JJBS

Volume 17, Number 1,March 2024
ISSN 1995-6673
Pages 163 – 171
https://doi.org/10.54319/jjbs/170116

Jordan Journal of Biological Sciences

Genetic Diversity and Relationship among and within the Bird of Paradise Plants of Strelitziaceae and Heliconiaceae

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Received: June 17, 2023; Revised: August 9, 2023; Accepted: August 26, 2023

Abstract

Bird of paradise plants belong to families Strelitziaceae and Heliconiaceae, two close-related families in the Zingiberales order. This study aimed to study the genetic diversity and relationship of species among and within Strelitziaceae and Heliconiaceae using molecular RAPD markers and compare them to morphological characters. Samples used comprised two species from Strelitziaceae and eight species from Heliconiaceae. Twenty OPA primers showed that 17 out of 20 OPA primers were successfully amplified. Primer OPA-10 was the most informative and recommended, followed by OPA-5, OPA-7, OPA-9, OPA-11, OPA-17, OPA-18, OPA-19, and OPA-20. Heliconiaceae and Strelitziaceae were high genetic diversity (P=100%, I=0.388±0.016, He=0.237±0.013) with low similarities (0.09-0.39). Heliconiaceae was more genetically diverse than Strelitziaceae. Heliconiaceae produced P=98.88%, I=0.409±0.017, and He=0.254±0.014. Strelitziaceae produced P=60.53%, I=0.366±0.049, and He=0.251±0.033. The tree topology based on the RAPD marker differs slightly from morphology. However, the morphological characteristics are important to support and complement it. Strelitziaceae was strongly separated from Heliconiaceae, which supports the monophyly of both families. Heliconiaceae was separated into two subclusters and paraphyletic. Subcluster 1 comprises subgenera *Heliconia* and *Stenochlamys*. Subcluster 2 comprises subgenera *Griggsia* and *Stenochlamys*. In conclusion, the molecular RAPD marker proved the powerful of classification at the family level but moderate at subgenus, section, and species levels. Nonetheless, it is considered a simple and valuable method to provide a primary reference for taxonomic delimitation, conservation and breeding efforts of the bird of paradise plants in the future.

Keywords: RAPD, Phylogenetic, Polymorphism, Molecular taxonomy, Variability

1. Introduction

Strelitziaceae and Heliconiaceae are close-related families belonging to the order Zingiberales (Simpson, 2006; Malakar et al., 2022) and are known as the bird of paradise plants. Strelitziaceae originated in South Africa. This plant was introduced to Europe in 1770, with distribution in Africa, Madagascar and South America (Prince and Kress, 2002; Vieira et al., 2012). Meanwhile, Heliconiaceae originated in Tropical America and Melanesia (Andersson, 1998). The diversity centers of *Heliconia* are located in tropic and subtropic regions of South America and Central America (Cronquist, 1981). However, the current distribution of *Heliconia* is found in many tropical countries in the world, including Brazil (Marouelli et al., 2010), Colombia (Isaza et al., 2012), Mexico (Avendaño-Arrazate et al., 2017), Indonesia (Hapsari et al., 2019). Several species of both families are used as ornamental plants for garden landscapes and cut flowers. They are utilized commonly for unique inflorescence and leaf characteristics; including size, shape, arrangement and color (Malakar et al., 2022).

The species member of Strelitziaceae is limited, consisting of three genera *Strelitzia*, *Phenakospermum* and *Ravenala*. The genus *Strelitzia* has five species of *S. alba*, *S. nicolai*, *S. caudata*, *S. reginae* and *S. juncea* (Cron et al., 2012). The genera *Phenakospermum* and *Ravenala* have only one species, i.e., *P. guyanense* and *R. madagascariensis*, respectively (Santos et al., 2009). Heliconiaceae has a single genus (monotypic), namely *Heliconia*, and has many species of approximately 250-300 (Kress, 1990). Furthermore, a cladistic morphological analysis divided *Heliconia* into five subgenera, i.e. *Heliconia*, *Taeniostrobus*, *Heliconiopsis*, *Stenochlamys* and *Griggsia*, and 23 sections (Kress, 1984; Kress, 1990).

Strelitziaceae and Heliconiaceae are flowering perennial herbaceous plants. They share many common morphological characteristics such as sympodial rhizomes, banana-shaped leaves and distichous inflorescences (Marouelli et al., 2010; Santos et al., 2009). However, the plant size of Strelitziaceae is larger than Heliconiacae, ranging from perennial herbs to trees. Strelitziaceae flowers enclose in a bract which is a leaf-like structure with two sheaths. Most species have large paddle-shaped leaves (Vieira et al., 2012). Likewise, the inflorescences of Heliconiaceae have the shape of boat-like or dangling

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bract. Previous morphology character analysis showed that both families were clearly distinguished. Strelitziaceae is differentiated due to its palm-like trunk and leaves with the appearance of a fan. The separation within Heliconiaceae was paraphyletic with overlapping morphology characteristics (Kholqiyah et al., 2022). Morphology approaches are considered less accurate due to subjectivity and environmental influences (Probojati et al., 2019; Amer et al., 2022). Therefore, molecular applications are very significant techniques to get more information about their genetic diversity and relationship to confirm the morphology result.

Previous genetic and diversity studies have been reported on Heliconiaceae and Strelitziaceae using some molecular applications. Isaza et al. (2012), using Amplified Fragment Length Polymorphism (AFLP), revealed 170 bands with an overall average of polymorphism was 34.34%. Two well-defined groups of *Heliconia* were produced, with *S. reginae* serving as an outgroup. Meléndez-Ackerman et al. (2005), focusing on the genetic population of *H. bihai* in the Caribbean islands, showed high levels of AFLP polymorphism. About 244 loci were detected, with an average polymorphic percentage up to 73%. Meanwhile, Kumar et al. (1998) was reported using Random Amplified Polymorphic DNA (RAPD) for phylogenetic and identification studies of 16 *Heliconia* cultivars. The results showed they were closely related and suggested that some accessions were the same genotype. Likewise, Sheela et al. (2006) and Marouelli et al. (2010) reported the high RAPD polymorphism and demonstrated the monophyletic of Heliconiaceae separated from Strelitziaceae and other families in the Zingiberales. Molecular applications using DNA sequencing methods also have been reported, including Kress et al. (2002), Cron et al. (2012); Iles et al. (2017), Hapsari et al. (2019), and others..

Nevertheless, the RAPD method is considered more economical and simple, yet accurate enough to study the genetic diversity and relationship of closely related families compared to other molecular methods (Probojati et al. 2019; Trimanto et al. 2023). RAPD is considered adequate genotype identification and analysis of genetic differences among induced mutants (Wahyudi et al. 2020). In addition to Heliconiaceae and Streltiziceae, RAPD has been popularly applied at inter- and intra- specific levels of various plant species, such as wheat and barley (Ghabeish et al., 2021), rice (Sholikhah et al., 2019), ramie (Mayerni et al., 2019), legumes (Purwanti et al., 2021), pea (Osman and Ali, 2021), orchid (Hartati and Samanhudi, 2022), kalanchoe (Al-Khayri et al., 2022), banana (Probojati et al., 2019; Wiguna and Pharmawati, 2021), ginger (Trimanto et al. 2023), and many more. RAPD is a DNA amplification technique based on PCR using random primers and generally consists of 10 arbitrary base sequences with a minimum content of G and C bases of 60%. The RAPD is an easy technique, a fast process, with no need for prior genomic information, and uses a small DNA quantity (approximately 0.5-50 ng). In contrast, the RAPD is a low repetition rate. It can be overcome with the appropriate consistency of PCR conditions, especially the temperature when the primer attaches to the DNA template (Agisimanto and Supriyanto, 2007; Probojati et al., 2019; Zulfahmi et al., 2023).

Hence, the objective of this present study was to study the genetic diversity and relationship of species among and within the bird of paradise plants of Strelitziaceae and Heliconiaceae using RAPD markers. In addition, this study also aimed to compare the genetic relationship tree between molecular RAPD and morphology from Kholqiyah et al. (2022). Understanding genetic diversity and the relationship of close-related plant species is essential for taxonomic purposes and also contributes to the conservation strategy setting and further breeding efforts (Trimanto et al., 2023). Meanwhile, studies on the genetic diversity, genetic relationship, and genetic distance among and within Strelitziaceae and Heliconiaceae from Asian countries using RAPD markers are still limited. The findings of this study would benefit as a primary reference in taxonomic delimitation, setting priority for genetic conservation and germplasm breeding, and support for further related research and development of the bird of paradise plants.

2. Material and Methods

2.1. Plant materials

Ten species of the bird of paradise plants belonging to Strelitziaceae (two species) and Heliconiaceae (eight species) were used, as presented in Figure (1) and Table (1). The plant's specimens were derived from the ex-situ living collection of Purwodadi Botanic Garden, National Research and Innovation Agency located in Pasuruan Regency, East Java Province, Indonesia. The plant collections had been obtained from exploration and collecting missions, material exchanges, and donations from other botanic gardens, communities, or personals (Lestarini et al., 2012). The species identification referred to books identification guides of Heliconiaceae and Strelitziaceae (Arnold 2013; Hintze 2014); protologues of some type species, including Flora Peruviana , Flora de Brasilia, Flora of Guatemala, Flora of Java, and Flora Mesoamericana; scientific journals, and online databases including *Heliconia* Society Puerto Rico (http://www.heliconiasocietypr.org/), Plants of the world online of Kew Science (http://www.plantsoftheworldonline.org), Smithsonian Tropical Research Institute (https://biogeodb.stri.si.edu/), Centre for Agriculture and Bioscience International (https://www.cabi.org/), and Fairchild Botanic Garden (https://www.fairchildgarden.org/).

Figure 1. Plant specimens of Strelitziaceae and Heliconiaceae examined. S1=R. madagascariensis, S2=P. guyannense, H1=H. bihai, H2=H. wagneriana, H3=H. collinsiana, H4=H. chartacea, H5=H. rostrata, H6=H. metallica, H7=H. hirsuta, H8=H. psittacorum.

Table 1. Species list of Strelitziaceae and Heliconiaceae examined

Code	Species name	Subgenus	Section	Collection source
S1	*Ravenala madagascariensis* Sonn.	-	-	Malagasy
S2	*Phenakospermum guyannense* (A.Rich.) Endl. ex Miq.	-	-	Brazil
H1	*Heliconia bihai* (L.) L.	*Heliconia*	*Heliconia*	East Java
H2	*Heliconia wagneriana* Petersen	*Heliconia*	*Heliconia*	East Java
H3	*Heliconia collinsiana* Griggs	*Griggsia*	*Pendulae*	Guatemala
H4	*Heliconia chartacea* Lane ex Barreiros	*Griggsia*	*Pendulae*	Brazil
H5	*Heliconia rostrata* Ruiz & Pav.	*Griggsia*	*Rostratae*	Trop. America
H6	*Heliconia metallica* Planch. & Linden ex Hook.	*Stenochlamys*	*Cannastrum*	Brazil, Venezuela
H7	*Heliconia hirsuta* L. f	*Stenochlamys*	*Zingiberastrum*	Seram Island, Mollucas
H8	*Heliconia psittacorum* L.f.	*Stenochlamys*	*Stenochlamys*	Malagasy

2.2. DNA extraction

The fresh leaf shoot of each species was mashed using a mortar and pestle in liquid nitrogen. The whole genome DNA was isolated using a DNA isolation kit (Promega Wizard®) following the guidelines for plants. The extracted DNA was patterned qualitatively using electrophoresis agarose gel 0.8% in TBE buffer and quantitatively to check the concentration and purity level using a spectrophotometer Nano-drop (ND-1000) at 260 and 280 nm of wavelengths.

2.3. RAPD-PCR

Twenty OPA primers from Operon Technologies were used, as presented in Table (2). The reaction mixture was carried out in a PCR tube with a 10 µL total volume consisting of 1 µL (25 ng) DNA sample, 1 µL (10 µM) primer, 5 µL DreamTaq DNA polymerase (2x DreamTaq Green Buffers; 0.4 mM dNTPs and 4 mM MgCl2), and 3 µL nuclease-free water. The thermal cycle protocol comprised 40 cycles of denaturation at 92^{o}C for 1 minute, followed by annealing temperature of each RAPD primer (Probojati et al., 2019), extension at 72°C for 2 minutes and post extension at 72°C for 7 minutes. The PCR products were confirmed using electrophoresis agarose gel 1% in TBE buffer and visualized on a Gel Doc/UV trans-illuminator (BioRAD) using a 100 bp DNA ladder marker.

Table 2. List of the RAPD primers used in this study

Primer	Sequence (5'-3')	MT(°C)	AT(°C)	GC Composition (%)
OPA 1	CAG GCC CTT C	36.40	41	70
OPA 2	TGC CGA GCT G	40.70	45	70
OPA 3	AGT CAG CCA C	34.30	39	60
OPA 4	AAT CGG GCT G	35.10	40	60
OPA 5	AGG GGT CTT G	32.60	37	60
OPA 6	GGT CCC TGA C	35.20	40	60
OPA 7	GAA ACG GGT G	33.20	38	60
OPA 8	GTG ACG TAG G	31.10	36	60
OPA 9	GGG TAA CGC C	37.40	42	70
OPA 10	GTG ATC GCA G	33.10	38	60
OPA 11	CAA TCG CCG T	36.70	41	60
OPA 12	TCG GCG ATA G	34.00	39	60
OPA 13	CAG CAC CCA C	37.70	42	70
OPA 14	TCT GTG CTG G	34.30	39	60
OPA 15	TTC CGA ACC C	34.20	39	60
OPA 16	AGC CAG CGA A	38.30	43	60
OPA 17	GAC CGC TTG T	35.70	40	60
OPA 18	AGG TGA CCG T	36.20	41	60
OPA 19	CAA ACG TCG G	34.20	39	60
OPA 20	GTT GCG ATC C	33.50	38	60

Notes: MT = Melting temperature, AT = Annealing temperature, GC = Guanine and Cytosine.

2.4. Data analysis

The amplified products of each primer are used to construct a binary matrix, as (0) if absent or (1) if present. They are used to estimate the level of DNA polymorphism, marker informativeness, genetic diversity, and the clustering pattern of genetic relationship. RAPD polymorphisms were analyzed including the total number of bands (TNB), number of polymorphic bands (NPB), and percentage of polymorphic bands (PB). The marker informativeness of each RAPD primer was analyzed using four parameters, i.e. polymorphic information content (PIC), effective multiplex ratio (EMR), marker index (MI), and resolving power (RP). The formulas of each parameter are as follows:

$$PIC = 2f(1-f)$$

Where, f is the frequency of present bands, and 1-f is the frequency of absent bands.

$$EMR = np(\frac{np}{n})$$

Where, n is the total number of present bands, and np is the number of polymorphic bands.

$$MI = PIC\ X\ EMR$$

$$RP = 1 - [2x(0.5-p)]$$

Where, p is the proportion of species containing present bands.

The derived data were fed into GenAlEx v.6.5 (Peakall and Smouse, 2012) to estimate the genetic diversity. Other genetic parameters, including the percentage of polymorphic loci (P), Shannon's index (I), expected heterozygosity (He), and unbiased expected heterozygosity (uHe) were calculated. Hierarchical clustering was determined by a genetic relationship tree using PAST3 (Hammer et al., 2001). RAPD binary matrix was treated as a single data, using the UPGMA algorithm and Jaccard index with 1000 bootstraps. Bootstrap support (BS) was categorized as high confidence if BS >85%, moderate confidence if BS = 70-85%, low confidence if BS = 50-69%, and very low confidence if BS <50% (Kress et al., 2002).

3. Results

3.1. RAPD polymorphisms and marker informativeness

RAPD amplifications showed that seventeen primers produced 97 bands through all studied ten samples with a range of 150-1400 bp, as shown in Figure (2). The average number of polymorphic bands was about six bands. Primer OPA-10 revealed the highest polymorphic bands (13), while OPA-2 and OPA-13 produced the most minor polymorphic bands (1) (Table 3). The seventeen primers of RAPD, as a result of primary screening, were considered efficient for amplifying Strelitziaceae and Heliconiaceae (Figure 2, Table 3).

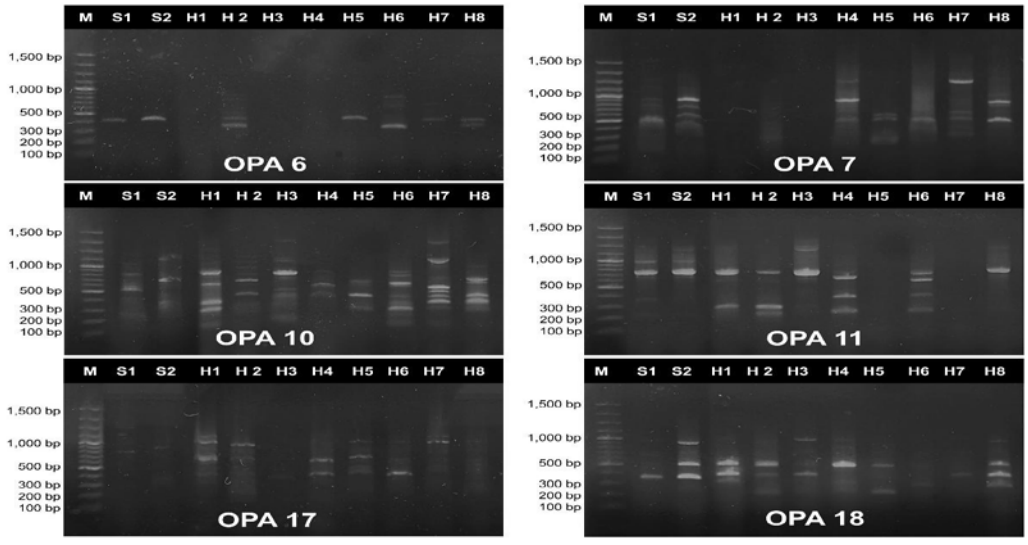

Figure 2. RAPD amplification profiles of OPA 6, OPA 7, OPA 10, OPA 11, OPA 17 and OPA 18.

PIC is a standard genetic parameter to evaluate the results of PCR amplification of genetic markers based on DNA bands (polymorphic). PIC value ranges from 0.00 to 1.00. The high value of PIC (>0.50) indicates that a primer is highly informative and discriminatory power, best for detecting the genetic variation (Anderson et al., 1993). PIC value results from this study ranged from 0.20-0.50, with an average of 0.37, which classified as moderatately informative power. The least PIC was scored 0.20 by the primer OPA-9. The highest value was 0.50 by the primer OPA-18. EMR is a genetic parameter used to determine the overall number of DNA bands formed in each primer and the number of polymorphic DNA bands (Medhi et al., 2014). The EMR values ranged from 1 to 169, with an average of 40.41 (Table 3). The primer OPA-2 and OPA-13 scored minimum EMR values (1), while the maximum EMR value scored 169 for the primer OPA-10 (Table 3). MI is a genetic parameter used to determine the index of a primer in producing polymorphic DNA bands (Varshney et al., 2007). MI values ranged from 0.32 to 76.05, with an average of 15.76. The least MI value was 0.32 for the primer OPA-2 and the highest was 7.05 by OPA-10 (Table 3). The RP is used to determine the most informative primers to differentiate DNA bands between genotypes (Prevost and Wilkinson et al., 1999). The values of RP ranged between 0.40 and 9.00, with an average of 3.16. The primer OPA-2 produced the least RP value, while the highest value was the primer OPA-10 (Table 3).

Table 3. RAPD polymorphisms and marker informativeness analysis results

Primer	TNB	NPB	PB	PIC	EMR	MI	RP
OPA 1	5	5	100	0.30	25	7.50	1.80
OPA 2	1	1	100	0.32	1	0.32	0.40
OPA 3	6	6	100	0.30	36	10.80	2.20
OPA 4	3	3	100	0.23	9	2.07	0.80
OPA 5	7	7	100	0.41	49	20.09	4.00
OPA 6	6	6	100	0.39	36	14.04	3.20
OPA 7	9	9	100	0.39	81	31.59	5.40
OPA 8	4	4	100	0.26	16	4.16	1.20
OPA 9	8	8	100	0.20	64	12.80	1.80
OPA 10	13	13	100	0.45	169	76.05	9.00
OPA 11	7	7	100	0.46	49	22.54	5.00
OPA 13	1	1	100	0.42	1	0.42	0.60
OPA 15	5	5	100	0.34	25	8.50	2.20
OPA 17	6	6	100	0.46	36	16.56	4.20
OPA 18	5	5	100	0.50	25	12.50	4.60
OPA 19	7	7	100	0.41	49	20.09	4.00
OPA 20	4	4	100	0.49	16	7.84	3.40
Total	97	97	1,700	6.33	706	275.28	53.40
Average	5.71	5.71	100	0.37	40.41	15.76	3.16

Notes: NB=Total number of present bands, NPB=Number of polymorphic bands, PB=Percentage of polymorphic bands, PIC=Polymorphic information content, EMR=Effective multiplex ratio, MI=Marker Index, and RP=Resolving Power.RAPD genetic diversity

The genetic diversity parameters analyzed from the RAPD results show that Heliconiaceae and Strelitziaceae have high genetic diversity. Heliconiaceae was more genetically diverse than Strelitziaceae in terms of polymorphic bands, Shannon index, and heterozygosity as shown in Table 4.

Table 4. RAPD genetic diversity analysis results. Data is mean value±standard error

Diversity parameters	Strelitziaceae	Heliconiaceae
No. of sample	2	8
No. of polymorphic bands	38	89
% polymorphic bands (P)	60.53	98.88
Shannon's index (I)	0.366±0.049	0.409±0.017
Expected heterozygosity (He)	0.251±0.033	0.254±0.014
Unbiased expected heterozygosity (uHe)	0.334±0.044	0.271±0.015

3.2. RAPD genetic relationship compare to morphology

Ten species of the bird of paradise plants examined in this study were clustered into two large groups following their families with low genetic similarities on both molecular RAPD and morphology methods, as shown in Figure 3. The range of genetic similarities based on RAPD was less than morphology, i.e., 0.09-0.39 and 0.11-0.74, respectively. Strelitziaceae (*R. madagascariensis* and *P. guyannense*) were separated as root (primitive relatives) at a similarity of 0.39 and supported by strong bootstrap values (100).

Furthermore, the tree topology within Heliconiaceae based on molecular RAPD (Figure 3A) was slightly different from morphology (Figure 3B). The molecular RAPD method produces two subclusters meanwhile the morphology method produces three subclusters. In the detail of RAPD genetic relationship within Heliconiaceae, cluster 1 comprised of subgenus *Griggsia* (*H. collinsiana*, *H. chartacea*, and *H. rostrata*) and subgenus *Stenochlamys* (*H. hirsuta* and *H. psittacorum*) at a similarity of 0.21; and

cluster 2 comprised of subgenus *Heliconia* (*H. bihai* and *H. wagneriana*) and subgenus *Stenochlamys* (*H. metallica*) at a similarity of 0.20. Cluster 1 of morphology clustering consisted of subgenus *Griggsia* at a similarity of 0.30; cluster 2 comprised of subgenera of *Heliconia* and *Stenochlamys* at a similarity of 0.35; and cluster 3 comprised of *H. metallica* at a similarity of 0.23 (Figures 3A-B). However, both methods revealed that the separation within the subgenera of Heliconiaceae was unclear and paraphyletic.

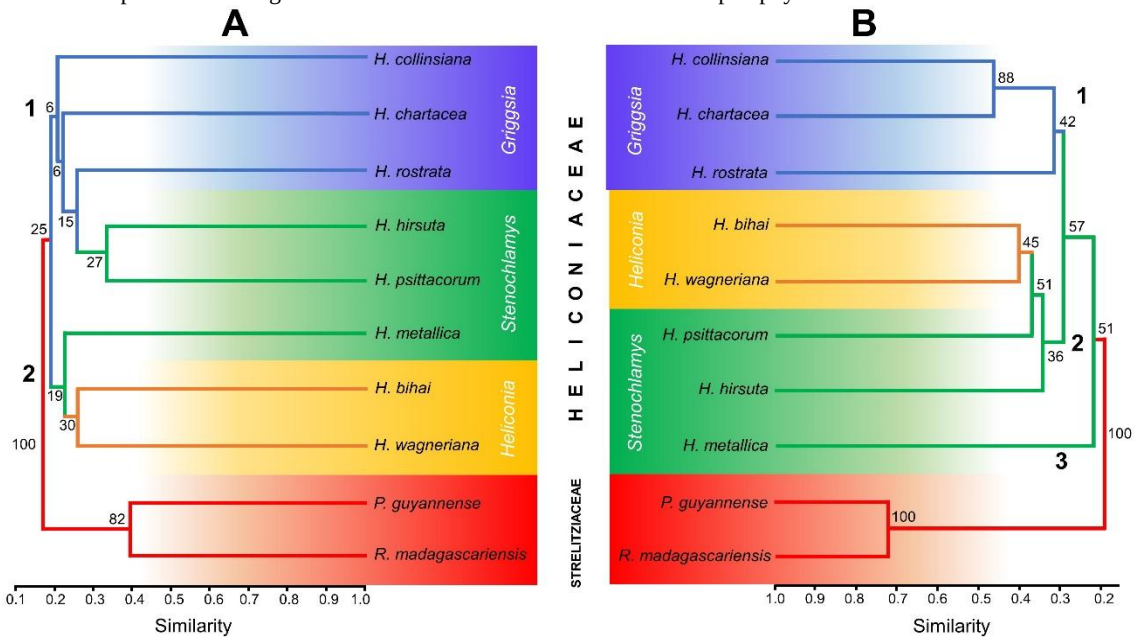

Figure 3. Dendogram relationship trees: A. based on molecular RAPD (this study); and B. based on morphology (Kholqiyah et al., 2022).

4. Discussion

The genetic diversity could be analyzed using the number of polymorphic bands. It categorizes as high if it is >50% (Hamrick and Godt, 1996). In this study, RAPD produced 100% polymorphic bands with different molecular positions across the samples. It indicates a high genetic diversity among and within species in Strelitziaceae and Heliconiaceae. Some of the species showed the absence of DNA bands (Figure 2). It may be because of a mismatch between samples and primer sequences during the annealing process. Another reason is the considerable molecular weight of the DNA fragments so that the bands fail to appear (Probojati et al., 2019; Agisimanto and Supriyanto, 2007).

A more detailed characterization of the DNA bands reveals that several primers produced unique bands at specific lengths. In this study, unique bands were found in both families. Primers OPA-4 (900 bp), OPA-11 (1000 bp) and OPA-19 (500, 600, and 1000 bp) resulted in unique bands that were only present in Strelitziaceae but absent in Heliconiaceae. Meanwhile, unique bands are found only in Heliconiaceae (subgenus *Heliconia*), namely primer OPA-3 (300 and 400 bp). The unique DNA bands from specific primers are considered as autapomorphic characters that could become a discriminant characters of that particular taxon (Assis, 2017).

Overall, RAPD marker informativeness analysis results showed that the most effective primer was OPA-10. The primer OPA-10 has high values at four parameters observed among other primers. The EMR, MI, and RP values of OPA-10 were the highest over the primers 169, 76.05, and 9.00, respectively. The PIC value of OPA-10 ranked fifth, with a high value of 0.45 (Table 3). Therefore, it could recommend the implication of OPA-10 as an effective RAPD primer for further similar genetic diversity studies on Strelitziaceae and Heliconiaceae, as well as Zingiberales in general. In addition, other effective primers recommended include OPA-5, OPA-7, OPA-9, OPA-11, OPA-17, OPA-18, OPA-19 and OPA-20.

The heterozygosity value for genetic diversity of the group of the taxon classified as high if He >0.20 (Hamrick and Godt, 1996). Shannon's information index values of Strelitziaceae (I=0.366±0.049) and Heliconiaceae (0.409±0.017) were classified as high. Hence, Heliconiaceae was more genetically diverse than Strelitziaceae. It is also supported by the fact that the species belonging to Strelitziaceae in the world are limited to only seven species (Santos et al., 2009; Cron et al. 2012), indicating that their genetic diversity is not as wide as that of Heliconiaceae which has reached 300 species. Furthermore, the genetic diversity of both families were found higher than previous studies on *Alpinia* spp. with I=0.350±0.040 (Basak et al., 2019) and *Musa* spp. with I=0.340±0.240 (Resmi et al., 2016), but lower than *Etlingera* spp. with I=0.417±0.011 (Trimanto et al., 2023) and *Curcuma* spp. with I=0.530±0.190 (Das et al., 2011).

Clustering analyses on molecular RAPD and morphology methods showed that Strelitziaceae were separated from Heliconiaceae as an outgroup and supported by strong bootstrap values of 100 (Figures 3A-B). An outgroup is important in phylogenetic inference to determine the polarity or direction of evolution (Kress et al., 2002). Therefore, this study supports the monophyly of both families, with Strelitziaceae as the primitive relative of Heliconiaceae in accordance to some previous phylogenetic studies (Sheela et al., 2006; Marouelli et al., 2010; Iles et al., 2017; Hapsari et al., 2019). Furthermore, both methods revealed that the separation within the subgenera of Heliconiaceae was unclear and paraphyletic. However, the previous study using RAPD markers showed

that subgenus *Heliconia* was monophyletic and subgenus *Stenochlamys* was polyphyletic (Marouelli et al., 2010). Compared to another AFLP marker study, Isaza et al. (2012) proved that the subgenus *Heliconia* is broadly monophyletic, while subgenus *Stenochlamys* falls in different clades and polyphyletic. Further, subgenus *Griggsia* could be paraphyletic, due to the presence of species from other subgenera in the same cluster. Thus, from this study, the RAPD application proved the power to classify at the family level (between Strelitziaceae and Heliconiaceae), but it is moderate at the subgenus, section, and species levels. Nonetheless, it is considered a simple and valuable molecular marker.

The relationship tree topology based on molecular RAPD differs slightly with morphology. However, the morphological characteristics are significant to support and complement it. The morphological characterization from the previous study (Kholqiyah et al. 2022; Figure 1) supports the tree separation pattern of the RAPD molecular (Figure 3A). *R. madagascariensis* and *P. guyannense* of the Strelitziaceae was separated from Heliconiaceae due to several synapomorphic characters, including banana-like (musoid) and palm-like leaf growth types, light green pseudostem without blotches and wax, fan-like petiole arrangements, light green ligule color and stiff-boat shaped bracts. Within Heliconiaceae, subcluster 1 comprises subgenera *Griggsia* and *Stenochlamys*. The three species of subgenus *Griggsia* morphologically have a distinguishing character of pendant inflorescence, whereas *H. hirsuta* and *H. psittacorum* are members of subgenus *Stenochlamys* have several morphological similarities, including distichous bracts with shallow and narrow boat shaped, the leaf not ripped and the ligule green with brown blotches and not waxy. Subcluster 2 comprises subgenera *Heliconia* and *Stenochlamys*. Two species of *H. bihai* and *H. wagneriana* had synapomorphic characters, including musoid leaf growth type, green pseudostem with brown blotches and waxy, oblique leaf base shape, green ligule with brown blotches, peduncle and rachis glabrous and deeply boat-shaped bracts. Meanwhile, *H. metallica* (subgenus *Stenochlamys*) was nested probably due to the presence of autapomorphic characters, namely pseudostem with brown-purplish blotches, Canna-like (cannoid) leaf growth type, the leaf blade is dark green-purplish (adaxial) and light green-purplish (abaxial), acute leaf base shape with dry and brown ligule.

The results from this study have provided important information on genetic diversity and relationship, among and within Strelitziaceae and Heliconiaceae. It could be utilized as a primary reference for the taxonomy, conservation and breeding efforts of Strelitziaceae and Heliconiaceae germplasm. Ex-situ conservation plays a role in maintaining the existence of valuable germplasm. It includes protecting species from extinction and environmental changes (Rachmat et al., 2020). In this study, Strelitziaceae with lower genetic diversity needs more ex-situ conservation efforts. The species member of this family is limited (only seven species recognized) (Santos et al., 2009; Cron et al., 2012) and rarely found in Indonesia (native to South Africa), thus material exchange between botanical gardens to increase the diversity of ex-situ collections is the most suitable strategy. Meanwhile, Heliconiaceae with high genetic diversity has a prioritization in conservation management. Several Heliconiaceae species comprised invasive species and weeds, including *H. bihai*, *H. wagneriana*, *H. latispatha* and *H. psittacorum*, so proper management of ex-situ collection is needed (Hapsari et al., 2019). In addition, propagation and further breeding efforts on *Heliconia* species suggested for ornamental purposes. Artificial hybridization through inter-species in *Heliconia*, particularly pairs of species with low genetic similarity as shown in Figure 3, makes it possible to produce hybrids with the desired characteristics (Malakar et al., 2022).

5. Conclusion

Molecular RAPD applications reveal that genetic diversity and relationship among and within bird of paradise plants Strelitziaceae, and Heliconiaceae had high polymorphism and low similarity. In this study, the characterization of DNA bands led to unique bands on several primers and base length (bp) in both families. The genetic relationship was separated Strelitziaceae from Heliconiaceae. Heliconiaceae split into two subclusters, and the division of subgenera is paraphyletic. The tree topology based on RAPD markers was slightly different with morphology. RAPD marker was powerful in classifying at the family level between Strelitziaceae and Heliconiaceae. However, it was moderate within families, subgenus, section, and species levels. Nine RAPD primers are recommended. Further study is suggested to increase the validity and significance by using taxonomically diverse species from both Strelitziaceae and Heliconiaceae.

Acknowledgements

The authors would like to acknowledge Purwodadi Botanic Garden, National Research and Innovation Agency for the plant materials examined; also thanks go to the Griya Sains Laboratory for the molecular facilities. All authors (SFK, DW, LH) contributed equally as main authors in this manuscript including research design, field sampling and laboratory works, data compilation and analysis, data interpretation, writing the original manuscript, and the review and editing of the final manuscript.

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