BIODIVERSITAS
Volume 24, Number 5, May 2023
Pages: 3004-3011

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240555

Interspecific hybridization of *Dendrobium mirbelianum* x *D. nindii* or *D. discolor*, in vitro seed germination, seedling growth and plantlet acclimatization

DESI MAULIDA[1,♥], YUSNITA YUSNITA[2], DWI HAPSORO[2], AGUSTIANSYAH AGUSTIANSYAH[2], AGUS KARYANTO[2]

[1]Department of Horticulture, Politeknik Negeri Lampung. Jl. Soekarno Hatta No. 10, Rajabasa, Bandar Lampung 35144, Lampung, Indonesia. Tel.: +62-721-703995, Fax.: +62-721-787309, ♥email: desi@polinela.ac.id

[2]Department of Agronomy and Horticulture, Faculty of Agriculture, Universitas Lampung. Jl. Prof. Dr. Soemantri Brojonegoro No. 1, Gedung Meneng, Bandar Lampung 35141, Lampung, Indonesia

Manuscript received: 19 February 2023. Revision accepted: 28 May 2023.

Abstract. *Maulida D, Yusnita Y, Hapsoro D, Agustiansyah A, Karyanto A. 2023. Interspecific hybridization of* Dendrobium mirbelianum *x* D. nindii *or* D. discolor*, in vitro seed germination, seedling growth and plantlet acclimatization. Biodiversitas 24: 3004-3011.* *Dendrobium mirbelianum* Gaudich. (P1), *D. nindii* W.Hill (P2), and *D. discolor* Lindl. (P3) as a parent crossing are potentially resulting a new type of orchid as the novel breeding achievement. This study aimed to identify orchid crossing compatibility and evaluate the most effective and efficient media for hybrid seed germination and growth. The study consisted of three experiments, namely interspecific hybridization (experiment 1), in vitro hybrid seed germination (experiment 2), and in vitro growth of the hybrid seedlings (experiment 3). The first experiment was conducted by a simple modified line×tester mating design, and all experiments used a completely randomized design, with five replications in the first experiment and three replications in the second and third experiments. The first experiment presented all of the crossing emerging seed pods, but only hybrid seeds from cross *D. mirbelianum* x *D. nindii* and its reciprocal cross that were potent to germinate in all tested media as the second experiment result. The third experiment showed FF+tryptone was the best media indicated by the highest and heaviest seedling after 8 months after sowing. All of the crossings resulted in seed pods, however, only *D. mirbelianum* x *D. nindii* and its reciprocal cross that able to germinate in all tested media. Foliar fertilizer is the most recommended media for germinating hybrid orchid seeds, and FF+tryptone is the best for seedling growth.

Keywords: *Dendrobium*, foliar fertilizer, incompatibility, interspecific hybridization, in vitro culture

INTRODUCTION

Orchid (Orchidaceae) is the largest family of flowering plants in monocots, having more than 28,000 species (Fay 2018; Li et al. 2018). Indonesia is the second largest diversity of orchids after Brazil, blessed with more than 5,000 orchid species (Hartini 2019). The genus *Dendrobium* is one of the most attractive, popular, and high interest, because of its flexible beauty as ornamental garden plants, pots, or cut flowers, diverse motifs, and relatively easy to grow. In 2000, Hawaiian orchid growers sold a million pots of *Dendrobium* valued at 5.6 million USD, as evidence, and its increase over time (De et al. 2014). There are about 1600 species worldwide and 275 of *Dendrobium* species can be found in Indonesia (Darmawati et al. 2018). It has been used in many breeding programs to produce either primary or complex hybrids. The hybridization has resulted in many hybrid off-springs that have extracted various novel characteristics from two and more species of *Dendrobium,* such as New Pink, Queen Pink, and Emma White (Devadas et al. 2016; Jangyukala and Hemenata 2021).

Certain *Dendrobium* species, such as *Dendrobium mirbelianum* Gaudich., *D. nindii* W.Hill, and *D. discolor* Lindl. have flowers with desirable aesthetics and are potential candidates for development through hybridization. *Dendrobium mirbelianum* is well-known as dark-stemmed antler orchid or mangrove orchid, which has yellow-brownish petals and sepals with slightly dark-red striped, the same color as the labellum with a white tongue in the center (Hartati et al. 2022). *Dendrobium nindii* is known as blue orchid, relatively tall posture with slender pseudostems. The long mature stems could bear emerge of 8-20 flowers. Its flowers have white or whitish with blue-tinge sepals and the same color of twisted petals, purplish-blue or dark-pink-wide-striped labellum (Jones 2006). *Dendrobium discolor* is known as golden antler orchid, brown antler orchid, and canary orchid has brown, twisted, and severe curly petals and yellow labellum with a yellowish brown tongue (Handini and Aprilianti 2020).

Interspecific hybridization generally encountered problems with unilateral incompatibility, hybrid inviability or hybrid lethality (hybrid seeds cannot germinate), sterility, and problems with gene linkage to undesirable characters (Acquaah 2012). The effect of viability constraints on orchid seeds from interspecific crosses can be fixed by in vitro asymbiotic seed germination (Darmawati et al. 2021; Jangyukala and Hemenata 2021). Hossain et al. (2013) and Udomdee et al. (2014) have extensively documented the use of distinct basal media for orchid seed germination in tissue culture. These media include Knudson C, Vacin and Went (VW), and Murashige and Skoog (MS) (1962).

Nugroho et al. (2019) reported that in vitro germination of *Dendrobium antennatum* Lindl. seeds can be carried out using Foliar Fertilizer (FF) with an N-P-K ratio of 10-55-10, supplemented with 10% coconut water and 50 g L^{-1} leek extract in the media. The addition of tryptone to three basal media might result in better protocorm growth than those without tryptone. The use of MS and VW or FF as basal media combined with tryptone might be used to overcome the potential problem of hybrid inviability.

To answer the challenge of interspecific crossing on orchids and to overcome the problems that might occur in the seeds resulting from these crosses, we conducted three consecutive experiments. These experiments aimed to investigate the effects of the pairing of parent plants on seed pod formation, and understanding the effects of basal media with tryptone on in vitro seed germination and seedling growth. In this study, *D. mirbelianum*, was reciprocally crossed with either *D. nindii* or *D. discolor* to produce primary hybrid off-springs to learn about cross-compatibility among chosen *Dendrobium* and for breeding well hybrid and studying genetic inheritance between parents. This study is not only provide information about cross-compatibility between *D. mirbelianum-D. nindii* and *D. mirbelianum-D. discolor*, but also give the most recommended media formulation for hybrid seed germination and growth.

MATERIALS AND METHODS

Plant materials

Three *Dendrobium* species from section Spatulata, namely *D. mirbelianum, D. nindii, D. discolor* from Merauke cultivated in Anarda Orchid, Lampung. The study was conducted at Anarda Orchid greenhouse and Plant Science Laboratory, Faculty of Agriculture, University of Lampung, Indonesia, from July 2019 to October 2020.

Procedures

Three consecutive studies were done, i.e., interspecific hybridization (experiment 1), in vitro hybrid seed germination (experiment 2), and in vitro growth of the hybrid seedlings (experiment 3).

Compatibility identification from crossing among *Dendrobium* parents

Dendrobium mirbelianum (P1), *D. nindii* (P2), and *D. discolor* (P3) (Figure 1) were chosen as parental plants. This study was conducted by four crosses, i.e., *D. mirbelianum* x *D. nindii* (P1×P2), *D. nindii* x *D. mirbelianum* (P2×P1), *D. mirbelianum* x *D. discolor* (P1×P3), and *D. discolor* x *D. mirbelianum* (P3×P1), as simple modified line×tester mating design (Muthoni and Shimelis 2020) with reciprocal crossing. *Dendrobium mirbelianum* is a main line, followed by *D. nindii* and *D. discolor* as a tester, arranged by completely randomized design repeated five times. Pollinations were carried out 6 days after anthesis between 7.00-10.00 AM. Pollinia from the anthers of male parents were attached to the pistils of the female parents using a toothpick. The percentage of fruit formation, fruit green-ripening time, pod length, pod diameter, and pod weight were observed every two weeks until 70-84 Days After Pollination (DAP) (Darmawati et al. 2021). It is possible to do interspecific crossing within species in orchids as reported by Devadas et al. (2016).

Hybrid seed germination on six treated media

The mature pods obtained from experiment 1 were harvested and sterilized by soaking and shaking them in a 1.5% NaOCl solution for 15 minutes, followed by three times of rinses with sterile water. The pods were dipped in 96% methanol and briefly burned, repeated once inside the laminar airflow cabinet. The pods were then longitudinally cut into two parts and planted on the media. Six different media of two factors were used,i.e., MS, VW, and FF, with or without 2 g L^{-1} tryptone. Basal MS medium contained macro and micro salts of Murashige and Skoog (MS) (1962), vitamins, and Myo-inositol. The VW medium contained VW macro and micro salts, the Foliar Fertilizer (FF) containing N:P:K (32:10:10), and various essential trace elements used, 2 g L^{-1}. All media were enriched with 20 g L^{-1} sucrose, 150 mL L^{-1} coconut water, and solidified with 8 g L^{-1} agar, adjusted to pH 5.8 before being boiled and dispensed into 250-mL-culture bottles, 30 mL per bottle. Then, each bottle was capped with a plastic sheet and autoclaved at 121°C, at a pressure of 1.2 kg $(cm^2)^{-1}$ for 15 minutes. Seeds were aseptically sowed on six different treatments. All cultures were placed in a culture room with continuous fluorescent light of approximately 2,000 lux and maintained at 25±2°C. This experiment was arranged by a completely randomized design with three replication. Each experimental unit consisted of five culture bottles, each of which was planted with approximately the same amount of seeds. The observed germinate seeds (well-grown into protocorms) with the conical tip as shoot-tip initials at 2 months After Seed Sowing (ASS).

Seedling performance on six treated media

Two-month-old protocorms from the previous experiment were cultured on the same medium for growing for 6 months. Subcultures were done 2, 4, and 6 months After Seed Sowing (ASS). In the first subcultures, individual seedlings (approximately 3-4 cm) were detached from the seedling clump and transferred to the first-used treatment media, with 10 seedlings per bottle. All cultures were incubated in the same culture room condition as previously described. The growth of seedlings (seedling heights and weights, total leaves and leaf length, also total roots and root length) was measured and arranged by a completely randomized design with three replication. After 8 months in cultures, seedlings were subcultured into FF medium enriched with 2 g L^{-1} activated charcoal for 3 months, then well-rooted, 6-7 cm seedlings with 5-6 leaves were removed from cultures, washed from the attached agar, and acclimatized in a shaded greenhouse.

Data analysis

Collected data were analyzed by variance using SPSS Statistic 20 software using 5% DMRT for experiment 1 and 5% HSD for experiments 2 and 3. Besides, we used Ms. Excel 2016 to proceed data into bar charts with their standard deviation.

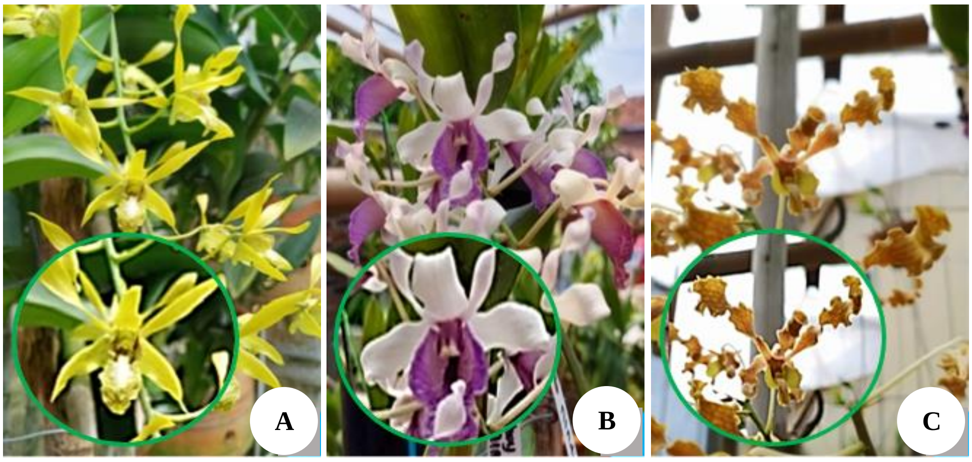

Figure 1. Parental plants used in the study. A. *Dendrobium mirbelianum*, B. *Dendrobium nindii,* C. *Dendrobium discolor*

RESULTS AND DISCUSSION

Compatibility identification from crossing among *Dendrobium* parents

The results showed that all (100%) of the four crosses formed fruit pods of different sizes, and the incompatibility phenomenon was not occurring. Ovaries enlarged into fruit pods that sized around 2.6-5.4 cm in length and 2.3-3.0 cm in diameter. A significant difference was observed in the sizes and ripening time of the pods. Crosses with *D. mirbelianum* (P1) as female parents produced pods that ripened earlier, at 83 days, whereas *D. nindii* (P2) and *D. discolor* (P3) were used as female parents at 115 and 93 days, respectively. It indicated that fruit pod ages to maturity were different and maternally affected. Furthermore, the weight of the fruit pod, and the P2×P1 cross was the heaviest seeds (Table 1). The hybrid *Dendrobium* seed shape was fusiform and the size of seeds was not uniform, some seeds were larger than others, ranging from 300 to 500 µm (Figure 2A), while the pattern of testa cells of the seeds that was elongated (Figure 2B).

Hybrid seed germination on six treated media

The germination ability from each cross was observed after 2 months of cultures. seeds from reciprocal crosses of *D. mirbelianum* × *D. nindii* (P1×P2 and P2×P1) were able to germinate on all media, while other crosses were not capable (Table 2). The germinated seeds were indicated by well-grown protocorms (rounded-green corm-like structures) with a conical tip as shoot-tip initials on all of the six tested media, while dead seeds were stagnant and turned brown. The representative appearance of protocorms of the hybrid seeds of *D. nindii* x *D. mirbelianum* (P2×P1) in all media is presented in Figure 3. Green color often becomes an indicator of living cells in juvenile plants, including in protocorms, due to the abundance of chloroplast organelles, while brown is the opposite condition (Gao et al. 2023).

Seedling performance on six treated media

Protocorms from the crosses of *D. mirbelianum* x *D. nindii* (P1×P2) and *D. nindii* x *D. mirbelianum* (P2×P1) were subcultured on the same six tested media as in experiment 2 until 8 months ASS. The seedlings formed were subcultured onto fresh media with the same treatments every 2-month interval. Results of these experiments showed that all protocorms grew into seedlings after 4 months ASS, indicated by the appearance of several leaves clumps. The average seedling heights and clump fresh weight on each tested medium and representative of the performance of hybrid seedling clumps at 4 months ASS are presented in Figure 4.

In vitro growth response of the reciprocal hybrid seedlings between *D. mirbelianum* and *D. nindii* (P1×P2 and (P2×P1) at 4 months ASS were significantly affected by media formulations as well as the mating parents (Figure 4). Supplementation of tryptone into the basal media generally increased seedling growth indicated by the increase in seedling heights and fresh weights (Figures 4A and 4B). Based on standard deviation, FF and FF+tryptone facilitated better nutrition resulting in the highest seedlings' performance compared to other treatments, while seedling in VW media was the shortest (Figure 4A). In addition, the FF medium resulted in relatively heavier seedling clump weight than either MS or VW, and it also affected greener leave clumps (Figures 4B and 4C). The seeds from P2×P1 produced well-grown seedling clumps rather than the reciprocal cross, indicated by the higher seedling height and clump weight on all formulations media, except for seedling weight on VW+tryptone and FF media.

The growth response of the reciprocal hybrid seedlings on in vitro media was also significantly affected by formulations media and the mating parents at 8 months ASS (Table 3). The FF medium consistently resulted in better seedling growth than other crosses. The hybrid seedlings of *D. mirbelianum* x *D. nindii* (P1×P2) on FF had a similar growth response to MS medium, while seedlings of *D. nindii* x *D. mirbelianum* (P2×P1) hybrids grew better on FF than MS medium, indicated by higher seedling heights and fresh weights. When *D. nindii* was a female parent, the seedlings significantly grew better than the reciprocal crossing, indicated by having taller and heavier seedlings, more leaves, and longer, as well as more roots and longer almost in all treated media at 8 months ASS (indicated by capital word “A” in *D. nindii* x *D. mirbelianum* (P2×P1) means better performance than capital word "B" in *D. mirbelianum* x *D. nindii* (P1×P2) based on HSD 0.05) (Table 3). VW produced poor root traits, whereas MS+tryptone resulted in the worst seedling height, weight, and number of leaves. Seedlings that lived on MS+trypton seemed stagnant compared to the previous observations. In addition, supplementation of tryptone into all basal media significantly increased seedling growth in both crosses rather than without tryptone. Overall, FF+tryptone was the best medium for growing seedling orchids almost in every observed trait, while VW and MS+tryptone could not support better seedling performance.

After being transferred to FF medium enriched with tryptone and 2 g L^{-1} activated charcoal for 3 months, seedlings from the two crosses grew well and most of them had 7-8 cm in length, 5-6 leaves, and well-rooted (Figure 5A). These plantlets were then hardened in a place at room temperature with diffuse light for 1 week before being removed from culture bottles and washed from the attached agar media. Seedlings were planted as a community pod of 10 plants per pot, then placed on a shaded bench in a greenhouse. After 3 months in ex vitro condition, 95% of plantlets were successfully acclimatized and grew well (Figures 5B and 5C).

Discussion

A common strategy to create new well-prized orchid varieties is through hybridization. Several species of orchids have been crossed in breeding programs to produce progenies having flowers with new patterns, shapes, sizes, and colors (Udomdee et al. 2014). *Dendrobium bigibbum* Lindl. from Maluku and *D. lineale* Rolfe from Papua, for instance, are Indonesian native orchid that had been crossed producing unique progeny flower pattern (Hartati et al. 2021). The success of the pollination is indicated by the withering of the flower ornaments and the swelling of the ovaries a few days after pollination, similar to what we observed in all crosses that have been made. Successful fertilization is shown by the growth and development of fruit pods, which continue to grow until ripening. Physiological ripening of fruits is characterized by slightly yellowish or bright color of the fruit pod and wider line of the fruit (Darmawati et al. 2021). The compatibility of the cross is influenced by environment, genetic and physiological factors, such as time of crossing (Pershina and Trubacheeva 2017).

Table 1. The pod quantitative traits resulted from crosses

Crosses	% fruit pod formation	Ripening time (DAP)	Pod length (cm)*	Pod diameter (cm)*	Pod weight (g)*
D. mirbelianum x *D. nindii*	100	83	3.9 b	2.5 b	3.0 b
D. nindii x *D. mirbelianum*	100	115	5.4 a	3.0 a	3.4 a
D. mirbelianum x *D. Discolor*	100	83	3.3 c	2.3 c	2.8 d
D. discolor x *D. mirbelianum*	100	93	2.6 d	2.4 bc	2.9 c

Note: *means in the same column followed by the same letter are not significantly different based on DMRT at 0.05 level

Table 2. Germination performance of hybrid orchid seeds from four crosses on all tested media at 2 months ASS

Crosses	Basal media					
	MS	MS+ tryptone	VW	VW+ tryptone	FF	FF + tryptone
D. mirbelianum x *D. nindii*	+	+	+	+	+	+
D. nindii x *D. mirbelianum*	+	+	+	+	+	+
D. mirbelianum x *D. discolor*	-	-	-	-	-	-
D. discolor x *D. mirbelianum*	-	-	-	-	-	-

Note: (+) Seeds germinated, (-) Seeds did not germinate, MS: Murashige and Skoog, VW: Vacin and Went, FF: Foliar Fertilizer

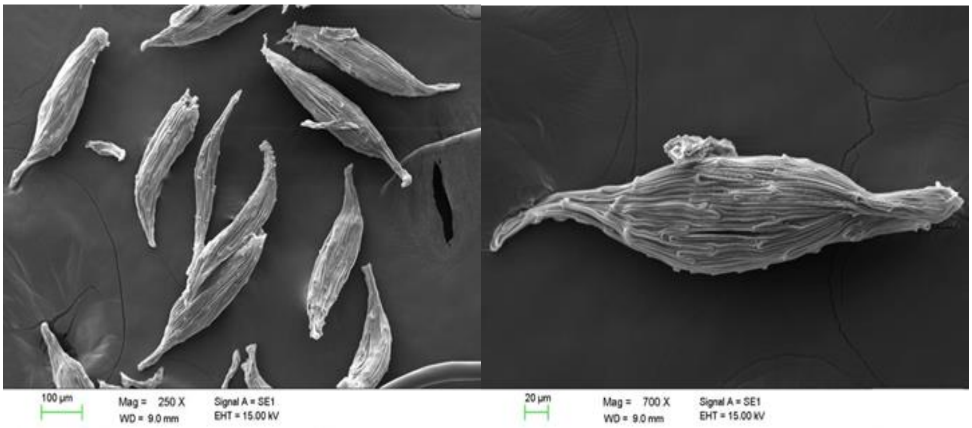

Figure 2. Scanning electron micrographs of hybrids orchid seed

According to Cardoso (2017), crossing in orchids should be carried out 7 days after blooming, because generally the stigma is still receptive. Furthermore, harvesting pods should be carried out when the pod reaches green stage maturity (early matured), so that the pods are easier to be sterilized before being sowed in vitro. The degree of maturity of the orchid seeds inside the pods affects the germination rate. Seed pods of *Dendrobium* usually require 3.3 to 4.7 months to mature (da Silva et al. 2015), almost similar compared to this study which needed time to mature in the range of 2.8 to 3.8 months.

The size of the ovary (ovule) determines the size of the fruit or pod, and it is maternal (Acquaah 2012). In line with *D. nindii* has a relatively larger ovary size than *D. mirbelianum* and *D. discolor* so that the pod size (length and weight) resulting from crosses where *D. nindii* as the female parent is significantly larger than the resulting pods crosses of *D. mirbelianum* and *D. discolor* as female parents (Table 1). The ovary size of *D. mirbelianum* is larger than that of *D. discolor* but smaller than that of *D. nindii*, so it has an intermediate pod size compared to other female parents. Meanwhile, the ovary size of *D. discolor* was relatively smaller compared to the other two species, resulting in the smallest pod size. That condition is well-informed for a long time, for example in olives, melons, and cucumbers (Rosati et al. 2009; Liu et al. 2020).

Barthlott et al. (2014) classified the orchid seed size, which refers to the length of the seeds into five categories, namely very small (100-200 µm), small (200-500 µm), medium (500-900 µm), large (900-2,000 µm), and very large (2,000-6,000 µm). The uniformity of the seeds indicated that the development of the zygotic embryos among seeds in one fruit pod was un-synchronous. Utami and Hariyanto (2020) reported *Dendrobium* seeds as fusiform in shape, light brown in color, very small in size, and simple in cellular organization confirmed by this study (Figure 2). The very small size of the seeds is one of the most distinctive features of the family Orchidaceae, that is why they are called “dust seeds”. The seeds consist of only an undifferentiated mass of embryonic cells without endosperm and are covered by testa (Utami and Hariyanto 2020).

Table 3. Growth response of *Dendrobium* hybrid seedling at 8 months ASS

Media	**Seedling height (cm)**	**Seedling weight (g)**		**Total leaves**		**Leaf length (cm)**		**Total roots**		**Root length (cm)**	
		P1×P2	**P2×P1**	**P1×P2**	**P2×P1**	**P1×P2**	**P2×P1**	**P1×P2**	**P2×P1**	**P1×P2**	**P2×P1**
MS	3.70 b	2.67 d	3.73 c	4.03 a	5.57 a	2.11 c	2.51 b	2.20 d	2.53 c	2.31 e	2.96 c
		B	A	B	A	B	A	A	A	B	A
MS+ tryptone	2.52 d	2.32 e	2.57 d	2.43 d	2.97 d	2.03 c	2.30 c	3.10 bc	3.70 b	3.36 c	3.52 b
		B	A	B	A	B	A	B	A	A	A
VW	3.31 c	2.69 d	3.55 c	3.47 b	3.97 c	2.09 c	2.35 bc	2.43 cd	2.83 c	2.93 d	3.07 c
		B	A	B	A	B	A	A	A	A	A
VW+ tryptone	3.96 b	3.74 b	4.18 b	3.63 b	4.57 b	2.44 b	3.35 a	3.53 b	4.23 b	2.27 e	3.01 c
		B	A	B	A	B	A	B	A	B	A
FF	3.85 b	3.35 c	4.14 b	2.97 c	3.23 d	2.00 c	2.54 b	5.23 a	5.67 a	4.53 a	4.61 a
		B	A	B	A	B	A	A	A	A	A
FF+tryptone	4.38 a	4.13 a	5.08 a	3.63 b	3.80 c	3.04 a	3.39 a	3.70 b	5.43 a	3.98 b	4.44 a
		B	A	A	A	B	A	B	A	B	A

Note: The number in the row followed by the same letter and capital letter under the number in the column for each observation variable shows no significant difference based on the results of the HSD test at 0.05

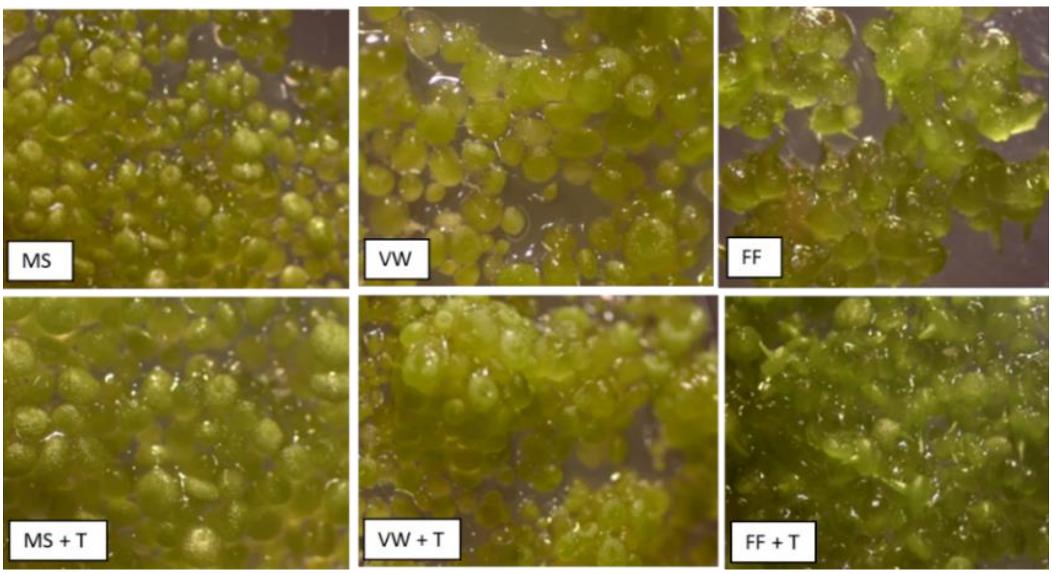

Figure 3. Representative performance of the hybrid protocorms of *D. nindii* × *D. mirbelianum* (P2×P1) using various media; bar: 100 mm

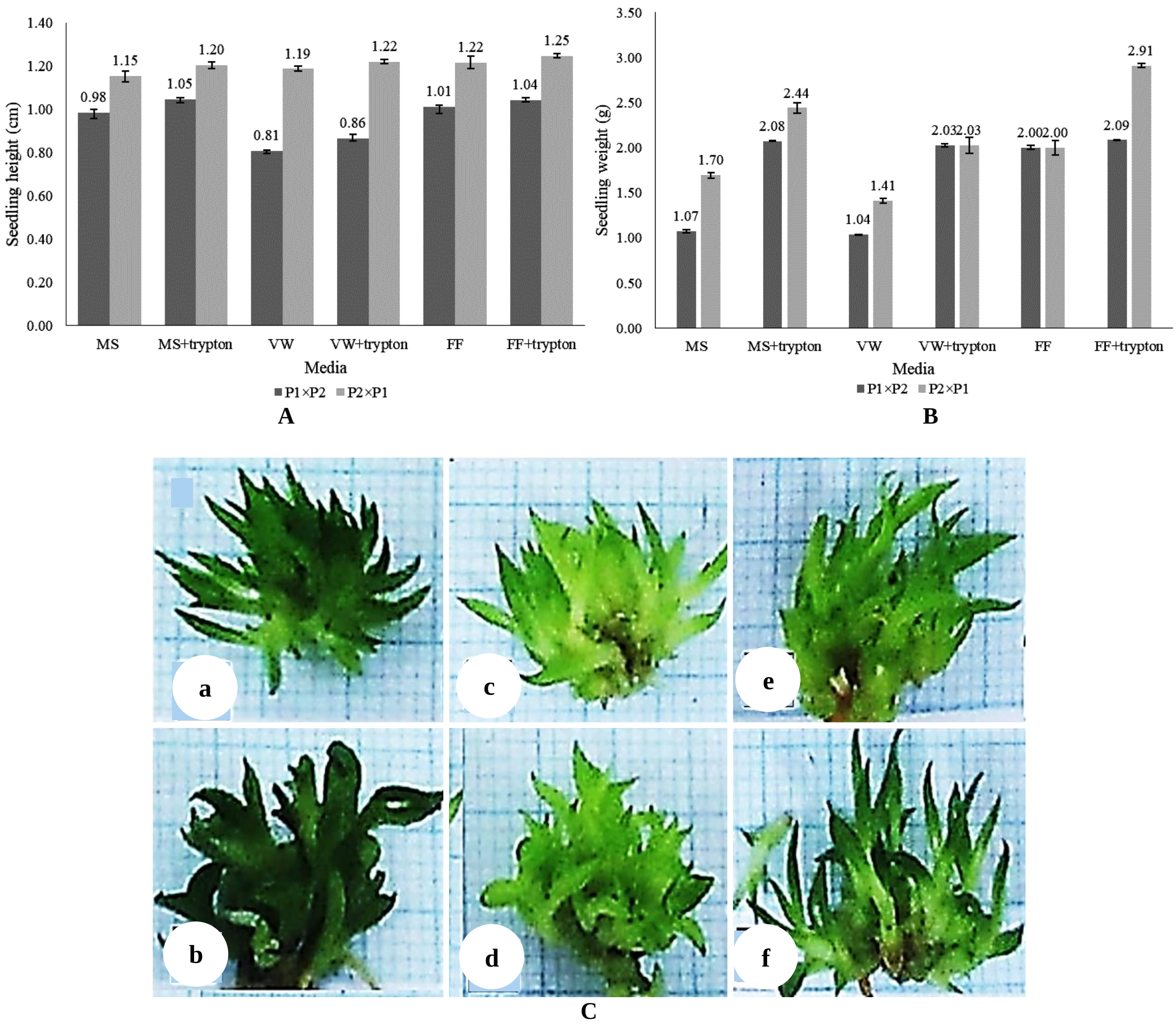

Figure 4. The performance of hybrid seedling clump on each tested medium at 4 months ASS: A. seedling height, B. seedling clump fresh weight, C. hybrid seedling clumps from P2×P1 cross. a. MS, b. MS+tryptone, c. VW, d. VW+tryptone, e. FF, f. FF+tryptone

Figure 5. The performance of hybrid seedling clump after 8 months ASS: A. Before acclimatization, B. After acclimatization, C. Individual pots

Physiologically mature seeds are characterized by the cessation of water and sugar transported into the seeds naturally, but there is no guarantee that all mature seeds are able to germinate (Acquaah 2012). Interspecific crosses with *D. discolor* (P3) either as female or as male parent, for instance, seemed to develop normally, but the seeds were non-viable. The inability to germinate seeds from any interspecific crosses could be due to (i) failure of embryonic development, (ii) post-fertilization incompatibility, resulting in a zygote that cannot divide or abort even before the first cell division but the fruit continues to develop, and (iii) a genetic interaction that results in lethal effects (Yunianti et al. 2010; Chu et al. 2016).

All treatment media tested, namely MS, VW, and FF supplemented with 20 g L^{-1} sucrose, coconut water, with or without 2 g L^{-1} tryptone, met the requirements for orchid germination, including adequate humidity, the availability of energy source and nutrients for germination, considering that orchid seeds do not have food reserves. The use of relatively economical media, namely FF without tryptone, could be recommended as the most economist and easy-to-prepare seed germination medium for *Dendrobium* hybrids, since there seemed no difference in protocorm growth performance in this medium, compared to other media tested. It is relevant to Hapsoro et al. (2018) and Nugroho et al. (2019) that reported foliar fertilizer is able to fulfill plant growth requirements, because it contains macro-essential nutrient needs for the plant, such as Natrium, Potassium, and Kalium.

All media can produce normal orchid growth, however, the best media capable of producing the most optimum almost in all vegetative traits until 8 months ASS was FF+trypton media. The addition of tryptone to each significant media resulted in better growth than without tryptone. Organic compounds such as HC, peptones, and tryptones, generally consisting of low molecular weight proteins, amino acids, vitamins, and plant growth substances, promote plant growth by providing plant cells with an available nitrogen source. Tryptone is a pancreatic digest amino acid that contains complex amino acids, which include arginine, aspartic acid, tyrosine, and others. The vitamins contained in tryptone include biotin, thiamin, pyridoxine, nicotinic acid, pyridoxine, and riboflavin. In addition, tryptone also contains nitrogen as the essential nutrient for cell division and chloroplast production in leaves (Wu et al. 2014). The result of this study was in line with Ferziana and Erfa (2013) and Zeng et al. (2016), that tryptone can support the growth of orchid plantlets because it contains complex nutrient needs.

In the treatment medium without tryptone, foliar fertilizer was able to produce a plantlet growth response that resembled MS and VW media, even better on several seedlings' observed variables until 8 months ASS. This is because foliar fertilizers contain complex macro elements as well as several microelements which are very necessary for the growth of orchid plantlets in general (Hapsoro et al. 2018; Nugroho et al. 2019). Foliar fertilizer can be used as an alternative germination and growth medium for *Dendrobium* hybrid plantlets because it is more economical.

In conclusion, crossing in *Dendrobium* may produce seeds or pods, but it may result in pods without normal embryos because of genetic and segregation factors. Even though, all crosses succeeded in resulting pods, only two series can germinate by in vitro culture. Normal embryos were only detected from the pairing of *D. mirbelianum* x *D. nindii* (P1×P2) and *D. nindii* x *D. mirbelianum*, but not in pods from *D. mirbelianum* x *D. discolor* (P1×P3) and *D. discolor* x *D. mirbelianum* (P3×P1). FF surprisingly can well-supported seedlings not only for germination, but also for growing them until acclimatization. Addition of tryptone as supplementary seedling nutrition is the best choice to support seedlings' growth, particularly on FF+tryptone media. It can provide complex essential elements, supporting both forage and root traits to be well-grown.

ACKNOWLEDGEMENTS

The authors sincerely thank the Owner of Anarda Orchids, Lampung, Indonesia for kindly providing some collections to be used as parent plants, and Laboratory of Plant Science, Faculty of Agriculture, Universitas Lampung, and Politeknik Negeri Lampung for accommodating most experiments of this research.

REFERENCES

Acquaah G. 2012. Principles of Plant Genetics and Breeding. Second Edition. Wiley Blackwell, Maryland.

Barthlott W, Große-Veldmann B, Korotkova N. 2014. Orchid Seed Diversity: A Scanning Electron Microscopy Survey. Botanischer Garten und Botanisches Museum, Berlin-Dahlem.

Cardoso JC. 2017. Ionocidium 'Cerrado 101': Intergeneric orchid hybrid with high quality of blooming. Onnam Hortic 23 (3): 351-356. DOI: 10.14295/oh.v23i3.1110.

Chu Y, Wu CL, Holbrook CC, Ozias-Akins P. 2016. Conditions that impact artificial hybridization of *Arachis hypogaea* L. Peanut Sci 43 (2): 106-115. DOI: 10.3146/PS16-11.1.

da Silva JAT, Tsavkelova EA, Ng TB, Parthibhan S, Dobránszki J, Cardoso JC, Rao MV, Zeng S. 2015. Asymbiotic in vitro seed propagation of *Dendrobium*. Plant Cell Rep 34 (10): 1685-1706. DOI: 10.1007/s00299-015-1829-2.

Darmawati IAP, Rai IN, Dwiyani R, Astarini IA. 2018. Analysis on relationship among *Dendrobium* spp. Bali based on characteristics of leaves anatomy. Intl J Biosci Biotech 5 (2): 111-117. DOI: 10.24843/IJBB.2018.v05.i02.p03.

Darmawati IAP, Astarini IA, Yuswanti H, Fitriani Y. 2021. Pollination compatibility of *Dendrobium* spp. orchids from Bali, Indonesia, and the effects of adding organic matters on seed germination under in vitro culture. Biodiversitas 22 (5): 2554-2559. DOI: 10.13057/biodiv/d220513.

De LC, Pathak P, Rao AN, Rajeevan PK. 2014. Commercial Orchids. De Gruyter Open Ltd, Warsaw/Berlin. DOI: 10.2478/9783110426403.

Devadas R, Pattanayak SL, Singh DR. 2016. Studies on cross compatibility in *Dendrobium* species and hybrids. Indian J Genet Plant Breed 76 (3): 344-355. DOI: 10.5958/0975-6906.2016.00052.3.

Fay MF. 2018. Orchid conservation: How can we meet the challenges in the twenty-first-century? Bot Stud 59: 16. DOI: 10.1186/s40529-018-0232-z.

Ferziana, Erfa L. 2013. The influence of tripton and active carbon on orchid *Phalaenopsis* in vitro seedling enlargement. Jurnal Penelitian Pertanian Terapan 13 (1): 45-51. DOI: 10.25181/jppt.v13i1.167. [Indonesian]

Gao X, Wang Y, Deng D, Luo Y, Shao S, Luo Y. 2023. Morphogenesis changes in protocorm development during symbiotic seed germination of *Dendrobium chrysotoxum* (Orchidaceae) with its mycobiont, *Tulasnella* sp. Horticulturae 9 (5): 531. DOI: 10.3390/horticulturae9050531.

Handini E, Aprilianti P. 2020. Lethal doses of LD20 and LD50 and gamma irradiation effect on protocorms of *Dendrobium discolor* Lindl. Buletin Kebun Raya 23 (3): 173-178. DOI: 10.14203/bkr.v23i3.631. [Indonesian]

Hapsoro D, Septiana VA, Ramadiana S, Yusnita Y. 2018. A medium containing commercial foliar fertilizer and some organic additives could substitute MS medium for in vitro growth of *Dendrobium* hybrid seedlings. Jurnal Floratek 13 (1): 11-22.

Hartati S, Muliawati ES, Syarifah ANF. 2021. Characterization on the hybrid of *Dendrobium bigibbum* from Maluku and *Dendrobium lineale* from Papua, Indonesia. IOP Conf Ser: Earth Environ Sci 724: 012011. DOI: 10.1088/1755-1315/724/1/012011.

Hartati S, Samanhudi, Cahyono O. 2022. Short Communication: Morphological characterization of five species of *Dendrobium* native to Indonesia for parent selection. Biodiversitas 23 (5): 2648-2654. DOI: 10.13057/biodiv/d230548.

Hartini S. 2019. Orchids diversity in the Sicikeh-Cikeh Forest, North Sumatra, Indonesia. Biodiversitas 20 (4): 1087-1096. DOI: 10.13057/biodiv/d200421.

Hossain MM, Rahi P, Gulati A, Sharma M. 2013. Improved ex vitro survival of asymbiotically raised seedlings of *Cymbidium* using mycorrhizal fungi isolated from distant orchid taxa. Sci Hortic 159: 109-116. DOI: 10.1016/j.scienta.2013.05.003.

Jangyukala M, Hemanta L. 2021. Review on advances in production of hybrids in orchids. Res Rev: J Crop Sci Technol 10 (3): 15-20.

Jones DL. 2006. A Complete Guide to Native Orchids of Australia Including the Island Territories. Reed New Holland, London.

Li J, Gale SW, Kumar P, Zhang J, Fischer G. 2018. Prioritizing the orchids of a biodiversity hotspot for conservation based on phylogenetic history and extinction risk. Bot J Linn Soc 186 (4): 473-497. DOI: 10.1093/botlinnean/box084.

Liu X, Pan Y, Liu C, Ding Y, Wang X, Cheng Z, Meng H. 2020. Cucumber fruit size and shape variations explored from the aspects of morphology, histology, and endogenous hormones. Plants 9 (6): 772. DOI: 10.3390/plants9060772.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bio assay with tobacco tissue cultures. Physiol Plant 15 (3): 473-497. DOI: 10.1111/j.1399-3054.1962.tb08052.x.

Muthoni J, Shimelis H. 2020. Mating designs commonly used in plant breeding: A review. Aust J Crop Sci 14 (12): 1855-1869. DOI: 10.21475/ajcs.20.14.12.p2588.

Nugroho JD, Arobaya AYS, Tanur EA. 2019. Propagation of *Dendrobium antennatum* Lindl via seed culture in vitro using simple medium: Fertilizer and complex organic based medium. Hayati 26 (3): 133-138. DOI: 10.4308/hjb.26.3.133.

Pershina LA, Trubacheeva NV. 2017. Interspecific incompatibility in wide hybridization of plants and ways to overcome it. Russ J Genet: Appl Res 7: 358-368. DOI: 10.1134/S2079059717040098.

Rosati A, Zipanćič M, Caporali S, Padula G. 2009. Fruit weight is related to ovary weight in olive (*Olea europaea* L.). Sci Hortic 122 (3): 399-403. DOI: 10.1016/j.scienta.2009.05.034.

Udomdee W, Kongsawad P, Darak R, Somrit P. 2014. Collection and evaluation on ex situ conservation of *Phaius* orchid. Khon Kaen Agr J 3: 529-534.

Utami ESW, Hariyanto S. 2020. Organic compounds: Contents and thier role in improving seed germination and protocorm development in orchids. Hindawi Intl J Agron 2020: 2795108. DOI: 10.1155/2020/2795108.

Wu K, Zeng S, Lin D, da Silva JAT, Bu Z, Zhang J, Duan J. 2014. In vitro propagation and reintroduction of the endangered *Renanthera imschootiana* Rolfe. PLoS ONE 9 (10): e110033. DOI: 10.1371/journal.pone.0110033.

Yunianti R, Sujiprihati S, Syukur M. 2010. Teknik Persilangan Buatan. Scientific Repository, IPB University. [Indonesian]

Zeng S, Huang W, Wu K, Zhang J, da Silva JAT, Duan J. 2016. In vitro propagation of *Paphiopedilum* orchids. Crit Rev Biotechnol 36 (3): 521-534. DOI: 10.3109/07388551.2014.993585.