSTRACT

Date palm (*Phoenix dactylifera* L.) is a flowering plant that is cultivated for its edible sweet fruit called dates. It is a dioecious plant species with unisexual flowers. In date farming, there is the need of a sufficiently large number of productive female trees with only a minimal number of male trees. However, due to phenotypic difficulties in sex determination before flowering, which takes 4 to 5 years, there is the need for an alternative genetic approach to curtail this problem. Therefore, this study was conducted to investigate the potential of random amplified polymorphic DNA (RAPD) technique as a genetic tool in sex determination of this plant at an early stage. Date palm seeds were planted until the young and tender leaves were grown. DNA was extracted from the leaves using Zymo DNA Mini Kit. Six different RAPD primers were used for polymerase chain reaction (PCR) amplification and the resulting PCR products were resolved on 2% agarose gel for 2 hours. The banding pattern on the gel was used to compare the results of the samples against a male positive control. The findings of this study showed clearly the differences in banding patterns between male and female seedlings. Hence, the use of RAPD as a routine genetic tool in fingerprinting date palm would aid in planting appropriate female date palm plants thereby reducing time wastage and increase in the return on investment.

Keywords: Date palm; Fingerprinting; Plant gender; RAPD

INTRODUCTION

Phoenix dactylifera L., commonly known as date or date palm (Qacif *et al.*, 2017) is a flowering plant species in the palm family, *Arecaceae*, cultivated for its edible sweet fruit called dates (Vayalil, 2012). The genus *Phoenix*, which includes 12–19 species of wild date palms, has *P. dactylifera* as its type species (Krueger, 2011). As a dioecious plant, flowers are unisexual where female plants only produce fruits, whereas male plants are used as pollinators. Although dates are naturally wind pollinated, they are fully pollinated manually in both traditional horticultural and contemporary commercial orchards. About equal

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https://dx.doi.org/10.4314/jagrenv.v20i1.20

numbers of male and female plants are needed for natural pollination to occur. However, with assistance, one male plant can pollinate up to 100 female plants (Siljak *et al.,* 1996). This enables the farmers to use their resources for many more fruit-producing female plants as the males are solely used as pollinators (Chao and Krueger, 2007).

Because of the difficulties to differentiate phenotypically between male and female plants before flowering (Biffi *et al.,* 2005; Hormaza *et al.,* 2004; Mulcahy *et al.,* 2002), which takes 5 to 10 years (Amjad *et al.,* 2022; Jani *et al.,* 2022; Adawy *et al.,* 2014; Daher *et al.,* 2010), sex determination in the seedling stage is very critical for breeding. Morphological identifications exploited include colour of flowers where males are yellow while females are white; leaves of males are narrow, while females are more comprehensive with more pores. Spikelet number is relatively higher in males than females (Amjad *et al.,* 2022; Bekheet and Hanafy, 2011). According to several cytological research, male date palms contain a heteromorphic chromosome pair that differs from females (Filatov, 2015; Cherif *et al.,* 2013; Siljak *et al.,* 1996). Recently, Mathew *et al.* (2014) published the first genetic map of date palm, while Al-Mssallem *et al.* (2013) published the *de novo* genome assembly of a female cultivar with a total length of approximately 600 Mb, covering more than 90% of the genome. More so, Torres *et al.* (2018) published the results of a full-length sequencing of male-specific sequences from a single male individual. Other organelle genomes published from date palm include mitochondrion (Fang *et al.,* 2012) and chloroplast (Khan *et al.,* 2018; Khan *et al.,* 2012; Yang *et al.,* 2010). Although there is paucity of genome mapping studies related to date palm sexing, certain DNA fingerprinting techniques have been considered as quick and reliable methods for genome analysis and sexing plant species (Yahaya *et al.,* 2022; Mohammed and Mohammed, 2019; Al-Qurainy *et al.,* 2018; Dhawan *et al.,* 2013; Bekheet and Hanafy, 2011; Sharma *et al.,* 2010; Gangopadhyay *et al.,* 2007). Random amplified polymorphic DNA (RAPD) marker is a simple, easy, and quick assay that has been adopted widely for plant genome analysis (Gaafar *et al.,* 2014; Abdel-Mawgood, 2012). Hence, the aim of this study was to investigate the suitability of RAPD-PCR as a simple molecular tool of determining the sex of date palm plant at an early stage of development thereby minimising redundant male date palm cultivation.

MATERIALS AND METHODS

Date Palm Seeds Collection and Planting

Date palm fruits were bought from Girei local market, Adamawa State, Nigeria. Five (5) seeds labelled a – e were surface-cleaned by washing with water and planted at Chevron Biotechnology Center's screen house, Modibbo Adama University (MAU) Yola, until fresh leaves were grown. A male date palm plant obtained from the University's horticultural garden was used as a positive control.

Leaf Processing and DNA Extraction

Individual leaf from the five seeds was cut, washed with distilled water, and weighed to obtain 40 mg. This was then grinded using pestle and mortar and added into a labelled 1.5 ml micro centrifuge tube for DNA extraction. DNA was extracted using Quick-DNA™ Miniprep Plus Kit (Zymo Research Corp; Catalog Nos. D4068 & D4069) following the manufacturer's instruction with minor modifications of incubation time for cell lysis and final

DNA elution volume. Briefly, to the 40 mg of ground leaf tissue, 95 µl distilled water, 95 µl solid tissue buffer, and 10 µl proteinase k were added and incubated at room temperature overnight for cell lysis. Subsequently, 400 µl genomic binding buffer was added and vortexed for 15 seconds. The mixture was transferred to a Zymo-spin IIC-XLR column in a collection tube and centrifuged at 14,000 rpm for a minute and the filtrate was discarded. The DNA was washed with 400 µl of DNA prewash buffer and 700 µl of genome DNA wash buffer. In a clean 1.5 ml micro centrifuge tube, the pure DNA was eluted from the column using 70 µl of DNA elution buffer. The concentration and purity of DNA was checked using a Nanodrop spectrophotometer (Thermo Scientific, USA).

PCR Amplification and Analysis

RAPD-PCR optimization was carried out as earlier described (Ja'afar *et al*., 2016), hence not reported in this study. Six different RAPD primers, OPJ-09 (5′TGAGCCTCAC3′), OPA-02 (5′TGCCGAGCTG3′), HKT-1 (5′TTCCGAACCC3′), HKT-2 (5′TCTGTGCGTG3′), ZA (5′GTGGATGCGA3′) and ZR (5′CAGGCCCTTC3′), synthesized at Inqaba Biotec™ West Africa Ltd were used. To a 0.2 ml PCR reaction tube, 1X *Taq* 2X master mix (New England BioLabs™), 40 ng/µl genomic DNA, 0.6 µM primer were added and made up to a final volume of 25 µl with distilled water. PCR thermal amplification was performed using SelectCycler™ II Thermal Cycler (Select Bioproducts, USA) with the following cycling profile: initial denaturation at 94^{o}C for 5 minutes; 40 cycles of denaturation at 94^{o}C for 30 seconds, annealing at 38^{o}C (OPJ), 34^{o}C (OPA, ZR and ZA) for 60 seconds and extension at 72^{o}C for 2 minutes. Final extension was at 72^{o}C for 10 minutes. PCR products were resolved using 1.2 % agarose gel in TAE buffer. Resolved products were visualized using a bench top UV transilluminator (UVP UV Transilluminator, USA). HKT-1 and HKT-2 did not produce any band during optimization reactions and were excluded from further studies.

RESULTS

To investigate the suitability of using RAPD technique in sexing date palm plant, four (4) out of six (6) RAPD primers provided different banding patterns that suggests differences in male and female date palm plants (Fig 1 – 4). The OPJ primer distinctly identified female date palm plants from the males, with three bands to one, respectively. In contrast, OPA primer only amplified the female date palms with about 10 bands each. ZA primer amplified a male and female date palm each, with four to three bands, respectively while ZR primer amplified the males and two female date palms with one to four bands each, respectively. Sexing of date palm with RAPD-PCR is therefore feasible when more than one RAPD primer is used.

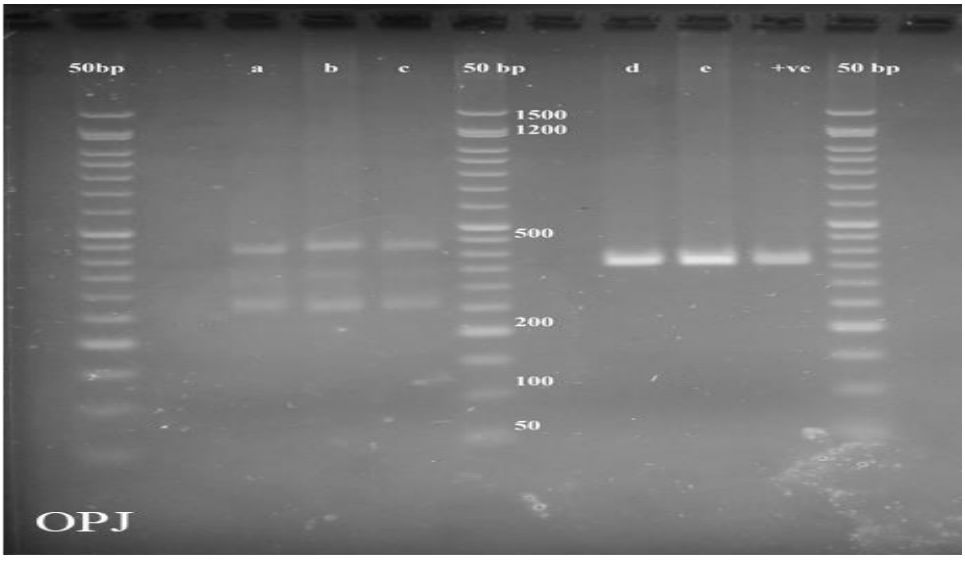

Figure 1: Ramdomly amplified polymorphic DNA Primer (OPJ09) of 5 date palm (A-E). Samples a, b and c are female date palm plants; d and e are male date palm plantlets; +ve is the male positive control; 50 bp are molecular size markers.

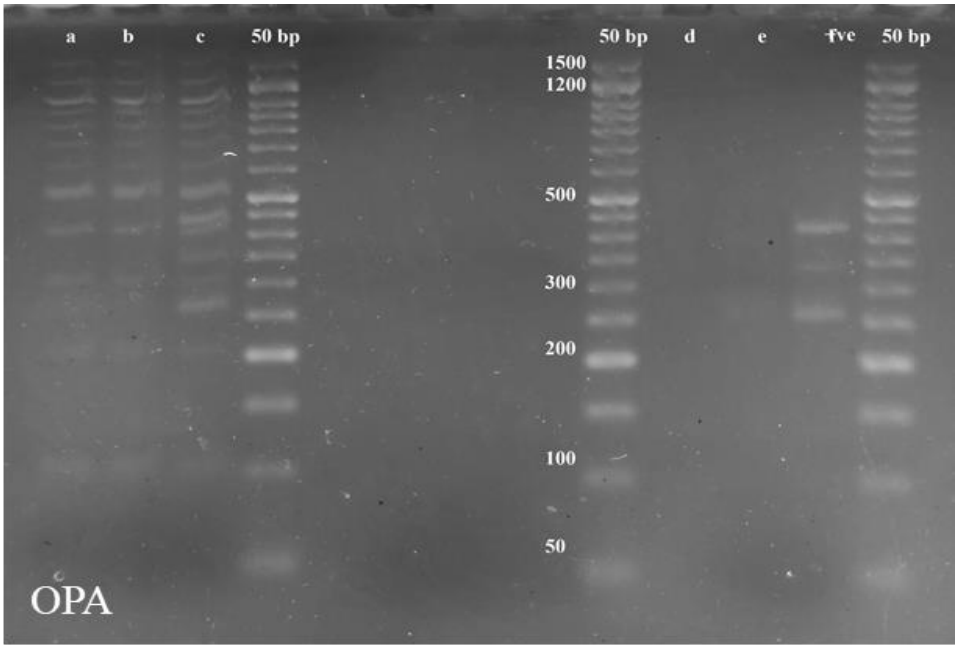

Figure 2: Ramdomly amplified polymorphic DNA primer (OPA02) of 5 date palm (a-e). Samples a, b and c are female date palms; d and e were not amplified; +ve is the male positive control; 50 bp are molecular size markers.

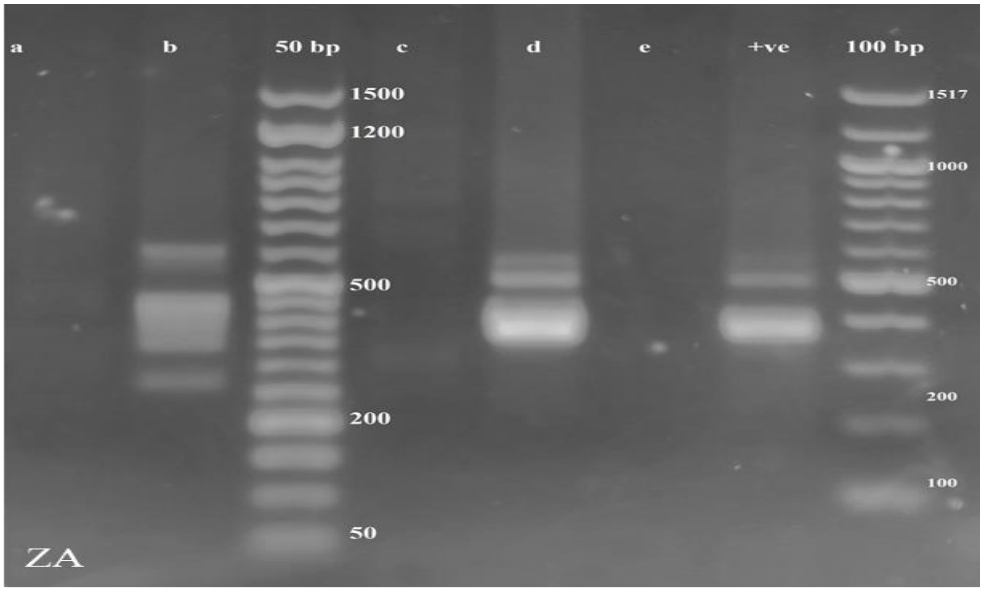

Figure 3: Ramdomly amplified polymorphic DNA primer (ZA) of 5 date palm (a-e). Sample b is a female date palm plantlet; d is a male date palm plantlet; a, c and e were not amplified; +ve is the male positive control; 50 bp and 100 bp are molecular size markers.

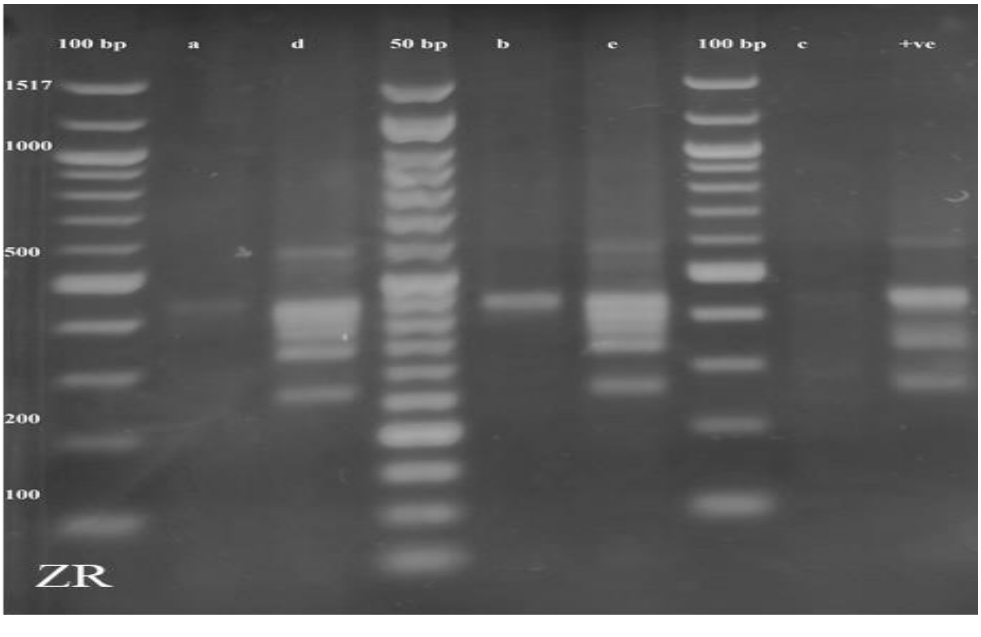

Figure 4: Ramdomly amplified polymorphic DNA primer (ZR) of 5 date palm (a-e). Samples a and b are female date palm plantlets; d and e are male date palm plantlets; +ve is the male positive control; 50 bp and 100 bp are molecular size markers.

DISCUSSION

In an effort to distinguish between male and female date palm, five unknown date palm plantlets were PCR amplified with four (4) RAPD primers and their banding patterns compared to a male positive control.

OPJ primer distinctively differentiated between the male and female date palms by amplifying unique sets of bands. In this study, consensus banding pattern was the goal rather than size of the bands as RAPD enables the amplification of independent genetic loci of the target genome (Ja'afar *et al.,* 2018). Samples a – c were believed to be females, while samples d and e are males. Similarly, OPA primer was able to identify samples a – c as females. The non-amplification of samples d and e was not surprising because with RAPD, complex banding patterns that are difficult to interpret or non-amplification has been previously reported (Ja'afar *et al.,* 2018; Ja'afar *et al.,* 2016; Demeke *et al.,* 1992; Echt *et al.,* 1992). ZA primer amplified one of the male samples, d, which had similar banding pattern with the male positive control. Dassanayake and Samaranayake (2003) had also suggested that the theoretical lack of amplification with some primers was due to their distance on the DNA template, being that a distance of more than 4 kbp by the primers on the 5′ and 3′ directions would yield no amplification. Lastly, ZR primer amplified two of the three females only (a and b). Although the banding pattern of samples d and e were somewhat different to the male positive control, they were still considered as males since they were completely different to the females.

As earlier mentioned, this study concentrated on the consensus banding patterns produced by the primers rather than the individual sizes of the bands. This is because the bands cannot ascertain if the plant is truly of a particular sex without sequencing the individual bands. Furthermore, RAPD bands are assumed to be unique, because the procedure does not amplify two distinct bands that co-migrate on gels owing to a similar size; however, this fact is not always true (Rafalski *et al*., 1991). As suggested by Betancor *et al.* (2004), RAPD band patterns were considered different when they differed by more than two bands. To buttress further, RAPD was developed as a simple and rapid fingerprinting and genetic mapping technique only (Lucia and Eliane, 2008; Williams *et al.* 1990). To benefit from its simplicity, multiple primers that are optimized is a pre-requisite. This is evident from this study and others were multiple primers had to be used to identify the different date palm types (Yahaya *et al.,* 2022; Mohammed and Mohammed, 2019; Younis *et al.,* 2008). However, with the recent sequencing of male and female genomes (Mssallem *et al.,* 2013; Torres *et al.,* 2018) more specific markers targeting the sex chromosome has been reported (Amjad *et al.,* 2022; Jani *et al.,* 2022; Intha and Chaiprasart, 2018; Jaskani *et al.,* 2016; Adawy *et al.,* 2014; Mahmoud *et al.,* 2012).

Other DNA-based fingerprinting assays include ISSR (Ameri *et al.,* 2016) and SSR (Elmeer and Mattat, 2012). Utilization of unique RAPD bands as sequence characterized amplified region (SCAR) markers for gender detection in date palm has also been reported (Mohei *et al.,* 2019; Al-Qurainy *et al.,* 2018; Kharb and Mitra, 2007). Other sex determination methods reported include gene-specific sex-linked markers (Ali *et al.,* 2018; Mohamed and Sami, 2015), *in vitro* culture (Khalifah *et al.,* 2006) and spectroscopy (Khan *et al.,* 2021). Other studies focused on date palm transcriptomics (Radwan *et al.,* 2015; Fang *et al.,* 2012), gene models annotation (Zhang *et al.,* 2012) and diversity and history (Hazzouri *et al.,* 2015; Gros-Balthazard *et al.,* 2017; Hadrami *et al.,* 2011).

CONCLUSION

In conclusion, the present study suggests that the use of multiple RAPD primers might contribute to the differentiation between male and female date palm at an early stage of development and breeding.

Additional molecular studies towards developing of a definitive SCAR marker for sex differentiation will permit rapid adoption of the method as a routine technique in date palm plantations.

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