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Hybridization technique of black orchid (*Coelogyne pandurata* Lindley) to enrich the genetic diversity and to rescue the genetic extinction

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Abstract

Hartati, S., Nandariyah, Yunus, A., Djoar, D. (2019). Hybridization technique of black orchid (*Coelogyne pandurata* Lindley) to enrich the genetic diversity and to rescue the genetic extinction. *Bulgarian Journal of Agricultural Science, 25*(4), 751–755

The existence of *Coelogyne pandurata* Lindley, an exotic and valuable orchid species from eastern Kalimantan is currently being endangered. The objectives of this study were to rescue the genetic extinction of the *Coelogyne pandurata* Lindley as well as to create new genetics through crossbreeding with other species. Crosses were performed at 4 to 6 flowering individuals. Pollens were transferred from the anther to the stigma by using sterile toothpicks, with following methods: crossing, reciprocal and self-pollination. The parameters observed included: percentage of success in crossing, percentage of fallen fruit, age of ripening fruit, time of protocorm formation.

The results showed that the abilities of crossing for each intersection were as follows: crossing (♀*Coelogyne pandurata* × ♂*Coelogyne rumphii*), reciprocal (♀*Coelogyne rumphii* × ♂*Coelogyne pandurata*) and selfing was 100%. The percentage of fallen fruit was as follows: crossing was 50%, reciprocal was 25% and self-pollination was 25%. Age of ripening fruit was as follows: crossing was 158 days, reciprocal was 191 days and selfing pollination was 155-201 days. Time of protocorm formation was as follows: crossing was 22 days, reciprocal was 48 days and selfing was 26-94 days.

Keywords: crossing; extinction; protocorm; reciprocal; self-pollination

Introduction

Group of orchid (*Orchidaceae*) is one of the largest families of flowering plants in the world that includes natural species and species from crosses (Xiang et al., 2003).

The genus of *Coelogyne* Lindl is one of more than 200 species with the deployment area of India, China, Indonesia and Fiji Island centering in Kalimantan, Sumatra and the Himalayas (Devi et al., 2012). One of the rare species of *Coelogyne* Lindl protected by the government of Indonesia is black orchid (*Coelogyne pandurata* Lindley). This species of orchid, naturally found in Eastern Kalimantan, is very exciting – it has large green flowers with a black tongue protruding from the centre. Therefore this orchid has high economic value. However the existence of black orchid is currently being endangered and now is hard to find it even in its native habitat, so the saving cultivation should be done before extinction occurs.

One of the possible action to save the genetic extinction is by crossing it to the other species. Selection of the parent having high compatibility to be crossed is very important in determining the success of a hybridization program.

One barrier in crossing success is that between the crossed parents should have a close genetic kinship. The previous research has been conducted in 2012 to select elders that have a genetic proximity to the black orchid (*Coelogyne*

pandurata Lindley) using morphological characterization and molecular characterization of RAPD (Random Amplified Polymorphic DNA). The study found that *Coleogyne rumphii* was the selected parent to be crossed with black orchid.

The cross of *Coelogyne pandurata* Lindley (Fig. 1) and *Coleogyne rumphii* (Fig. 2) was done in the present research. The seeds obtained from the crosses were grown *in vitro* using tissue culture.

By crossing of *Coelogyne pandurata* having big green flower and black tongue and rarely flowering (2-3 times a year) and *Coelogyne rumphii* having small yellow flower with brown tongue but simultaneous and almost every months flowering, was expected to obtain new variants having combination characters and to enrich genetic diversity.

Materials and Methods

Materials are *Coelogyne pandurata* and *Coelogyne rumphii* (collection of Bogor botanical garden, Indonesia). Place of experiment is the Center Bogor botanical garden plant conservation. Methods – there were three kinds of crosses:

• Crossing: ♀ *Coelogyne pandurata* × ♂ *Coelogyne rumphii*;

• Reciprocal: ♂*Coelogyne pandurata* × ♀ *Coelogyne rumphii*;

• Selfing: pollen transfer to the stigma of a flower on one plant.

Crossing was conducted in the morning (07:00 to 10:00 am) and performed at 4 flowering orchids. Pollens are transferred from the anther to the stigma by using sterile tooth picks.

After harvesting the seeds sterilized with 70% alcohol for 5 min. The pods were rinsed 4 times with sterile distilled water before being transferred to a laminar box. Seeds were cultured in medium with basic media of Knudson C + coconut milk (150 ml/l) + bean extract (150 g/l) + gelatine (7 g/l) + activated charcoal (1g/l), pH 5.6.

Observed parameter: percentage of crossing success, age of ripen fruit, the percentage of fruit loss, and time of protocorm (seed germination).

Results and Discussion

Based on the study of Hartati et al. (2013), morphological characters of black orchids (*Coelogyne pandurata*) are epiphytic with sympodial growth type, lanceolate breech-shape leaf, star-shape flower. The positions of the flower is at the basal, its colour is pale green with black tongue, about 9.1 cm long and 10.35 width, the dorsal sepal is 5.37 cm long and 2 cm width, the lateral sepals is 4.38 cm long and 1.46 cm width, the petal is 5.1 cm long and 1.3 cm width. The curve of the lip is at the threshold and the cross section flipped into the transverse lip. Each node contains big pseudo bulb, 11.4 cm long, 2.78 cm width and 4.5 cm thick. The numbers of pollinia are 4, number of florets per stem are 7-8, length of blooming duration is 6 days, the number of flower stalks is 1-2, not simultaneous flowering and only 2-3 times flowering season per year.

Fig. 1. *Coelogyne pandurata,* Bogor botanical garden, Indonesia, 2012

Fig. 2. *Coelogyne rumphii*, Bogor botanical garden, Indonesia, 2012

Coelogyne rumphii orchids are epiphytic orchids with the type of growth: sympodial, lanceolate breech shape leaf, star-shape flower, light yellow flowers with brown tongue, flowering position in tip, flower stalk length of 30 cm, length of flowers is 4.4 cm, width of 5.1 cm, dorsal sepals is 4.36 cm × 1.42 cm, lateral sepals of 4.44 cm × 1,1 cm, petal of 4.2 cm × 3.5 cm, the location of the curve of the lips is at the basal, reverse lip cross section is very deep, large pseudo bulb size, 10.5 cm long, 3.9 cm width, and 3.1 cm thick. The number of pollinia is 4, number of florets per stem is 1, length of blooming duration is 8 days and the number of flower stalks is 3-5, flowering simultaneously and almost every month.

Percentage of Crosses Success

Several days after that petal and crown of the female flower will withered, dry and fallen, then emerge the small lengthwise fruit. Sivanaswari et al. (2011) also stated that if crosses are made less than a week after flower bloom, the surface of the stigma is receptive to pollen. After 2 weeks, the flower closes and pollen becomes brownish and receptive. It is important to reach flowering in different individuals at the same time for pollination through artificial hybridization.

This study showed that whatever the method of pollination is, it succeeds to form fruit (Table 1). Selection of parent to be crossed is very important in determining the success of a cross breeding program.

Study of Sivanaswari et al. (2011) showed that *Aerides odorata* as female resulted successful crosses of 0-60%, while in the reciprocal cross of *Aerides odorata* as male resulted 25-62%. Described by Chaturvedi and Shonali (2010) that the morphology of the orchid flower is slightly complicated by stem structure called column, and at the apical section of the column whose anther has pollen inside called pollinarium. The stigma lies in the sub-apical column called rostellum. The success of pollination occurs when pollinarium can be incorporated into the rostellum.

Percentage of fruit loss

The orchid pollination usually takes place with an assistance of insects/bees. The flower of *Coelogyne* species is dominated by yellow-green (Clayton, 2002 in Cheng et al., 2009). The study of Tremblay et al. (2005) in Cheng et al. (2009) showed a failure to form fruit if the pollination is done on the same flower (autogamy) or another flower on the same plant (geitonogamy).This is due to a mismatch in the Orchidaceae plants.

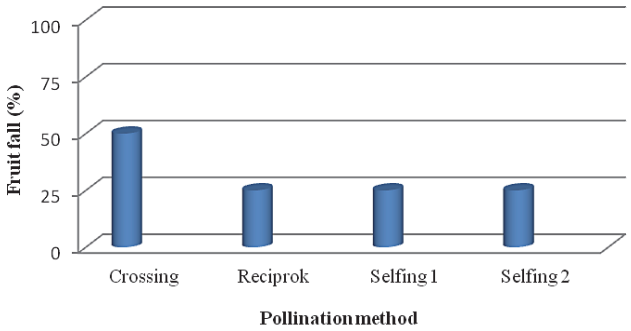

Fig. 3. Effect of pollination method to the percentage of fruit fall

Semiarti et al. (2007) stated that the conventional breeding methods through crossing, such as interspecific hybridization is a common way to create new varieties. Based on the classification of Wang (1963) there are generally three groups of compatibility which are: compatible (above 20% success), incompatible majority (10-20%) and fully incompatible (below 10%). Research results in Table 1 show that all methods are compatible crosses (100%).

The study done by Hartati (2010) stated that a cross between *Phalaenopsis* and *Vanda tricolor* sp is compatible, but to produce seeds *Phalaenopsis sp* as a male parent and a female parent *Vanda tricolor* as likely to be greater than the reciprocal (Fig. 3).

Time of fruit maturity

The percentage of harvested fruit set is determined by the number of total fruit and fall fruit. On cross of ♀*Coelogyne pandurata* × ♂*Coelogyne rumphii* fruit ripening took place for 158 days and the reciprocal crosses of ♀*Coelogyne rumphii* × ♂*Coelogyne pandurata* took place for longer time, 191 days (Table 2).

Table 1. Percentage of successful crosses, level of compatibility and time of fruit emergence

No	Parental crosses	Pollination method	Number flower of crossed	Successful crosses (%)	Compatibility level	Time of fruit emergence (days)
1	♀ *C. pandurata* × ♂ *C. rumphii*	Crossing	4	100	Compatible	4
2	♀ *C. rumphii* × ♂ *C. pandurata*	Reciprocal	4	100	Compatible	4
3	*Coelogyne pandurata*	Selfing 1	4	100	Compatible	6
4	*Coelogyne rumphii*	Selfing 2	4	100	Compatible	5

Table 2. Mean time of fruit maturity and time of protocorm emergence

No	Parental crosses	Pollination method	Percentage of fallen fruit (%)	Time of fruit maturity (days)	Time of protocorm emergence (days)
1	♀ *C.pandurata* × ♂ *C. rumphii*	Crossing	50	158	22
2	♀ *C. rumphii* × ♂ *C. pandurata*	Reciprocal	25	191	48
3	*Coelogyne pandurata*	Selfing 1	25	155	26
4	*Coelogyne rumphii*	Selfing 2	25	201	94

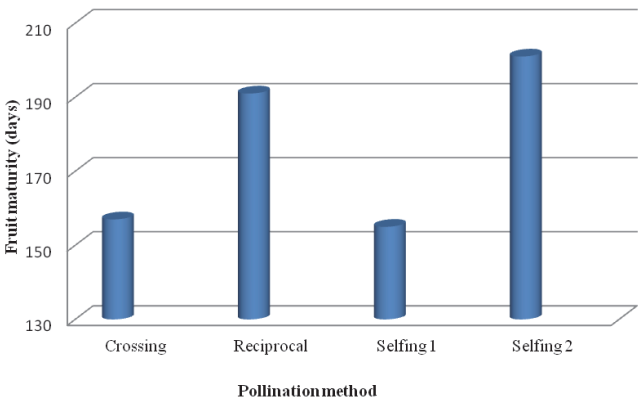

Fig. 4. Effect of pollination methods to the time of fruit maturity

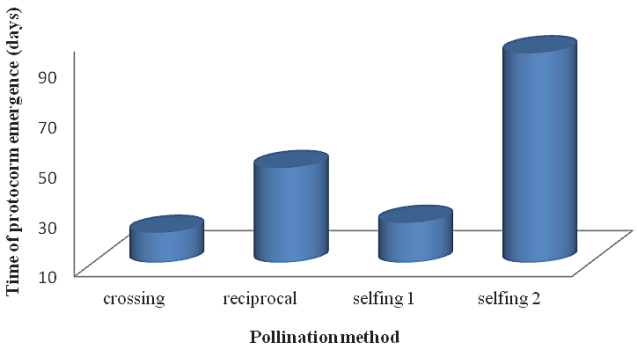

Fig. 5. Effect of pollination method to the protocorm emergence

Table 2 indicated that time of fruit maturity in self-pollination (selfing) was longer than if the plant crosses in the crossing or in the reciprocal.

Compared to the study by Sivanaswari et al., (2011) showed that the time of fruit maturity on the crossing of *Aerides odorata* as the female parent ranged from 0-179 days and the reciprocal cross, *Aerides odorata* as the male parent, ranged from 116-184 days (Fig. 4).

Protocorm Emergence

The study showed that the crossing methods affected the protocorm emergence. The crossing of ♀*Coelogyne pandurata* × ♂*Coelogyne rumphii* protocorm emerged at 22 days, the reciprocal cross of ♀*Coelogyne rumphii* × ♂*Coelogyne pandurata* emerged at 48 days, while the selfing in *Coelogyne pandurata* emerged at 26 days and *Coelogyne rumphii* at 94 days (Table 2).

Invitro orchid seed germination is influenced by several factors such as the age of the seeds, nutrient media and sources of organic carbon (Mohanty et al., 2012). Furthermore Arditti and Ernst (1993) mentioned that in certain orchid species, after 20 days on germination medium, the cells in the basal part of the embryo will divide and accumulate tannins (Fig. 5).

According to Kauth et al. (2008) development to chid plantlets from the seeds could be directly or indirectly through secondary protocorm (PLBs). In addition, PLBs differentiated embryonic tissue that can develop bipolar two different structures, namely, the shoot and root meristems. Thus this structure can grow into plantlets when grown on appropriate nutrient medium (Ng & Saleh, 2011).

Conclusions

A cross of ♀*Coelogyne pandurata* × ♂*Coelogyne rumphii* in the methods of crossing, selfing and reciprocal cross are compatible and 100% successful. Time of fruit maturity at the cross of ♀*Coelogyne pandurata* × ♂*Coelogyne rumphii* is 158 days, at the reciprocal cross of ♀*Coelogyne rumphii* × ♂*Coelogyne pandurata* is 191 days and at the selfing is 155-201 days.

Time of protocorm at the cross of ♀*Coelogyne pandurata* × ♂*Coelogyne rumphii* is 22 days and at the reciprocal cross of ♀*Coelogyne rumphii* × ♂*Coelogyne pandurata* is 48 days while at the selfing of *Coelogyne pandurata* and *Coelogyne rumphii* ranged from 26-94 days.

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