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Short Communication: Genetic variation of *Coelogyne pandurate, C. rumphii* and their hybrids based on RAPD markers

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Abstract. *Hartati S, Muliawati ES. 2020. Short Communication: Genetic variation of* Coelogyne pandurata, C. rumphii *and their hybrids based on RAPD markers. Biodiversitas 21: 4709-4713.* One effort to increase the genetic diversity of orchids is by crossing. This research aims to assess the genetic variation of *Coelogyne pandurata, C. rumphii* and their hybrids based on RAPD markers. In this research, both parents were analyzed in three replications, while the hybrid was done in 10 replications. The study was conducted by analyzing DNA bands using RAPD markers with six primers, i.e. OPA 02, OPA 07, OPA 13, OPB 12, OPB 17, and OPD 08. Identification of the parents and their F1 hybrids showed 95.83% polymorphic bands with 43 bands measuring 200-2100 bp. The parents of ♂*C. pandurata*, ♀*C. rumphii*, and their hybrids presented a similarity range of 0.16-1.00. The dendrogram generated by the UPGMA cluster analysis separated the parents and hybrids in two large clusters, i.e. the first cluster consisted only of *C. pandurata* and the second cluster consisted of *C. rumphii*, together with all hybrid individuals. It means that the hybrids of the crossing of ♀*C. rumphii* and ♂*C. pandurata* follow the parent ♀*C. rumphii*.

Keywords: *Coelogyne*, genetic variation, hybrid, orchid, RAPD

INTRODUCTION

Coelogyne pandurata Lindl. as known as the black orchid is one of the most fascinating orchids which has characteristics of large green flowers and a contrasting black tongue. The species is found naturally in India, China, Indonesia, and Fiji Island, with distribution centers in Borneo, Sumatra, Malaya, and the Philippines. *C rumphii* Lindl. is another orchid species with beautiful flowers. It has greenish-yellow to yellowish cream sepals and petals, lip cream to whitish with orange-yellow at the base, lateral lobes tinged red to orange-brown inside with red-brown lines junction of epichile and hypochile. The species is distributed in Moluccas (Maluku) mainly in Buru, Ambon, and Ceram (Gravendeel and de Vogel 2000).

The charm of the black orchids challenges breeders to cross the orchid for new hybrid orchids and to create new genetic diversity. The selection of the parent species is very important in determining the success of a hybridization program, particularly in regard to the genetic similarity of the parents (Hartati 2017).

RAPD (*Random Amplified Polymorphic* DNA) is one method that can be used to analyze the genetic relationships of interspecies and intraspecies. Parab and Krishnan (2008) and Sulistianingsih et al. (2010) stated that the RAPD method can highlight differences among orchid species. The advantages of RAPD markers are simple in preparation for other molecular markers, such as Inter Simple Sequence Repeats (ISSR). The other advantages of RAPD markers are easy to employ for examining the differentiation of organisms. Several studies that have used RAPD techniques include the study of genetic diversity in *Dendrobium* (Xue et al. 2010), *Aerides* (Sivanaswari et al. 2012), *Vanda* (Tanee et al. 2012), *Cattleya* (Pinheiro et al. 2012), and *Coelogyne* (Hartati et al. 2014).

Genetic diversity analysis between parents and their hybrids has been conducted using the RAPD analysis on *Paphiopedilum* (Chung and Choi 2012), *Grevillea* (Pharmawati and Macfarlane 2013), and *Dendrobium* (Choopeng et al. 2019). Furthermore, RAPD analysis can distinguish wild orchids, hybrids, and species from one another by identifying different patterns.

Coelogyne has an enchanting beautiful flower so that it has a high economic value. However, unlike other orchids, the genus has not developed much of its genetic diversity. Based on ISSR markers, *C. pandurata* and *C. rumphii* have the closest genetic relationship that is 70%. Additionally, cytological studies have shown that the karyotype of *C. pandurata* is 2n=56, *C. rumphii* is 2n=72 and their hybrid is 2n=54 (Hartati et al. 2017). A second study showed that based on the morphological identification of these two species, they have a morphological similarity of 87% (Hartati et al. 2019a). Furthermore, the crosses of *C. pandurata* and *C. rumphii* are 100% successful using selfing, crossing, and reciprocal methods (Hartati et al. 2019b).

This study aims to assess the genetic variation of the parent species *Coelogyne pandurata*, *C. rumphii*, and their hybrids using RAPD markers.

MATERIALS AND METHODS

Plant materials

The species used in this study were *Coelogyne pandurata*, *C. rumphii*, and their F1 hybrids (Table 1). Both parent plants were obtained from the Bogor Botanic Gardens, LIPI and this is the site where the crosses were manually carried out. The seeds from the crosses were grown in tissue culture during research conducted at the Tissue Culture Laboratory at the Faculty of Agriculture, Sebelas Maret University from May until August 2019. In this study, the parent species *C. pandurata* and *C. rumphii* were each used in three replications, whereas the F1 hybrid was used in as many as 10 replications.

Procedures

DNA from the orchids was extracted using a method in which CTAB was modified with the addition of RNAse until the end concentration of 250 ug ml^{-1} was reached (Poerba and Ahmad 2013). Six RAPD primers were used from the original 12 primers (Operon Technology Ltd.) that were proven to produce polymorphic bands in orchids. Those six primers were OPA 02, OPA 07, OPA 13, OPB 12, OPB 17, and OPD 08. Each PCR tube contained 7.5 µl Green master mix (Promega), 5 pmol of single primer as much as 1.5 µl, and 1 µl of DNA sample. The first heating was carried out at 94 ºC for 5 minutes and this was followed by 35 cycles. Each cycle consisted of a denaturation phase at a temperature of 94 ºC for 1 minute, an attachment phase at a temperature of 50 ºC for 45 seconds, and an elongation phase at a temperature of 72 ºC for 2 minutes. After 35 cycles were completed, the PCR amplification process ended with an elongation phase at 72 ºC for 5 minutes and cooling at 25 ºC.

The amplification product was then separated by electrophoresis using 1.5% agarose gel in TAE (Tris-acetic acid- EDTA) at 100 volts for 120 minutes. Finally, DNA fragments were visualized with UV transilluminator gel documentation. A standard use 100 bp DNA Ladder was used to determine the band size of the DNA amplification results.

Data analysis

The amplification products were analyzed by marking their presence (1) or absence (0) for each DNA fragment generated. The data obtained were analyzed with the NTSYS-PC (Numeral Taxonomy and Multivariate Analysis Sistem) version 2.02. Genetic similarity was calculated according to Dice Coefficient using SIMQUAL (Similarity for Qualitative Data). The UPGMA (Unweight Pair Group Method with Arithmetic Averages) clustering method was used to construct a dendrogram (Rohlf 1998).

Table 1. Plant materials used in the study

Acc. species	Source of origin	Note
C pandurata	East Kalimantan	Single individual (3 replications)
C rumphii	South Sulawesi	Single individual (3 replications)
F1 Hybrid	♀*C. rumphii x* ♂*C. pandurata*	Single individual (10 replications)

Figure 1. The flower of *Coelogyne pandurata* (A) and *C. rumphii* (B) (Photo: Bogor Botanic Gardens 2012)

RESULTS AND DISCUSSION

The intensity of DNA bands in each primer amplification product is affected by the purity and concentration of the DNA template. It means that not all RAPD markers can be amplified in the hybrid plant as well as in their parents (Inthawong et al. 2006). In this study, it could be found at the OPD 08 primers contained 3 bands of 3 replicates of ♂ *C. pandurata* at 800 bp and 900 bp which decreased in 50% individual hybrids at replicates of 7, 8, 9, 10,11. While the other 3 bands from replication of 4,5,6 came from ♀ *C. rumphii* at 700 bp reduced individual hybrids by 50% in replication of 12, 13,14,15,16 (Fig. 2).

The OPB 12 primers contained 3 bands of 3 replicates of ♂ *C pandurata* which reduced 50% in individual hybrid 800 bp at replicates of 7, 8, 9, 10, 11. While the other 3 bands from replicates of 4,5,6 came from ♀ *C rumphii* at 350 bp and 1000 bp reduced 100% of all individual hybrids at 10 replications of 7,8,9,10,11,12,13,14,15,16 (Fig. 3).

Some DNA bands that appear in hybrids but are not found in the parents likely occur due to recombination or mutation. Nucleotide sequence variability can identify each interspecific hybrid and specific cultivar to distinguish it from the parent species and original species. Conversely, crossing chromosomes during meiosis can cause loss of primary sites so that the primers are amplified in the parent but not amplified in F1 (Tyagi et al. 1992). When cross-crossing of all compatible, it was found that none of them could produce hybrids (Inthawong et al. 2006). It suggests that certain species can be used as the only female parent plants and cannot be used as pollen donors.

The results of DNA amplification using six primers are presented in Table 2. Polymorphism is a picture of amplification obtained from differences in observed DNA fragments. Identification of the results of crossing in this study using six RAPD primers showed that 95.83% of the bands were polymorphic. The total number of bands produced was 43 bands, with the bands ranging in size from 200-2100 bp with an average amplified band of 7.17 bands per primer.

RAPD analysis on orchids present varied of amplified and polymorphic bands, such as *Rhynchostylis retusa* produced 76.13% polymorphic bands (4.38), with amplified bands of 5.79 bands per primer (Parab and Krishnan 2008), five species of *Dendrobium*, i.e. *D. bellatulum*, *D. densiflorum*, *D. fimbriatum*, *D. nobile*, and *D. apphyllum* using six primers produced in a total of 124 amplified bands, resulted in 122 polymorphic bands percentage of 98.39% and DNA length of 250 to 800 bp (Chattopadhyay et al. 2012), *Dendrobium nobile*, *D. moniliforme*, and their hybrids showed a total of 286 bands, polymorphic bands for each primer was 1-4 with an average of 2.27 bands per primer, and sizes ranging from 150 to 500 bp (Feng et al. 2013)..

UPGMA cluster analysis produced a dendrogram describing the genetic relationship among 16 replication studied (3 replications of *C. pandurata*, 3 replications of *C. rumphii*, and 10 replications of the hybrids). The results of crossing between ♀*C. rumphii* and ♂*C. pandurata*, and F1 hybrid have a similarity range of 0.16-1.00. A similarity of 0.49 the crossing of ♀*C. rumphii* and ♂*C. pandurata* is shown in two large clusters. The first cluster consisted only of ♂*C. pandurata* and the second cluster consisted of the ♀*C. rumphii* together with all hybrids, with genetic similarity in the hybrids obtained 77% or produce a genetic variation of 23% (Fig. 4). According to Lu et al. (2011), the closer the similarity coefficient between one species orchid, the greater the similarity and genetic distance.

The dendrogram shows that the hybrids of the crossing of ♀*C. rumphii* and ♂*C. pandurata* follow the parent ♀*C. rumphii*. It means that female parent is more dominant in giving their characteristics to their hybrid compared to male parent. This result supports Inthawong et al. (2006) that female parent is also more dominant than male parent in *Dendrobium* hybrids. The crossing results between ♀ *C. rumphii* x ♂ *C. pandurata* showed that RAPD analysis has been used successfully in *Coelogyne*.

Information on genetic relationships between individuals inter and intraspecies has been used for plant breeding. Furthermore, hybrid combinations in different hybrid lines show different compatibility (Yuping et al. 2012). The genetic relationship from the results of the RAPD analysis above still needs to be re-tested through crosses. It shows that certain species can be used as the sole mother plant and cannot be used as a pollen donor.

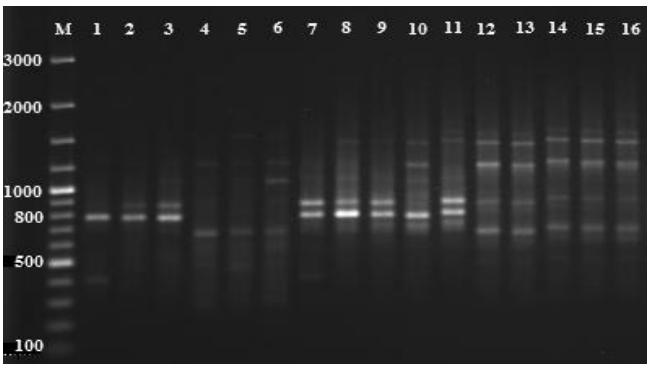

Figure 2. RAPD banding patterns of *C. pandurata* (lane 1-3), *C. rumphii* (lane 4-6), F1 Hybrid ♀*C. rumphii x* ♂*C. pandurata* (lane 7-16), generated by OPD-08 primer

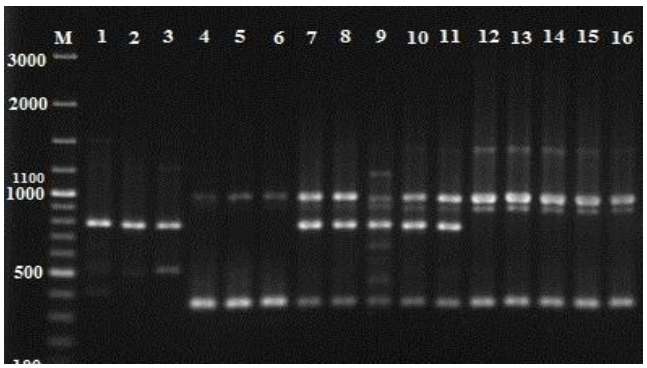

Figure 3. RAPD banding patterns of *C. pandurata* (lane 1-3), *C. rumphii* (lane 4-6), F1 Hybrid ♀*C. rumphii x* ♂*C. pandurata* (lane 7-16), generated by OPB-12 primer

Table 2. Primer sequence, polymorphic bands, and percentage of polymorphism in RAPD analysis

Primer	Sequence of nucleotides (5'-3')	Size (bp)	Total bands	polymorphic bands	Percentage of polymorphism
OPA-02	TGCCGAGCTG	450-1900	8	7	87.5
OPA-07	GAAACGGGTG	350-1100	7	7	100
OPA-13	CAGCACCCAC	400-2000	7	7	100
OPB-12	CCTTGACGCA	350-1000	6	6	100
OPB-17	AGGGAACGAG	200-2100	8	7	87.5
OPD-08	GTGTGCCCCA	700-1500	7	7	100
		Total	43	41	575
		Average	7.17	6.83	95.83

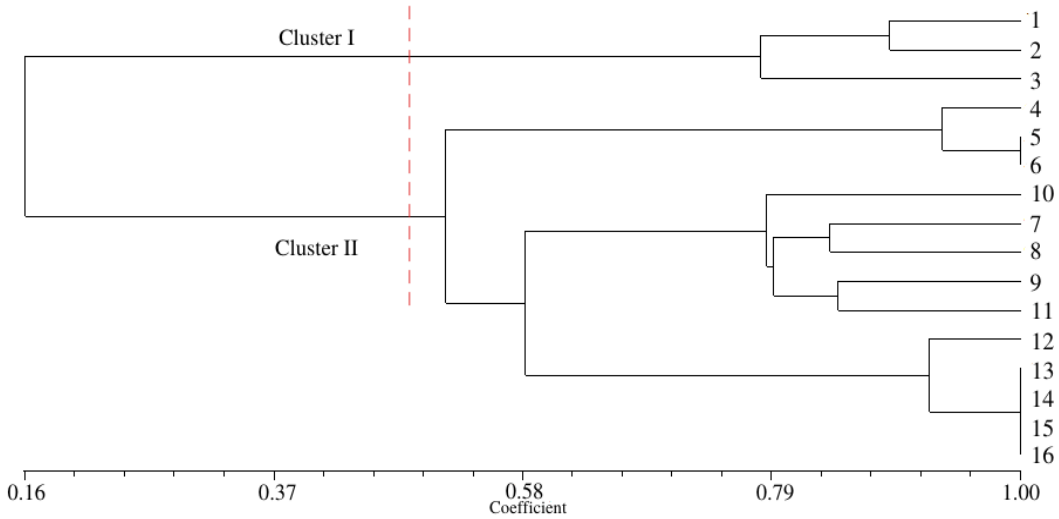

Figure 4. A dendrogram of *Coelogyne* generated by the UPGMA cluster analysis. individual parent of ♂*C. pandurata* (1-3), ♀*C. rumphii* (4-6), and the hybrids (7-16)

In conclusion, RAPD analysis has successfully to detect genetic variation of ♀ *C. rumphii*, ♂ *C. pandurata*, and their F1 hybrids. Six RAPD primers generated 95.83% polymorphic bands, with 43 bands in an average of 7.17 bands per primer. The parents and their F1 hybrid have a similarity range of 0.16-1.00. The dendrogram generated by the UPGMA cluster analysis separated the parents and hybrids in two large clusters, i.e. the first cluster consisted only of *C. pandurata* and the second cluster consisted of *C. rumphii*, together with all hybrid individuals. It means that the hybrids of the crossing of ♀*C. rumphii* and ♂*C. pandurata* follow the parent ♀*C. rumphii*.

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REFERENCES

Abbas B, Dailami M, Listyorini FH, Munarti. 2017. Genetic variations and relationships of Papua's endemic orchids based on RAPD markers. Nat Sci 9 (11): 377-385

Arya V, Yadav S, Yadav JP. 2011. Intraspecific genetic diversity of different accessions of *Cassia occidentalis* by RAPD marker. Genet Eng Biotechnol J 1 (22): 1-8.

Chattopadhyay P, Banerjee N, Chaudhary B. 2012. Genetic characterization of selected medicinal *Dendrobium* (Orchidaceae) species using molecular markers. Res J Biol 2: 117-125.

Choopeng S, Te-chato S, Khawnium T. 2019. The use of RAPD marker for verification of *Dendrobium* hybrid. Intl Agric Technol 15 (3): 399-408.

Devi BC, Sahaya SB, Servin WP. 2012. In vitro regeneration of *Coelogyne stricta* direct somatic embryogenesis. J Trop Med Plants 13 (2): 153-161.

Feng S, Zhao H, Lu J, Liu J, Shen BO, Wang H. 2013. Preliminary genetic linkage maps of Chinese herb *Dendrobium nobile* and *D. moniliforme*. J Genet 92: 205-212. DOI: 10.1007/s12041-013-0246-y

Gravendeel B, Vogel EF. 2000. Revision of Coelogyne section Verrucosae (Orchidaceae): A New section Delimitation based on morphological and molecular evidence. In Reorganising the Orchid genus *Coelogyne* Chapter 6. ISBN 90-71236-48-X

Hartati S, Nandariyah, Yunus A, Djoar DW. 2014. Genetic diversity of orchid *Coelogyne* spp. by molecular RAPD (Random Amplified Polymorphic DNA) markers. Int Appl Agric Res 9: 147-154.

Hartati S, Nandariyah, Yunus A, Djoar DW. 2017. Cytological studies on black orchid hybrid (*Coelogyne pandurata* Lindley). Biodiversitas 18 (2): 555-559. DOI: 10.13057/biodiv/d180216.

Hartati S. 2017. Study of genetic diversity on six species of Indonesian *Coelogyne* spp. based on ISSR Markers. Pak J Biol Sci 20 (11): 577-583. DOI: 10.3923/pjbs.2017.577-583.

Hartati S, Muliawati ES, Pardono, Cahyono O, Yuliyanto P. 2019a. Morphological characterization of *Coelogyne* spp. for germplasm conservation of orchids. Rev Ceres Viç 66 (4): 265-270.

Hartati S, Nandariyah, Yunus A, Djoar DW. 2019b. Hybridization technique of black orchid (*Coelogyne pandurata* Lindley) to enrich the genetic diversity and to rescue the genetic extinction. Bulgarian J Agric Sci 25 (4): 751-755.

Inthawong S, Weenun B, Nuttha K, Pimchai A. 2006. Analysis of intersectional hybrid of *Dendrobium* by RAPD technique. Kasetsart J 40 (2): 456-461.

Lu J, Hu X, Liu J, Wang H. 2011. Genetic diversity and population structure of 151 *Cymbidium sinense* cultivars. J Hortic For 3 (4): 104-114.

Parab GV, Krishnan S. 2008. Assessment of genetic variation among populations of *Rhynchostylis retusa* an Epiphytic orchid from Goa, India using ISSR and RAPD marker. J Biotechnol 7 (17): 313-319.

Pharmawati M, Macfarlane IJ. 2013. The genetics relationships of *Grevillea* hybrids determined by RAPD marker. Hayati J Biosci 20 (4): 196-200. DOI: 10.4308/hjb.20.4.196.

Pinheiro LR, Rabbani ARC, da Silva AVC, Lédo AdS, Pereira KLG, Diniz LEC. 2012. Genetic diversity and population structure in the Brazilian *Cattleya labiate*. Plant Syst Evol 298: 1815-1825. DOI: 10.1007/s00606-012-0682-9.

Poerba YS, Ahmad F. 2013. Genetic variation analyses of *Musa balbisiana* Colla based on RAPD and ISSR markers. Berita Biologi 12: 259-267. [Indonesian]

Rohlf. 1998. NTSYS-pc Numerical Taxonomy and Multivariate Analysis Syersion Version 2,02. Exerter software, New York.

Sivanaswari C, Thohirah LA, Fadelah AA, Abdullah NAP. 2011. Hybridization of several *Aerides* species and in vitro germination of its hybrid. Afr J Biotechnol 10 (53): 10864-10870.

Sulistianingsih R, Semiarti E, Purwantoro A, Mangoendidjojo. 2010. Analysis of genetic diversity of the orchid *Phalaenopsis amabilis* L. Blume mutants by RAPD. Proceedings of the National Seminar on Biology UGM Yogyakarta. 24-25 September 2010

Tanee T, Chadmuk P, Sudmoon R, Chaveerach A, Noikotr K. 2012. Genetic analysis for identification, genomic template stability in hybrids and barcodes of the *Vanda* species (Orchidaceae) of Thailand. Afr J Biotechnol 11 (55): 11772-11781. DOI: 10.5897/AJB11.3992

Tyagi BR, Ahmad T, Bahl JR. 1992. Cytology, genetics and breeding of commercially important *Mentha* species. Curr Res Med Arom Plant Sci 14: 51-56.

Xue D, Feng S, Zhao H, Jiang H, Shen B, Shi N, Lu J, Liu J, Wang H. 2010. The linkage maps of *Dendrobium* species based on RAPD and SRAP marker. J Genet Genom 37 (3): 197-204. DOI: 10.1016/S1673-8527(09)60038-2.

Yuping Z, Wang W, Li X, Zhao X. 2012. Study on interspecific compatibility of different combinations inner (inter) lily hybrids. J Sci Res 11 (5): 567-574.