

# Genetic similarity analysis of *Vanda tricolor* Lindley var. *Suavis* orchids using OPU 03 and OPU 16 RAPD marker

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**Abstract.** The purpose of this research is to understand the genetic similarity level of *Vanda tricolor* Lindley var. *Suavis* orchid plants that were regenerated from seeds of the same fruit/pod produced by self-pollination. The seeds were sown on New *Phalaenopsis* (NP) *in vitro* culture medium supplemented with 15 % coconut water (CW). Genetic similarity analysis was performed by PCR-based Random Amplification of Polymorphic DNA (PCR-RAPD) technique. The analysis was carried out at 16 months old regenerated plants after sowing (mas). Seven DNA samples were isolated using a modified Castillo method. It was then used to amplify (PCR) OPU 3 and OPU 16 primers. The electropherogram of PCR result was then converted to binary data and analysed using NTSYSpc software version 2.02. The genetic analysis result showed that the genetic similarity of 7 tested plants ranged between 0.4-0.8.

## 1. Introduction

*Vanda tricolor* Lindley var. *Suavis* has a beautiful colour and fragrant flowers. Two petals and sepals of the flower are white with the brownish-red and purple spot, whereas the lip (labellum) is purple with gradation to pink [1]. *V. tricolor* can be found in several regions in the world, one of these is on Merapi mountain [2]. Exploitation by humans and Merapi eruption are the main cause of the endangered plant. To propagate the plant, people usually planted self-pollinated seeds through *in vitro* culture. Self-pollinated of wild-type plants were done to plants with high genetic character similarity. *Ex situ* conservation of *V. tricolor* orchids can generally be carried out by maintenance in the garden or in the laboratory using *in vitro* culture techniques [3]. Although the use of self-pollination technique is assumed to yield genetically homogenous *Vanda* plants, the similarity level has never been measured.

## 2. Materials and Methods

The seeds of *V. tricolor* derived from the same fruit of self-aged results 12 bst collection from the Laboratory Plant Tissue Culture Department of Biology Education FMIPA UNY were grown *in vitro*. New *Phalaenopsis* (NP) + coconut water 150 ml.L<sup>-1</sup> was used as *in vitro* culture medium. Materials for DNA isolation consisted of Castillo extraction buffer (CTAB 10% + EDTA + Tris-HCl + NaCl), polyvinylpyrrolidone (PVP), chloroform: isoamyl alcohol 24: 1, isopropanol, RNase A solution (Thermo Scientific). Materials for DNA amplification consisted of Go Taq® Green PCR Master Mix



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(Promega), OPU-03 primer (5'CTATGCCGAC3 ') and OPU-16 primer (5'ACCTTGCGG3'). Materials for electrophoresis were agarose, loading dye, fluorosafe, buffer Tris Buffer EDTA (TBE) 0.5x and 100 bp marker plus DNA ladder (Vivantis). Equipment used for the manufacture of *in vitro* culture media consisted of analytical scales, 1 L Erlenmeyer, 250 ml measuring cup, hot plate + magnetic stirrer, pH stick, clock bottle and autoclave. The tools used for the *in vitro* planting process consisted of Laminar Air Flow (LAF), tweezers, spatulas, Petri dishes and spirit lights. The equipment used for DNA isolation consisted of mortar and stamper, 15 ml tube, centrifuge machine (Kokusan), pipette, water bath and thermometer. Tools for DNA amplification consisted of thermocycler machine (Eppendorf Mastercycle Hamburg), Eppendorf 0.2 µl tube, micropipette and tips. Tools for electrophoresis were electrophoresis chamber (Mupid e-Xu), parafilm, gel moulds, gel braces and tools for documentation in the form of cameras. UV Vis Spectrophotometer (Genesys) was used to measure the purity of DNA.

The descriptive explorative method was used in this research. The study was conducted in three stages of *in vitro* culture, DNA isolation and DNA amplification by PCR-RAPD technique. *In vitro* cultures were performed to obtain plant samples with subculture and incubation processes. DNA isolation was performed by Castillo method with modification at a 3,500 rpm centrifugation rate [4]. The plant sample was crushed with the addition of 0.1 gram of PVP and 6 ml of Castillo buffer then incubated in a water bath for 30 min at 65°C. The extraction was done by adding 6 ml of CI solution and centrifuged at 3,500 rpm for 20 min. The obtained supernatant was then extracted again. The supernatant on the second extraction added 5 ml of cold isopropanol and incubated at 4 °C. for one night (± 16 hours). The solution is then centrifuged at 3,500 rpm for 20 min. The obtained pellets were then resuspended with aquadest and RNase degradation was performed with the addition of RNase and then incubated at 37°C for one night (± 16 hours). For DNA amplification used composition as much as 1x Go Taq PCR Mix, 10 ng DNA templates, 5 µM primer with total volume 25 µl. The PCR process was carried out at 95°C. for 3 minutes for pre-denaturation followed by 45 repeating cycles consisting of denaturation at 95°C. for 30 seconds, annealing at 27°C for OPU-03 and 36 °C primers for primary OPU -16 for 30 seconds and extension at 70°C for 25 seconds. Final extension is carried out at 70°C for 5 minutes. The collecting of qualitative data of DNA was done by an electrophoresis method. The DNA concentration data were obtained by using Qubit Fluorometer, while for DNA purity was calculated using the UV Vis spectrophotometer at an absorbance of λ260 and λ280 by the formula:

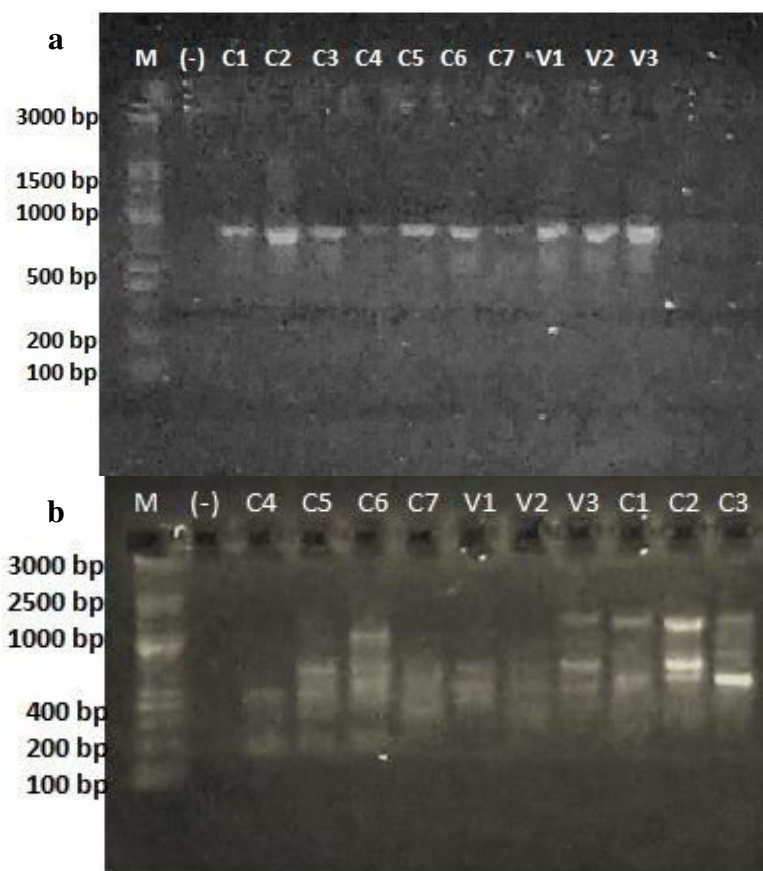
$$\frac{\lambda 260}{\lambda 280}$$

RAPD data analysis is done by scoring on the amplification result. The emerging band is scored 1 while the one that does not appear is given a score of 0. The data is then incorporated into the NTSYS 2.0.2 program to calculate the coefficient of simple matching equations and create a dendrogram using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) method [5].

### 3. Results and Discussion

The successful DNA amplification results using OPU 03 and OPU 16 primers was indicated by the appearance of the bands in all samples including three samples of orchid plants *V. tricolor in vivo* used as a benchmark for the identification of genetic uniformity. Band patterns generated from the amplification process with primers OPU 03 and OPU 16 indicated the presence of genetic variation. The amplified DNA band with OPU 03 primer exhibited a monoformic band, while the amplified DNA band with OPU 16 primer showed a polymorphic band (**Figure 1**). Hoon *et al.* (1999) show a high polymorphism band on the amplification results using OPU 03 primer in some species of the genus *Vanda* orchid [6]. This indicates that primer OPU 03 is better at showing polymorphism at the level of interspecies, whereas based on the primary visualization results of OPU 16 it is better to show polymorphisms at the level of intraspecies. The successful amplification of genomic DNA using

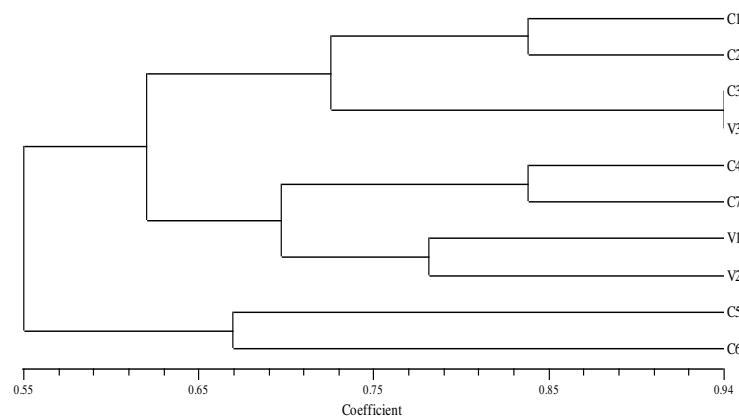
RAPD is largely determined by the sequence of primary bases used and their quality or primer content in each reaction.



**Figure 1.** Electropherogram of DNA amplification using primer OPU 03 (a) and OPU 16 (b); (C) *in vitro* plants 2, (C3) plants *in vitro* 3, (C4) plants *in vitro* 4, (C5) *in vitro* 6, (C6) *in vitro* plants 7, (V1) *in vivo* plants 1, (V2) plants *in vivo* 2, (V3) plants *in vivo* 3.

Analysis of genetic uniformity using NTSYSpc 2.0.2 software resulted in the orchid dendrogram of *V. tricolor* (**Figure 2**). The value of genetic similarity coefficient on orchid samples *V. tricolor* ranged between 0.4-0.8. These values indicate that the genetic similarity in the seven plants (C1-C7) has a considerable range. The range of coefficient values of genetic similarity far enough signifies that genetic diversity was present in all seven analysed plants. The genetic diversity that arises can be generated by plants derived from the seeds of non-homozygous pollination. Such diversity can naturally be attributed to Mendelian diversity due to the segregation of both parent genes (heterozygous). Although the RAPD technique used in this study has advantages in terms of ease, it also lacks the ability to identify heterozygotes [7]. The genetic diversity needs to be identified again at the molecular level using a marker other than RAPD capable to identify heterozygotes. *V. tricolor* orchid plant *in vitro* which has the highest similarity level was shown by C1 with C2 and C4 with C7 which has the value of genetic equation coefficient of 0.8. This means that the sample has a genetic similarity rate of 80%. While both sample C2 and C5 have the lowest level of genetic similarity of 0.4. This means that the level of similarity C2 with C5 is only about 40%. The orchid plant *V. tricolor in vivo* used as a comparator is also known to have genetic diversity. If *in vitro* and *in vivo* samples are

compared, it can be observed that the highest genetic similarity coefficient was shown by sample C3 with V3 of 0.9, while the smallest distance coefficient value was shown by C6 with V3 of 0.5.



**Figure 2.** Dendrogram of seven orchid plants *V. tricolor* grown *in vitro* and three orchid plants *V. tricolor* grown *in vivo* (C1-C7) *in vitro* samples; V1-V3 sample *in vivo*.

In addition to the internal factors of individual plants caused by heterozygous parent mating, the genetic variation that appears in the seven plants (C1-C7) can be caused by external factors that occur during *in vitro* cultures. Genetic variation that emerged *in vitro* cultures is a constraint in the effort of uniform plant propagation. These constraints arise as a result of the use of chemicals that at certain levels can function as mutagenic such as the use of colchicine and 2,4-D with high concentrations. In addition, the use of inappropriate techniques *in vitro* cultures allows for variation by either gene mutation or activation of the jump element that allows insertion, deletion and duplication. Variations occur mainly due to repetitive and uncontrolled subcultures resulting in high rates of multiplication [8].

#### 4. Conclusions

Genetic similarity analysis was successfully performed using PCR-based Random Amplification of Polymorphic DNA (PCR-RAPD) technique. Based on the research, it can be concluded that the results analysis of genetic similarity on seven orchid plants *V. tricolor* (C1-C7) self-pollination grown *in vitro* ranged from 0.4-0.8.

#### 5. References

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