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Sex-Specific Bands in Dioecious Plant Jernang Rattan (*Daemonorops draco*) Based on RAPD Markers

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Abstract

Background: *Daemonorops draco (Willd.) Blume, or dragon's blood rattan, is a dioecious species that produces high-value resin but is increasingly threatened by habitat degradation. Effective cultivation and conservation require early identification of male and female plants, which remains challenging at the seedling stage. Although RAPD markers have been used for sex identification in dioecious plants, studies on D. draco are still limited. Therefore, this study aims to screen sex-specific bands in D. draco using the RAPD approach.* **Methodology:** *Samples of six male and six female individuals collected from Jambi were analyzed, with DNA isolated using a modified CTAB protocol and amplified using 44 RAPD primers. The products were separated by 2% agarose gel electrophoresis. Bands were considered sex-specific if they consistently appeared only in one sex and were absent in the other.* **Findings:** *Out of the 44 primers tested, 39 successfully amplified D. draco DNA, generating a total of 662 bands. Among them, primer OPA-14 (455 bp) and OPC-19 (310 bp) produced male-specific bands that were consistently present in all male individuals, while primer OPB-10 (850 bp) generated a female-specific band detected in all female individuals. The male-specific 455 bp band from OPA-14 and 310 bp from OPC-19, and the female-specific 850 bp band from OPB-10 were identified. These bands are recommended for development into SCAR markers for early sex identification in D. draco.* **Contributions:** *The findings support the provision of male and female seedlings to enhance conservation and cultivation programs.*

Keywords: *Daemonorops draco*; *Dioecious Plant; RAPD; Sex-Specific Band*

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INTRODUCTION

Jernang Rattan (*Daemonorops draco*) is a species of the Arecaceae family that produces resin from its fruit skin (Asra et al., 2019). Traditionally, the Anak Dalam tribe (SAD) uses this resin to as medicine for thrush, diarrhea, headache, and ulcers (Mairida et al., 2016; Harnov et al., 2016). This resin is also used as an antibacterial, antifungal, antiaging, antioxidant (Jura-Morawiec & Tulik, 2016; Purwanti et al., 2019), and anticancer agent (Park et al., 2022). In addition to medicine, *D. draco* resin is also used as a natural dye (Mairida et al., 2016). These benefits are due to *D. draco* being rich in primary and secondary metabolites, namely flavonoids and terpenoids, as well as saponins, glycosides, and steroids/triterpenoids (Wulandari et al., 2021). These benefits and contents have led to dragon's blood resin being considered a forest product with high economic value, reaching IDR 8,000,000 - 10,000,000 in the export market (Fitriandi et al., 2020).

This species is only distributed in several provinces, namely Jambi, Riau, and South Sumatra (Rustiami et al., 2004). However, its habitat has begun to degrade due to land conversion and overexploitation (Lestari et al., 2017). To address this problem, efforts such as the conservation and cultivation of *D. draco* have been done (Mairida et al., 2016; Harnov et al., 2016). Despite these efforts, significant obstacles remain, particularly the limited availability of seeds and the challenge of identifying male and female seedlings in this dioecious plant. Morphologically, male and female individuals cannot be distinguished before entering the generative phase, which typically occurs when the plant is 4–5 years old (Asra et al., 2012), and no secondary sexual characteristics are present at the seedling stage. In general, dioecious plants do not have clearly differentiated sex chromosomes, making them difficult to distinguish cytologically (Choong & Wickneswari, 2016).

Molecular approaches are among the most effective strategies for overcoming the challenge of early sex identification, as they enable sex determination indepent regardless of the plant's reproductive age (Sarmah et al., 2016). One of the simplest and most cost-effective molecular markers is Random Amplified Polymorphic DNA (RAPD) (Saputro et al., 2016). The advantages of RAPD over other techniques are that it is efficient, can be performed without prior knowledge of the target DNA, and produces polymorphism bands (Promkaew et al., 2024).

RAPD has also been successful as a first step in identifying the sex of dioecious plants, particularly in the Arecaceae family, namely the OPAU-02 primer in *C. travancoricus* (Priya et al., 2019), and the OPC-06, OPB-18, and OPA-02 primers in *P. dactylifera* (Al-Qurainy et al., 2018; Intha & Chaiprasart, 2018; Mohammed & Mohamed, 2019), primers OPO-17 and OPAP-20 in *Salacca edulis* (Ediwirman, 2015). Based on the effectiveness of the RAPD technique in identifying the sex of plants from the Arecaceae family, primers were selected to get the specific-band related to sex in *D. draco*. The aim of this study was to screen primers producing sex-specific bands in *D. draco*. This research represents an important preliminary step and is the first to report sex-specific bands in *D. draco* for early molecular sex identification, supporting the optimization of its conservation and cultivation programs.

METHOD

This research was carried exploratory and descriptively using RAPD (Random Amplified Plolymorphic DNA) technique following Qurainy et al., (2018). This research was conducted at the Genetic and Biomolecular Laboratory, Faculty of Mathematic and Sciences, Universitas Andalas in September 2024 - April 2025.

Sample Collection

Samples of young *D. draco* leaves consisting of six male and six female individuals were collected from two populations in Jambi Province, Sumatra, Indonesia, namely Sako Besar Village (Batanghari Regency) and Bukit Dua Belas National Park (Sarolangun Regency). The sampling sites within the national park included Sungai Kuning, the Jernang Demonstration Plot, and Ujung Kutai, which are located in lowland tropical forest areas at approximately 50–200 m above sea level. Geographically, these locations lie within the central part of Sumatra and are characterized by relatively dry lowland forest conditions with moderate canopy cover and seasonal moisture availability.

DNA Isolation and Extraction

The isolation and extraction method used was a modified CTAB method (Doyle & Doyle, 1987). Leaf powder was placed in a 1.5 ml microtube, then 750 µl of 2% CTAB solution (consisting of 5% CTAB, 5 M NaCl, Tris-HCl, Na_2EDTA, and distilled water) was added. The suspension was homogenized with a vortex and incubated at 65°C for 45 minutes, with vortexing every 10 minutes. The mixture is centrifuged at 12,000 rpm for 10 minutes at 27°C, then the supernatant is transferred to a new microtube and chloroform:isoamyl alcohol (24:1) is added, followed by centrifugation again. The extracted supernatant is mixed with an equal volume of isopropanol, centrifuged at 12,000 rpm for 5 minutes at 4°C. The resulting pellet is washed twice with cold 70% ethanol, dried for 1–2 hours, then dissolved in 50 µl of nuclease-free water and stored at –20°C. DNA concentration and purity were measured quantitatively using a NanoDrop spectrophotometer with an absorbance ratio at a wavelength of 260/280 nm. Good quality DNA is indicated by ratio purity of ~1.8 (Hassan et al., 2015) and a concentration above 100 ng/µL (Aboul-Maaty & Oraby, 2019).

DNA Amplification

PCR amplification was performed using 44 RAPD primers were selected randomly and based on previous studies about identification of sex-specific bands in Arecaceae (Tabel 1). The PCR reaction consisted of 12.5 µL of MyTaq™ Red Mix Bioline, 2 µL of primer, 6.5 µL of nuclease-free water, and 4 µL of DNA. The PCR program included an initial denaturation at 95°C for 2 minutes, followed by 45 cycles of denaturation for 2 minutes, annealing at 34°C for 1 minute, extension at 72°C for 2 minutes, and a final extension at 72°C for 7 minutes. The amplification products were separated using 2% agarose gel electrophoresis in 10× TBE solution for 2 hours. Band visualization was performed with EtBr (0.5 µg/mL) and documented using the Uvitec

Gel Documentation system. A 100 bp DNA ladder was used as a fragment size marker.

Tabel 1. Selected RAPD Primers for PCR Amplification in *D. draco*

No.	Primer	Sequence (5' - 3')	Reference
1	OPA-01	CAGGCCCTTC	
2	OPA-02	TGCCGAGCTG	Dhawan et al., (2013); Mohammed and Mohamed, (2019)
3	OPA-03	AGTCAGCCAC	
4	OPA-04	AATCGGGCTG	
5	OPA-05	AGGGGTCTTG	
6	OPA-06	GGTCCCTGAC	
7	OPA-07	GAAACGGGTG	
8	OPA-08	GTGACGTAGG	
9	OPA-09	GTGATCGCAG	
10	OPA-10	GTCATCGCAG	Hassan et al., (2020)
11	OPA-11	CAATCGCCGT	Asra et al., (2018)
12	OPA-12	TCGGCGATAG	Younis et al., (2008)
13	OPA-13	CAGCACCCAC	
14	OPA-14	TCTGTGCTGG	
15	OPA-16	AGCCAGCGAA	
16	OPAU-1	GGGATGGAAC	
17	OPAU-2	CCAACCCGCA	Priya et al., (2019)
18	OPAU-3	ACGAAACGGG	
19	OPAU-4	GGCTTCTGTC	
20	OPAU-5	GAGCTACCGT	
21	OPAD-03	TCTCGCCTAC	
22	OPAD-09	TCGCTTCTCC	
23	OPO-10	TCAGAGCGCC	Asra et al., (2018)
24	OPO-17	GGCTTATGCC	Ediwirman, (2015)
25	OPC-06	GAACGGACTC	Al-Qurainy et al., (2018)
26	OPC-19	GTTGCCAGCC	Hassan et al., (2020)
27	OPB-08	GTCCACACGG	
28	OPB-10	CTGCTGGGAC	
29	OPB-18	CCACAGCAGT	Hassan et al., (2020)
30	OPE-18	GGACTGCAGA	Asra et al., (2018)
31	OPF-01	ACGGATCCTG	Hassan et al., (2020)
32	OPAP-20	CCCGGATACA	Ediwirman, (2015)
33	OPAQ-05	ACGGAGCTGA	Asra et al., (2018)
34	OPD-10	GGTCTACACC	Younis et al., (2008)
35	OPJ-09	TGAGCCTCAC	Mohammed & Mohamed, (2019)
36	OPW-18	TTCAGGGCAC	Promkaew et al., (2024)
37	OPZ-13	GACTAAGCCC	Asra et al., (2018)
38	UBC-499	GGCCGATGAT	Asra et al., (2018)
39	OPAC-11	CCTGGGTCAG	Li et al., (2017)
40	OPAC-12	GGCGAGTGTG	

No.	Primer	Sequence (5' - 3')	Reference
41	OPAC-15	TGCCGTGAGA	
42	OPAC-16	CCTCCTACGG	
43	OPAA-01	AGACGGCTCC	
44	OPAH-06	GTAAGCCCCT	

Data Analysis

Band scoring was performed based on the presence of amplified fragments as determined from band size estimation using the Uvitec Gel Documentation system. Bands with identical molecular sizes across samples were considered the same locus and evaluated visually. A band was considered sex-specific when it consistently appeared only in all male or only in all female individuals and was absent in the opposite sex under amplification with a given RAPD primer.

RESULT AND DISCUSSION

DNA Isolation Result

Based on the Nanodrop measurements, the average DNA concentration and purity of the *D. draco* isolate were 105 ng/µL and 1.8, respectively. A concentration of 105 ng/µL is sufficient for RAPD amplification, and A260/280 ratio of 1.8 indicates acceptable DNA purity. Ratios below this value generally suggest the presence of protein, phenol, or other contaminants. The CTAB method used during the lysis step of DNA extraction is effective for removing polysaccharides from plant cell samples when perform correctly (Xia et al., 2019). The abundant polysaccharides in plant cells can cause DNA to become physically dirty and affect the purity of DNA (Lopez et al., 2025).

DNA Amplification Analysis

Of the 44 RAPD primers screened, 39 successfully amplified *D. draco* DNA (Table 2), generating a total of 662 bands. Among these, 4 bands were specific to male individuals and 2 bands were specific to female individuals, while 63 bands were shared by both sexes and 593 bands were polymorphic. The primer that produced the highest number of bands was OPAU-01, generating 41 bands ranging from 300 to 3100 bp, followed by OPAU-03, which produced bands ranging from 170 to 1900 bp. All bands amplified from the polymorphic RAPD primers ranged from 40% to 100% polymorphic. The variation in DNA band sizes reflects differences in the length of target genomic regions amplified by the primers (Hefzi et al., 2023). In addition, sex-specific bands were identified from primers OPA-02, OPA-14, OPC-19, OPB-10, and OPD-10 (Table 1), indicating their potential for further development into sex-linked molecular markers.

Based on previous research on *Phoenix dactylifera*, three primers screened from 30 RAPD primers were successfully used for sex identification, one primer from screening 300 RAPD primers (Al-Qurainy et al., 2018), and one primer from screening 100 RAPD primers (Dhawan et al., 2013). Four primers that produced sex-specific bands were also found from screening 6 primers (Mohammad & Mohamed, 2019), and one primer from screening 44 RAPD primers (Promkaew et al., 2024). This study

used a primer selection approach that had previously successfully identified sex in the Arecaceae family, so that sex-specific bands in *D. draco* could be obtained with fewer RAPD primer screenings.

Table 2. Amplified bands with RAPD primers in male and female *D. draco* individuals

No	Primer	Number of Band	Size Range (bp)	Number of Specific Band			Number of Polymorphic Band	Poly-morphic (%)
				Male	Female	Both		
1	OPA-01	6	512-2098	0	0	0	6	100,0
2	**OPA-02**	**9**	**444-1116**	**1**	**0**	**2**	**6**	66,7
3	OPA-03	9	249-1326	0	0	4	5	55,6
4	OPA-05	10	288-1914	0	0	4	6	60,0
5	OPA-07	6	578-1266	0	0	0	6	100,0
6	OPA-08	7	427-1427	0	0	3	4	57,1
7	OPA-09	7	248-815	0	0	1	6	85,7
8	OPA-10	33	162-1726	0	0	0	33	100,0
9	OPA-11	24	50 – 2478	0	0	0	24	100,0
10	OPA-12	8	26-1218	0	0	0	8	100,0
11	OPA-13	9	505-1311	0	0	5	4	44,4
12	**OPA-14**	**7**	**475-1025**	**1**	**1**	**0**	**5**	71,4
13	OPAU-1	41	300-3100	0	0	0	41	100,0
14	OPAU-2	32	376-1440	0	0	0	32	100,0
15	OPAU-3	35	170-1900	0	0	0	35	100,0
16	OPAU-4	33	306-2830	0	0	0	33	100,0
17	OPAU-5	31	244-2371	0	0	0	31	100,0
18	OPAD-03	7	513-1593	0	0	1	6	85,7
19	OPAD-09	18	387-1438	0	0	0	18	100,0
20	OPO-10	22	281-3500	0	0	0	22	100,0
21	OPC-06	23	281-2269	0	0	2	21	91,3
22	**OPC-19**	**28**	**310-2010**	**1**	**0**	**1**	**26**	92,9
23	OPB-08	14	152-2293	0	0	5	9	64,3
24	**OPB-10**	**9**	**280-1059**	**0**	**1**	**2**	**6**	75,0
25	OPB-18	18	478-1641	0	0	2	16	88,9
26	OPE-18	28	240-1332	0	0	0	28	100,0
27	OPF-01	12	207-1511	0	0	3	9	75,0
28	OPAP-20	10	433-1074	0	0	1	9	90,0
29	OPAQ-05	23	308-1553	0	0	0	23	100,0
30	**OPD-10**	**5**	**785-1023**	**1**	**0**	**2**	**2**	40,0
31	OPJ-09	17	409-1486	0	0	1	16	94,1
32	OPW-18	6	317-1065	0	0	2	4	66,7
33	OPZ-13	26	312-1331	0	0	0	26	100,0
34	UBC-499	27	204-1771	0	0	3	24	88,9
35	OPAC-11	27	258-1581	0	0	3	24	88,9
36	OPAC-12	10	76-982	0	0	4	6	60,0
37	OPAC-15	3	362-2113	0	0	1	2	66,7
38	OPAA-01	9	337-1670	0	0	4	5	55,6
39	OPAH-06	7	208-1168	0	0	4	3	42,9
	Band Number	656		4	2	6	590	

Note: Bolded entries indicate RAPD primers that produced sex-specific bands

The primers with the highest potential for sex identification in dioecious *D. draco* were OPC-19, OPA-14, and OPB-10. It is because as shown in Table 3, primers OPA-14 and OPC-19 amplified all male individuals with 100% consistency, producing bands of 455 bp and 310 bp, respectively (Table 3; Figure 1 and Figure 2). In addition, primer OPB-10 amplified all female individuals with 100% consistency at 850 bp (Table 3; Figure 3). Such consistent and sex-specific amplification patterns indicate that these fragments are likely linked to genomic regions associated with sex determination. Previous studies have demonstrated that RAPD bands showing strict sex-specificity are often genetically linked to sex-determining loci and can be used as reliable markers for early sex identification (Qurainy et al., 2018; Zeng et al., 2024). Although RAPD markers are dominant and not strictly locus-specific, each band is generally treated as representing a DNA fragment from a particular genomic region, which may be closely associated with target traits such as sex (Rajesh et al., 2014; Patole et al., 2025).

Previous studies reported that primer OPC-19 generated male-specific bands of 882 bp and 748 bp (Hassan et al., 2020). However, to date, there have been no reports documenting the successful application of primers OPA-14 and OPB-10 for sex identification in dioecious plants. Therefore, the identification of sex-specific bands using these primers in *D. draco* represents a novel contribution and provides promising candidates for molecular-based sex determination in this species.

Table 2. Percentage of specific sex bands from amplification with RAPD primers

Primer	Male	Amplified Specific Band (%)	Female	Amplified Specific Band (%)
OPA-02	444 bp	83.33	-	-
OPA-14	455 bp	100	501 bp	83.33
OPC-19	310 bp	100	-	-
OPD-10	880 bp	83.33	-	-
OPB-10	-	-	850 bp	100

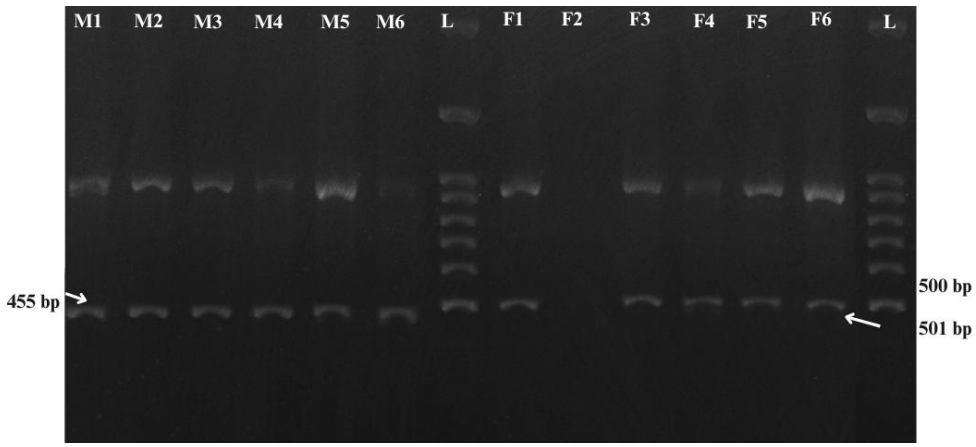

Figure 1. DNA fragments profile of *D. draco* with OPA-14. M1-M6: Male; F1-F6: Female; L: Ladder 100 bp

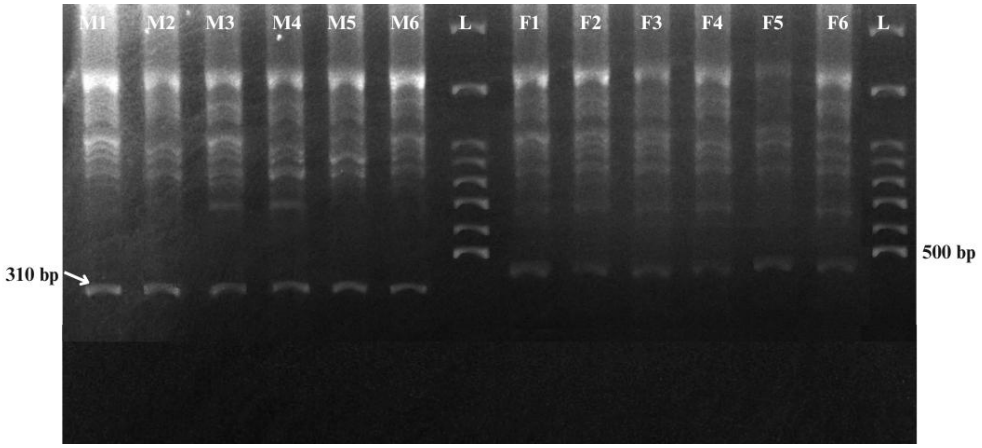

Figure 2. DNA fragments profile of *D. draco* with OPC-19. M1-M6: Male; F1-F6: Female; L: Ladder 100 bp

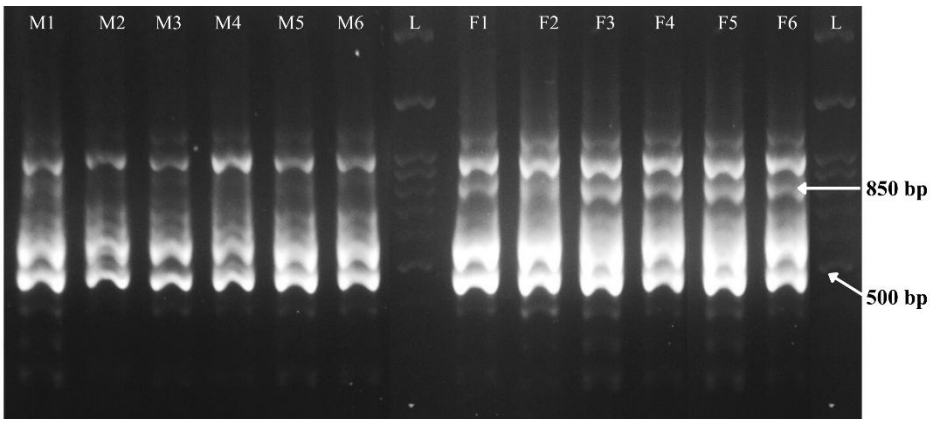

Figure 3. DNA fragments profile of *D. draco* with OPB-10. M1-M6: Male; F1-F6: Female; L: Ladder 100 bp

Sex-specific markers in dioecious plants play a crucial role in conservation and cultivation strategies by enabling balanced sex ratios and improving productivity (Choong & Wickneswari, 2016). In *D. draco*, morphological sex identification requires approximately 4–5 years until reproductive maturity (Asra et al., 2012), highlighting the importance of early molecular-based sex determination (Vaidya et al., 2014). The identification of male- and female-specific RAPD bands in this study provides a valuable foundation for the development of Sequence Characterized Amplified Region (SCAR) markers (Amiteye, 2021). Therefore, further research is required to clone and sequence these candidate bands, followed by their conversion into SCAR markers, in order to enhance the specificity, reproducibility, and accuracy of sex identification at the seedling stage of *D. draco*.

CONCLUSION

From the screening of 44 RAPD primers, 39 successfully amplified a total of 656 bands. Four male-specific and two female-specific bands were identified; however, only OPA-14 (455 bp) and OPC-19 (310 bp) showed 100% consistency in males, while

OPB-10 (850 bp) was consistent in females. These bands are recommended for development into SCAR markers for accurate sex identification at the seedling stage of *D. draco*. This study represents the first report of sex-specific bands in *D. draco* and provides a basis for improving its conservation and cultivation programs.

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