BIODIVERSITAS
Volume 24, Number 6, June 2023
Pages: 3550-3558

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240654

Morphological relationships and cross compatibility of seven *Dendrobium* species in Indonesia

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Manuscript received: 7 March 2023. Revision accepted: 27 June 2023.

Abstract. *Lestari NKD, Deswiniyanti NW, Sari NKY, Murna IM, Rizqy AN. 2023. Morphological relationships and cross compatibility of seven* Dendrobium *species in Indonesia. Biodiversitas 24: 3550-3558.* The development of *Dendrobium* hybrid orchids in Indonesia has received less attention. This study aimed to obtain the morphological characteristics and taxonomical relationships of *Dendrobium* species, their interspecific and intersectional compatibility for hybridization, as well as the fertility of hybridized *Dendrobium* orchids. Seven *Dendrobium* species from several sections were used, namely *D. phalaenopsis, D. lineale, D. stratiotes, D. macrophyllum, D. nobile, D. heterocarpum* and *D. bracteosum*. We characterized the seven species based on morphological traits and investigated the taxonomical relationships using hierarchical cluster analysis by dendrogram charts. We then crossed the seven species to produce hybrids and the hybrids were then cultured in vitro. The hybridization and in vitro culture data were analyzed using an ANOVA test with Duncan's post hoc test when the results significantly differed. The results showed that the taxonomical relationship was clustered based on the sections of each *Dendrobium* species. The highest percentage of hybridization compatibility was found in *D. phalaenopsis* and *D. lineale* with 61.9% while the lowest was in *D. nobile,* and *D. bracteosum* (0%). Germination percentages in in vitro culture ranged from 40% to 100% for both interspecific and intersectional crosses, except for intersectional crosses of *D. nobile,* which failed to germinate. The findings of this study suggest that the results of interspecies and intersectional crossing showed that the highest compatibility was indicated by *D. phalaenopsis* and *D. lineale*. Therefore, they are highly recommended as the parents of hybrid orchids.

Keywords: Characterization, compatibility, dendrogram, morphological, orchid

INTRODUCTION

Dendrobium is a genus of orchids (Orchidaceae family) with diverse variations in the shape and color of the flowers. The *Dendrobium* genus is epiphytic and has unique characteristics with segmented stems and pseudo tubers (Dehgahi and Alireza 2017). In Indonesia alone, there are 275 species from the genus of *Dendrobium*. Many species of orchids from this genus have the potential as a source of breeding for ornamental plants, both as cut flowers and potted plants, and are prospective for medicinal purposes (Teixeira et al. 2014; Li et al. 2020).

The genus of *Dendrobium* is divided into several sections based on the shape of the flowers and stem. The sections that are widely used as parents to produce popular hybrids in Indonesia include Ceratobium/Spatulata, Palaenanthe, Eugenanthe, and Callista. The Ceratobium or Spatulata section originates from eastern Indonesia and includes *Dendrobium antennatu* Lindl., *D. bicaudatum* Reinw. ex Lindl., *D. stratiotes* Rchb.f., and *D. lineale* Rolfe. (Clement 2014; Metusala 2019; Arobaya et al. 2022).

Improving the quality of *Dendrobium* orchids can be achieved through hybridization techniques by paying attention to the characters of the flowers of the cross parents. The determination of parental orchids for hybridization is based on the desirable traits to be inherited into the hybridized generation of new orchid plant varieties. Nonetheless, the use of native orchid species as cross parents has not been optimal due to the lack of studies on the inheritance of characteristics of native Indonesian orchid species and their relationships (Hartati et al. 2022).

Hybridization or crossing techniques are used to produce new cultivars, which is the main goal in developing new orchid varieties with desirable traits such as plant shape, flower shape, flower color, size, bloom resistance, aroma, quantity of flower number, and resistance to pests (Mahfut et al. 2021). Among the sections in *Dendrobium*, Spatulata is recommended as the male parent since it demonstrates the desirable characteristics in terms of frequent flowering, fragrant flowers, pest resistance and abundant quantity of florets (Indraloka et al. 2019). The hybridization of *Dendrobium* orchids that have been carried out includes interspecies hybridization between *D. phalaenopsis* Fitzg and *D. lineale* Rolfe., and its reciprocal crosses.

The selection of male and female parents to be crossed requires a comprehensive understanding of the morphological characteristics of the two parents, including dominant traits, such as flower size, color and flower shape, which will be inherited by their offspring (Hartati 2021). The success of hybridization is also determined by the level of

closeness of the relationship or kinship between species. In general, the further the relationship, the lower the level of cross-compatibility (Jean et al. 2022). The relationship between *Dendrobium* orchid species needs to be understood in breeding programs, and morphological relationships can be used to assemble more potential genotypes. Thus, an in-depth study on the potential of genetic diversity and its relationship through morphological characterization is necessary to support the assembly of new genotypes in hybridization. This study aimed to determine the taxonomical relationships based on morphological traits, compatibility of hybridization and in vitro culture of seven species of *Dendrobium* orchids in different sections. The seven species included *D. phalaenopsis* Fitzg., *D. lineale* Rolfe, *D. stratiotes* Rchb.f., *D. macrophyllum* A.Rich, *D. nobile* Lindl., *D. heterocarpum* Wall.ex Lindl. and *D. bracteosum* Rchb.f.. We expect the results of this study can be used to inform the most compatible species as cross parents in hybridization.

MATERIALS AND METHODS

Study area and period

The research was carried out from August 2022 to February 2023. The morphological characterization of the parental of *Dendrobium* orchids was carried out at the Science and Technology Laboratory of Dhyana Pura University, while the hybridization was performed at the greenhouse of an orchid house in Peguyangan, Denpasar, Bali. The in vitro culture of the seeds was carried out at the in vitro culture laboratory of the Agency of Certification and Monitoring of Seeds of Food and Horticultural Crops (*UPTD Balai Perbenihan Pengawasan Sertifikasi Tanaman Pangan Hortikultura dan Perkebunan*) in Luwus, Tabanan, Bali.

Plant material and research tools

This study used seven species of *Dendrobium*, namely *D. phalaenopsis* Fitzg., *D. lineale* Rolfe, *D. stratiotes* Rchb.f., *D. macrophyllum* A.Rich, *D. nobile* Lindl., *D. heterocarpum* Wall.ex Lindl., and *D. bracteosum* Rchb.f. This study used the following tools: a ruler, toothpick, culture bottle, tweezers, scalpel, measuring cup, autoclave, laminar air flow, Petri dish, beaker glass, electric stove, tissue, aluminum foil, bunsen, and microscope. For laboratory analysis, we used MS medium, sterile aquadest, activated charcoal, glucose, agarose, sodium hypochlorite, 70% alcohol and denatured alcohol.

Experimental procedure

The selection of orchid species was done through a purposive sampling method based on the different characters of sections in *Dendrobium* orchids. Hybridization or crossing technique used Randomized Group Design with three replications. In vitro culture of hybridized seeds used Complete Randomized Design with five replications. The details of each step of the experiment are detailed below.

Characterization

The characterization of *Dendrobium* orchid species as male and female parents were carried out morphologically using direct observation techniques. Morphological characterization was carried out to plant appearance, leaf and flower characters, both qualitatively and qualitatively. The quantitative data were based on 10 morphological characters and the qualitative data were based on 13 morphological characters. Quantitative measurements were performed using a ruler, while flower color was determined using a RHS color chart (red-purple group, red group, violet-blue group, white group and yellow-green group). Qualitative data were scored in the range of 1-12 following the guidelines for characterizing ornamental plants issued by the Secretariat of the National Germplasm Commission and RHS color chart. The data were analyzed with a dendrogram to determine the kinship between the parents of the *Dendrobium* orchids.

Hybridization

The hybridization technique was carried out by taking *Dendrobium* pollen as the male parent and placing it in the pistil above the labellum of the female parent. Crossing or hybridization was carried out starting at 07.00 a.m., with three replications conducted for each hybridization treatment. The measured parameters included the percentage of compatibility, time of fruit formation, fruit length, and fruit harvest time.

Culture media preparation

The culture media were prepared by weighing 7 grams of MS media, 20 grams of glucose, 8 grams of agarose, and 1 gram of activated charcoal. Subsequently, one liter of sterile distilled water was added, and stirred homogeneously. Five ml of media solution was poured into each culture jar, which was subsequently closed tightly and sterilized using an autoclave for 15 minutes with a pressure of 150 psi.

In-vitro culture of the hybrids

The first stage of in vitro culture was the sterilization of the pod. Sterilization was carried out by washing the pod with soap under running water for five minutes. The pod was then soaked in sodium hypochlorite with a concentration of 30% and 15% for 15 minutes each. Subsequently, the fruit was sterilized in 70% alcohol for 15 minutes and placed into the laminar air flow. The pod was washed with sterile aquadest and burned using Bunsen burner for five seconds. Then, the pod was cut into half using a scalpel in a Petri dish and the seeds were taken using tweezers and sown on top of the culture medium. The culture jars were then closed and labeled for each hybridization treatment. The culture jars were stored in culture racks with eight hours of light. Observations were made every week to observe the development of seed growth. The observed parameters in vitro culture results included the percentage of seed fertility and germination time.

Data analysis

In morphological characterization, the qualitative data were converted into quantitative data and subsequently analyzed using the dendrogram cluster test with SPSS version 22.0. Quantitative data were presented in tables, while the compatibility and in vitro culture data were analyzed using the ANOVA test. If the results were significantly different, the Duncan test with SPSS version 22.0 was carried out. The results of the qualitative data were described descriptively.

RESULTS AND DISCUSSION

Morphological characterization

Morphological characterization was carried out to plant appearance, leaf and flower characters, both qualitatively and qualitatively. The quantitative data were based on 10 morphological characters and the qualitative data were based on 13 morphological characters as presented in Table 1.

The morphological characters data show the different characteristics of the seven species of *Dendrobium* orchids in terms of leaf and flower morphology. Based on morphological characterization results, the data were subsequently analyzed using the Dendrogram analysis as presented in Figure 1.

Based on the dendrogram analysis as shown in Figure 1, the seven species of *Dendrobium* orchids were divided into 2 main clusters. The first cluster was further divided into 4 sub-clusters, namely (i) *D. lineale*, and *D. stratiotes*; (ii) *D. phalaenopsis*; (iii) *D. heterocarpum*; (iv) *D. nobile*, and *D. macrophyllum.* Meanwhile, *D. bracteosum* separated as the second cluster.

Based on dissimilarity analysis, *D. phalaenopsis* had the lowest dissimilarity with *D. stratiotes* (26.48%) and had the highest dissimilarity with *D. bracteosum* (77.256%). *D. lineale* had the lowest dissimilarity with *D. stratiotes* (17.832%) and had the highest dissimilarity with *D. bracteosum* (74.683%). *D. heterocarpum* had the lowest dissimilarity with *D. nobile* (16.234%) and had the highest dissimilarity with *D. lineale* (43.946%). *D. macrophyllum* had the lowest dissimilarity with *D. nobile* (16.234%) and had the highest dissimilarity with *D. bracteosum* (53.247%) (Table 2).

Table 1. Morphological characters of seven species of *Dendrobium*

Morphological character	*D.phalaenopsis*	*D.stratiotes*	*D. lineale*	*D.macrophyllum*	*D.nobile*	*D.heterocarpum*	*D.bracteosum*
Stem shape	Simpodial	Simpodial	Simpodial	Simpodial	Simpodial	Simpodial	Simpodial
Growth type	Evergreens	Evergreens	Evergreens	Evergreens	Decidous	Deciduous	Deciduous
Stem length (cm)	16.5	156.1	112.1	45	17	17.1	15
Stem circumference	3	6	6	4	3	3	3
Leaf shape	Elliptical	Elliptical	Elliptical	Oval	Oblong	Elliptical	Elliptical
Leaf length (cm)	8	11	10	12	16	8	12
Leaf width (cm)	2.3	4.1	3.8	9	4.2	2	1.5
Number of leaves	5.1	26	28	4	7.8	3.2	6
Flower shape	Round	Horned	Star	Notched star	Curved round	Star	Bud
Petal color	Deep Purplish Pink (73A)	Brilliant Yellow Green (149A)	Light Violet (91A)	Brilliant Greenish Yellow (151D)	Deep Purplish Pink (N66C)	Yellowish White (155D)	Strong Reddish Purple (72C)
Label color	Strong Reddish Purple (N74A)	Vivid Purplish Red (61C)	Dark Violet (N92B)	Light Olive (152B)	Brilliant Yellow Green (150C) and White (NN155B)	Brilliant Yellow Green (154B)	Deep Purplish Red (59B)
Sepal color	Deep Purplish Pink (73A)	White (NN155B)	Light Violet (91A)	Brilliant Greenish Yellow (151D)	Deep Purplish Pink (N66C)	Yellowish White (155D)	Strong Reddish Purple (72C)
Petal shape	Round	Twisted	Straight	Heart	Round	Ovals	Tapered
Label shape	Round	Heart	Round	Heart	Round	Tapered	Round
Sepal shape	Round	Twisted	Twisted	Heart	Round	Tapered	Tapered
Petal length (cm)	2.5	5	3	1	2.3	3.5	2
Label length (cm)	1.7	4	2	1	2	3	1
Sepal length (cm)	1.7	2	4	1.2	2	3	1.8
Flower scent	No	No	Yes	No	Yes	Yes	No
Pollinia form	Beans	Beans	Heart shape	Heart shape	Beans	Beans	Beans
Flower resistance	20	40	60	7	5	5	150
Number of florets	7	14	21	4	10	7	25
Hair on flowers	No	No	No	Yes	Yes	No	No

Table 2. Dissimilarity matrix of morphological characters of seven *Dendrobium*

Species	*D.phalaenopsis*	*D. lineale*	*D. stratiotes*	*D.heterocarpum*	*D. nobile*	*D.macrophyllum*	*D.bracteosum*
D. phalaenopsis	0.000	32.438	26.483	31.969	33.026	28.053	77.256
D. lineale	32.438	0.000	17.832	43.946	36.724	47.056	74.683
D. stratiotes	26.483	17.832	0.000	34.413	23.780	35.795	53.212
D. heterocarpum	31.969	43.946	34.413	0.000	21.353	42.832	39.041
D. nobile	33.026	36.724	23.780	21.353	0.000	16.234	28.625
D. macrophyllum	28.053	47.056	35.795	42.832	16.234	0.000	53.247
D. bracteosum	77.256	74.683	53.212	39.041	28.625	53.247	0.000

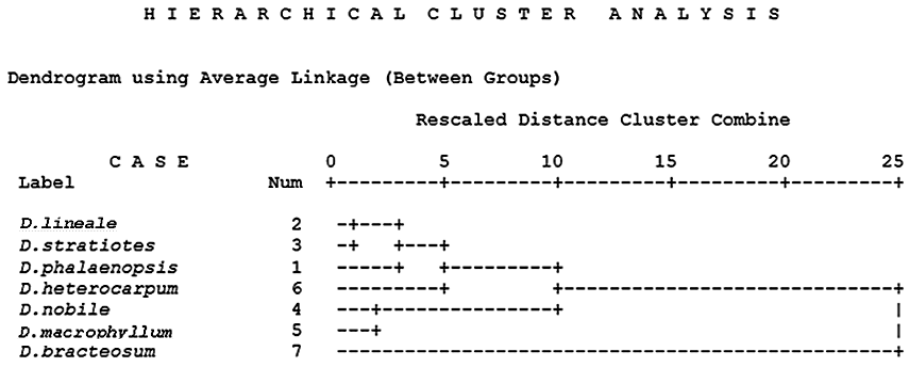

Figure 1. Dendrogram of morphological characters of seven *Dendrobium* species

The clustering patterns showed that the seven orchids were divided based on their section. In the orchid classification system, the *Dendrobium* has enormous diversity with strong variation in flower characters in terms of habitat, size, shape of pseudobulb and leaves that botanists decided to divide into sections (Aleksandra et al. 2022). In the seven observed *Dendrobium* orchid species, they were divided into the Latouria section, Eugenanthe section, Phalaenanthe section, *Dendrobium* section, Pedilonum section and Spatulata section. The *Dendrobium* section Latouria species consisted of *D. macrophyllum*, the Eugenanthe section consisted of *D. nobile*, while the Phalaenanthe section consisted of *D. phalaenopsis.*

Based on the cluster dendrogram, *D. lineale* and *D. stratiotes* are in the same cluster and have characteristic of flowers which shaped like horns. The Spatulata section, also known as the antelope flower type, has a particular flower in which the sepals and petals tend to twist. In addition, it has a pseudobulb shape that tends to be upright. This section is known as a horn orchid due to the shape of its long, twisted petals (Arobaya et al. 2022). Based on morphological characterization, *D. lineale* has a distinctive fragrant aroma and small blue flowers, whereas *D. stratiotes* is a large flower with a unique labellum that can reach 10 cm in length, but is generally about 5 cm.

D. phalaenopsis, which is locally called Anggrek Larat, has a round flower shape, purple petals on all parts of the flower, thinner sepals and petal strands, and small flower stalks, hence the lack of a strong appearance (Setiari 2017). *D. heterocarpum* is a subcluster closely related to *D. phalaenopsis* from the Phalaenanthe section. This section has a peculiarity in that the flowering type is a single or solitary flower with growing points on the segments or in the axils of the leaves. The flowers also produce an extremely fragrant smell. Meanwhile, D. *nobile* and *D. macrophyllum* have a close kinship even though they are in different sections due to the similarity of the characters of the flowers and stems. *D. nobile* is a *Dendrobium* orchid belonging to the Eugenanthe section which has a fleshy pseudobulb and the thickening occurs throughout the pseudobulb which grows hanging or upright. Flowers usually emerge from the pseudobulb nodes after all the leaves have fallen off. The flower shape tends to be round, and the large size varies in purple, pink, white and yellow colors. *D. macrophyllum* belongs to the Latouria section which has a slender lower part of the pseudobulb, and an enlarged second segment towards the top. The flowers are arranged like bunches and their color varies from green to yellow-brown with a lip pattern like batik. The two well-known species in the Latouria section are *D. macrophyllum* and *D. spectabile*.

In the dendrogram cluster, *D. bracteosum* forms its own cluster, which is included in the Pedilonum section. The same results were obtained by Hartati et al. (2022) in which *D. bracteosum* formed a separate cluster. The distinctive character of this species is the type of flowering in which the point of flower growth is in the segments or in the axils of the leaves. *D. bracteosum* has inflorescence in many nodes (points), has a few flowers, and the lips do not have side plates with striking lip color.

The results of the clustering of seven *Dendrobium* orchid species can be used as potential parents in assembling more potential orchids. Hartati et al. (2019) described that characterization data are extremely useful

not only for resource management and conservation of individual species, but also for orchid breeders or cultivating farmers. The obtained characteristics are prerequisites for scientific studies in crossbreeding, propagation, and also for germplasm conservation and genetic improvement.

Hybridization compatibility

The compatibility of primary crosses or hybridization of between *Dendrobium* orchids, both interspecies and intersectional crosses are presented in Table 3. Compatibility percentage (%) was calculated as the number of crosses that formed pod divided by the total number of crosses.

Based on the results of Table 3, interspecies crosses had a higher compatibility percentage than intersectional crosses. *D. nobile* and *D. bracteosum* had a compatibility percentage of 0%. The required time for *Dendrobium* orchid pod formation ranged from 5 to 8 days and the pod length ranged from 2.1 to 7.1 cm. The pod harvest time for seven types of *Dendrobium* orchids ranged from 60 to 100 days.

Table 3. Results of compatibility of primary crosses/hybridization between *Dendrobium* species

Hybridization (♀x ♂)	Compatibility percentage (%)	Time to form pod (day)	Pod length (cm)	Harvest time (day)
D. phalaenopsis x *D. phalaenopsis*	100	5	3	90
D. phalaenopsis x *D. stratiotes*	100	5	3.3	90
D. phalaenopsis x *D. lineale*	100	5	2.6	85
D. phalaenopsis x *D. macrophyllum*	66.7	6	2	85
D. phalaenopsis x *D. heterocarpum*	33.3	8	3.4	85
D. phalaenopsis x *D. nobile*	33.3	6	3.1	60
D. phalaenopsis x *D. bracteosum*	0	0	0	0
D. stratiotes x *D.phalaenopsis*	100	7	5.8	100
D. stratiotes x *D. stratiotes*	100	7	7.1	100
D. stratiotes x *D. lineale*	100	7	6	90
D. stratiotes x *D. macrophyllum*	66.7	7	5	90
D. stratiotes x *D. heterocarpum*	0	0	0	0
D. stratiotes x *D. nobile*	0	0	0	0
D. stratiotes x *D. bracteosum*	0	0	0	0
D. lineale x *D. phalaenopsis*	100	6	2.5	80
D. lineale x *D. stratiotes*	100	6	2.6	80
D. lineale x *D lineale*	100	7	2.5	80
D. lineale x *D. macrophyllum*	100	7	2.1	80
D. lineale x *D. heterocarpum*	0	0	2.4	80
D. lineale x *D. nobile*	33.3	6	2.5	60
D. lineale x *D. bracteosum*	0	0	0	0
D. macrophyllum x *D. phalaenopsis*	66.7	8	2.4	90
D. macrophyllum x *D. stratiotes*	100	8	2.6	90
D. macrophyllum x *D. lineale*	100	8	2.4	90
D. macrophyllum x *D. macrophyllum*	100	7	3.4	75
D. macrophyllum x *D. nobile*	0	0	0	0
D. macrophyllum x *D. heterocarpum*	0	0	0	0
D. nobile x *D. phalaenopsis*	0	0	0	0
D. nobile x *D. stratiotes*	0	0	0	0
D. nobile x *D. lineale*	0	0	0	0
D. nobile x *D. macrophyllum*	0	0	0	0
D. nobile x *D. heterocarpum*	0	0	0	0
D. nobile x *D. nobile*	0	0	0	0
D. nobile x *D. bracteosum*	0	0	0	0
D. heterocarpum x *D. phalaenopsis*	0	0	0	0
D. heterocarpum x *D. stratiotes*	0	0	0	0
D. heterocarpum x *D. lineale*	0	0	0	0
D. heterocarpum x *D. macrophyllum*	0	0	0	0
D. heterocarpum x *D. heterocarpum*	100	7	5	80
D. heterocarpum x *D. nobile*	0	0	0	0
D. heterocarpum x *D. bracteosum*	0	0	0	0
D. bracteosum x *D. phalaenopsis*	0	0	0	0
D. bracteosum x *D. stratiotes*	0	0	0	0
D. bracteosum x *D. lineale*	0	0	0	0
D. bracteosum x *D. macrophyllum*	0	0	0	0
D. bracteosum x *D. heterocarpum*	0	0	0	0
D. bracteosum x *D. nobile*	0	0	0	0
D. bracteosum x *D. bracteosum*	0	0	0	0

Table 4 shows that the highest percentage of compatibility was in *D. phalaenopsis* and *D. Lineale*, followed by *D. stratiotes*, *D. macrophyllum*, and the lowest was in *D. bracteosum* and *D. nobile* (0%). The longest pod formation time was shown by *D. macrophyllum* and *D. stratiotes* and while the fastest was indicated by *D. phalaenopsis*. For the pod length parameter, the highest average fruit length was demonstrated by *D. stratiotes* and *D. heterocarpum*, and the lowest was demonstrated by *D. liniale*, while the fastest harvest time was achieved by *D. liniale* and D. *phalaenopsis*, and the longest harvest time was achieved by *D. stratiotes* (Figure 2).

The results of orchid crossing showed that *D. phalaenopsis* and *D. lineale* had a high level of compatibility compared to other *Dendrobium* orchids, followed by *D. stratiotes* and *D. macrophyllum*. These species of *Dendrobium* can be recommended as a cross parents with a high level of compatibility both in interspecies and intersectional crossing compared to *D. heterocarpum*, *D. nobile* and *D. bracteosum*. Hartati et al. (2017) stated that successful compatibility in crosses is indicated by the formation of the fruits. Fertilization success in orchids is more influenced by the stigma receptivity period and fertility of pollinia. According to Christine et al. (2019), many factors can affect the success rate of crossing, including pollen condition and degree of compatibility. Compatibility in crossing occurs since there is a match between the pistil and stamen so that the fruit is formed.

In the study results, there was a low number of interspecies and intersectional crosses, namely *D. bracteosum*. Barriers to interspecies hybridization are caused by environmental factors, nutrition, and pollen failure to germinate on foreign stigmas due to incompatibilities caused by genetic factors or physiological barriers by substances secreted by the stigma (Pershina and Trubacheeva 2017). For intersectional crossing, due to differences between sections with relatively distant relationships, it was difficult to cross, resulting in *D. bracteosum* forming its own clusters with quite distant kinship relations (Figure 1). In addition, the differences in pollen size and stigma are considerable, which can also affect pollen germination. Hidayati et al. (2016) stated that the further the relationship between the species, the lower the success of the cross. However, the possibility of obtaining a superior genotype is greater if the cross is successful. The more genetic diversity, the greater the possibility of superior genotypes. Jean et al. (2022) stated that the low crossbreeding success is also influenced by the asynchronous flowering time between parents (males and females). In addition, there are several factors, such as plant failure to flower, buds, and flowers falling before or after fertilization, low pollen production, unviability, male infertility, and self-incompatibility. The results of this study indicated that the highest intersectional crosses were found in crosses between the Spatulata and Phalaenthae sections, as well as the Spatulata and Latouria sections.

In this study, *D. nobile*, *D. herocarpum* and *D. bracteosum* had the lowest cross-compatibility, but self-crossed *D. heterocarpum* showed a high compatibility percentage of 100% (Table 3). Similar results were shown by Darmawati et al. (2021), in which the results of crossing *D. macrophyllum* x *D. macrophyllum* and *D. heterocarpum* x *D. heterocarpum* had a high compatibility of 100% and the lowest was in *D. secundum* (0%), which was still included in the same section with *D. bracteosum*.

For the parameter of the time of fruit formation, the average result of crosses indicated that fruits were formed in 5-8 days. According to Hartati et al. (2017), the day from pollination to the time of fertilization in orchids varies depending on the species, environment and nutrients. In the fruit size parameter, the longest fruit pod was indicated by *D. stratiotes*, followed by *D. heterocarpum*, and the smallest fruit size was indicated by *D. lineale*. The fastest harvest time was indicated by the cross between *D. phalaenopsis* and *D. nobile* in 60 days. The same result was obtained in the study by Darmawati et al. (2021), in which *Dendrobium* orchid fruit from crosses was harvested in 66-109 days.

In vitro culture of the hybrids

After harvesting the *Dendrobium* orchids, a total of 20 hybrids/crosses were obtained, and the in vitro culture results are presented in Table 5. Based on the study results, the germination percentage ranged from 40% to 100% (Table 5), except for the results of the crossing between *D. phalaenopsis* and *D. nobile* since the fruit had broken and a large number of seeds were contaminated before germinating. The fruit burst because the harvest time was faster than the other hybridizations. Contamination of orchid seeds and media greatly affects the success of in vitro culture (Abdalla et al. 2022). The germination time parameters varied depending on the type of *Dendrobium* crosses, which ranged from 19.8-29.1 (Table 5). The germination time of orchid seeds varies depending on various factors such as media, nutrition, seed viability, and fruit ripening time (Salazar and Botello 2020).

Table 4. Duncan's test analysis to test the difference in parameters of hybridization compatibility between *Dendrobium* species

***Dendrobium* species**	**% Compatibility**	**Time to form pod (day)**	**Pod length (cm)**	**Harvest time (day)**
D. phalaenopsis	$61.9^{c} \pm 40.5$	$5.8^{b} \pm 1.1$	$2.9^{b} \pm 0.5$	$82.5^{bc} \pm 11.2$
D. stratiotes	$52.3^{bc} \pm 50$	$7^{cd} \pm 0$	$5.9^{d} \pm 0.8$	$95^{d} \pm 11.7$
D. lineale	$61.9c \pm 48$	$6.4^{bc} \pm 0.5$	$2.4^{b} \pm 0.1$	$76^{b} \pm 8.9$
D. macrophyllum	$52.3^{bc} \pm 50.3$	$7.7^{d} \pm 0.5$	$2.7^{b} \pm 0.4$	$86.2^{c} \pm 7.5$
D. nobile	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$
D. heterocarpum	$14.2^{ab} \pm 37.7$	$7^{cd} \pm 0$	$5^{c} \pm 0$	$80^{bc} \pm 0$
D. bracteosum	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$

Note: The mean number followed by different letters shows a significant difference according to Duncan's test ($\alpha = 0.05$)

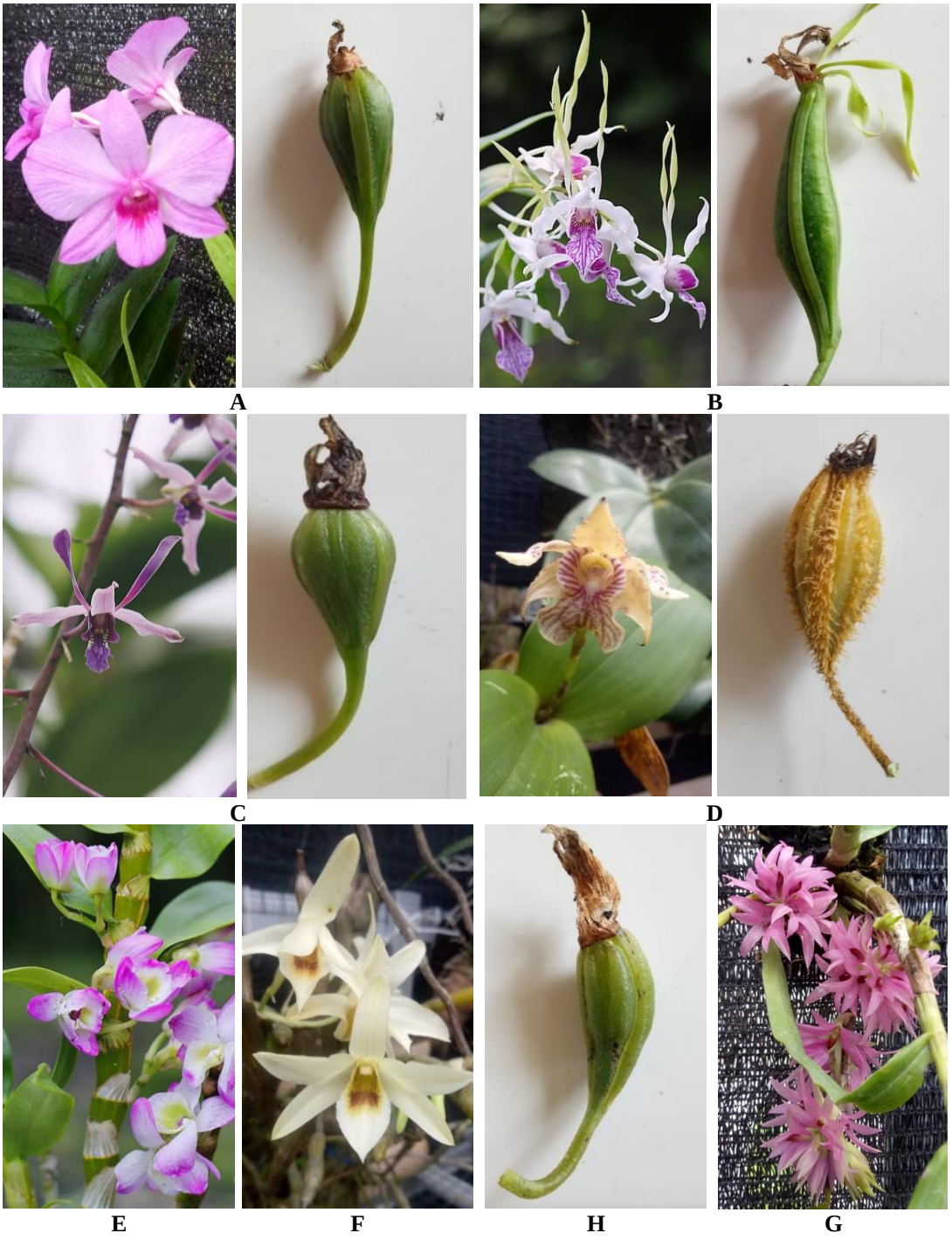

Figure 2. The flowers and pod of: A. *D. phalaenopsis*; B. *D. stratiotes*; C. *D. lineale*; D. *D. macrophyllum*; E. *D. nobile*; F. *D. heterocarpum*; G. *D. bracteosum*

One of the most significant applications of biotechnology for the propagation and conservation of orchid diversity is in vitro or tissue culture. In vitro culture is carried out based on sustainable use and prevention of over-exploitation by communities living around the forest. In addition, propagation by this method is the most potential technique for mass propagation, especially for rare orchid species. The tissue culture method will produce uniform clones for ornamental purposes and medicinal potential. Orchid germination using in vitro method is also needed to support orchid conservation efforts, especially for rare species. In their natural habitat, orchid seedlings often require the presence of fungal symbionts for ex-situ growth efficiency. In vitro techniques aimed at the germination, propagation, and multiplication of orchids on a large scale have been widely used as a conservation and commercial effort for various types of orchids (Teixeira et al. 2014).

Table 5. Results of in vitro culture of the seeds of the hybrids

Hybridization	No. of jar	Germination (jar)	Germination percentage (%)	Germination time (day)
D. phalaenopsis x *D. phalaenopsis*	5	5	$100^{c} \pm 0.0$	$29.8^{b} \pm 1.6$
D. phalaenopsis x *D. stratiotes*	5	5	$100^{c} \pm 0.0$	$31.2^{bcd} \pm 2.1$
D. phalaenopsis x *D. lineale* 'blue'	5	5	$100^{c} \pm 0.0$	$31^{bcd} \pm 0.0$
D. phalaenopsis x *D. macrophyllum*	5	4	$80^{c} \pm 44.7$	$32.4^{cde} \pm 3.1$
D. phalaenopsis x *D. nobile*	5	0	$0^{a} \pm 0.0$	$0^{a} \pm 0.0$
D. phalaenopsis x *D. heterocarpum*	5	4	$80^{c} \pm 44.7$	$32.2^{cde} \pm 1.1$
D. stratiotes x *phalaenopsis*	5	5	$100^{c} \pm 0.0$	$39.2f \pm 1.1$
D. stratiotes x *D. stratiotes*	5	5	$100^{c} \pm 0.0$	$35.2^{e} \pm 1.4$
D. stratiotes x *D. lineale*	5	5	$100c \pm 0.0$	$35.4^{e} \pm 4.0$
D. stratiotes x *D. macrophyllum*	5	3	$60^{bc} \pm 54.7$	$33.8^{cde} \pm 3.1$
D. lineale x *D. phalaenopsis*	5	4	$80^{c} \pm 44.7$	$33.8^{cde} \pm 2.7$
D. lineale x *D. stratiotes*	5	5	$100^{c} \pm 0.0$	$34.2^{de} \pm 3.9$
D. lineale x *D lineale*	5	5	$100^{c} \pm 0.0$	$39.2^{f} \pm 2.6$
D. lineale x *D. macrophylum*	5	3	$60^{c} \pm 54.7$	$33.9^{cde} \pm 1.6$
D. lineale x *D. nobile*	5	0	$0^{a} \pm 0.0$	$0^{a} \pm 0.0$
D. macrophyllum x *D. phalaenopsis*	5	2	$40^{ab} \pm 54.7$	$31.4^{bcd} \pm 0.5$
D. macrophyllum x *D. stratiotes*	5	3	$60^{bc} \pm 54.7$	$34.4^{de} \pm 3.5$
D. macrophyllum x *D. lineale*	5	3	$60^{bc} \pm 54.7$	$31.2^{bcd} \pm 0.4$
D. macrophyllum x *D. macrophyllum*	5	5	$100^{c} \pm 0.0$	$33.8^{cde} \pm 3.8$
D. heterocarpum x *D. heterocarpum*	5	5	$100^{c} \pm 0.0$	$30.4^{b} \pm 1.3$

Note: The mean number followed by different letters shows a significant difference according to Duncan's test ($\alpha = 0.05$).

In conclusion, the clustering of seven species of *Dendrobium* orchids resulted in the division into six sections, namely Spatulata, Phalaenanthe, *Dendrobium*, Eugenanthe, Latouria and Pedilonum section. The results of interspecies and intersectional crossing showed that the highest compatibility was indicated by *D. phalaenopsis* and *D. lineale*. Therefore, they are highly recommended as the parents of hybrid orchids. Interspecies crosses resulted in a higher success rate than intersectional crosses. For intersectional crosses, the highest compatibility was indicated by the Spatulata section and its reciprocal (*D. lineale* and *D. stratiotes*) with the Phalaenanthe section (*D. phalaenopsis*) as well as the Spatulata and Phalaenanthe section with the Latouria section (*D. macrophyllum*).

ACKNOWLEDGEMENTS

Acknowledgements to Dhyana Pura University, Bali, Indonesia and the Dhyana Pura University Research and Service Institute for sponsoring this research with the grant contract number: 034/UNDHIRA-LPPM/ST/VIII/2022.

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