Molecular Identification of Several Orchid Species Based on OPA10 and OPA18 RAPD Marker

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Abstract. The purpose of this study was to determine the genetic characteristics of nine orchids plants collected by Biology Gardens of Mathematics and Science Faculty of UNY based on OPA10 and OPA18 markers, to detect their genetic relationship, and determining the exact RAPD markers for identification of intraspecies and interspecies orchids. The analysis was carried out by isolating DNA from the leaves of 9 species of orchids (Rhynchostylis retusa, Vanda tricolor, Dendrobium aphyllum, Dendrobium fimbriatum, Dendrobium moschatum, Dendrobium crumenatum, Dendrobium antenatum, Dendrobium anosmum Gigantea Alba and Dendrobium anosmum Gigantea) using TIANGEN DNA healing plant kit. Molecular identification was analysed based on the PCR-RAPD technique using OPA 10 and OPA18 primers. Electrophoregraph of PCR results are converted into binary data and analysed with NTSYSpc 2.02 software. The similarity index (kinship level) was analysed based on UPGMA dendrogram. The results of the analysis showed that there was genetic diversity in 9 orchid plants tested. Intra-species orchids show higher similarity than inter-species. Primer OPA 10, OPA18, and a combination of both shows polymorphism in intra-species orchids.

1. Introduction

Indonesia has around 5000 of the 25,000 orchid species in the world that are scattered throughout the island [1]. High commercial values in the fields of medicine, food, ornamental plants, and cut flowers, make orchids as one of the flower plants with the highest trade rank globally. CITES (Convention on the International Trade in Endangered Species of Wild Fauna and Flora) reports that the world orchid trade is dominated by orchids that have beautiful, fragrant, long-lasting flower colors, shapes and patterns. Some genera found in the market are Cymbidium, Dendrobium, and Phalaenopsis [2].

At present 736 genera are recorded in Orchidaceae. New discoveries continue to occur every year and the orchid genus is described as reaching 13 per year [3]. Updates to the classification of plants can occur due to new findings. Morphological identification, in general, is an accepted basis for describing new taxa. At present, there are many descriptions of new genera that involve molecular analysis (DNA). The description of the new species currently also involves molecular DNA studies to show its peculiarities [4]. The development of molecular techniques for genetic analysis in the past decade has increased information on orchid genetic diversity. Molecular techniques have been used to study variations in DNA sequences among orchid species and cultivars [5]. According to [6] descriptions of orchid taxa at any level must include molecular studies and morphological studies.
PCR-based molecular markers are more widely used because they require low concentrations of DNA, are able to amplify DNA sequences from stored tissue, are simple, and relatively inexpensive [7]. Among the molecular markers that have been found, RAPD is a molecular marker that is widely used to identify genetic characters in plants. The RAPD technique is based on DNA amplification with a single primer that targets random sequences. Many current research publications use the RAPD technique to determine genetic equations in orchid plants such as *Vanda tricolor* which uses OPA10 primers to detect some clonal variation in propagation through in vitro culture [8].

At this time the Biology Garden of Faculty of mathematics and science of UNY collects various types of orchids for conservation purposes as well as germplasm sources in the development of varieties. Morphological identification has been carried out, but molecular identification has not been done. The genetic characteristics of these orchids have not been identified so that the research is important to support data on a collection of orchid species (*Rhynchostylis retusa, Vanda tricolor, Dendrobium aphyllum, Dendrobium fimbriatum, Dendrobium moschatum, Dendrobium crumenatum, Dendrobium antennatum, Dendrobium anosmum* Gigantea Alba and *Dendrobium anosmum* Gigantea).

2. Method

Nine orchids (*Rhynchostylis retusa, Vanda tricolor, Dendrobium aphyllum, Dendrobium fimbriatum, Dendrobium moschatum, Dendrobium crumenatum, Dendrobium antennatum, Dendrobium anosmum* Gigantea Alba and *Dendrobium anosmum* Gigantea) were collected from Biology Gardens Faculty of Mathematics and Natural Science UNY. DNA isolation was carried out using TIANGEN DNA isolation plant kit. The source of DNA comes from the leaves of 9 orchid plants analysed. The subsequent DNA isolation is visualized through electrophoresis. The concentration that is used is 0.8% which is added with 5µl DNA staining (fluorosafe). The electrophoresis process is carried out at a voltage of 100 V for 20 minutes. The reading and documentation of the visualization results are done using the 1012 Series Gel Documentation System. The isolated DNA was amplified using the OPA10 (5’CTGATCGCAG3’) and OPA18 (5’AGGTGACCGT3’). The amplification process was carried out using the Variti Thermal Cycler tool. The results of the amplification process were then migrated on agarose gel with a concentration of 0.8%. Migration is carried out on electrophoresis devices with a voltage of 50 V for 60 minutes. Electrophoresis was continued by visualization using the 1012 Series Gel Documentation System.

Analysis of RAPD data was done by scoring the amplification results. The bands that appear are given a score of 1 and those that do not appear are given a score of 0. Subsequent data is applied to the NTSYSpc2.0.2 program to calculate the equation coefficients and make a dendrogram using the Unweighted Pair-Group Method using Arithmetic Average (UPGMA).

3. Result and Discussion

3.1. Result

The results of genomic DNA amplification from nine orchids plants tested using RAPD OPA10 and OPA18 markers showed a diversity of genetic (polymorphism) (Figure 1). The diversity is indicated by the number and variation in the size of the fragments produced (Table 1).

| Sample | OPA10 | OPA18 |
|--------|-------|-------|
| Σ fragmen | Measurement (bp) | Σ fragmen | Measurement (bp) |

Table 1. Number and measure of DNA fragments
|   | 5 | 1531, 1052, 839, 626, 200 | 7 | 1739, 1397, 1282, 1168, 1026, 826, 541 |
|---|---|------------------------|---|------------------------|
| 2 | 4 | 998, 839, 572, 359       | 4 | 997, 826, 427, 228      |
| 3 | 4 | 1078, 918, 492, 359     | 3 | 1054, 826, 456         |
| 4 | 3 | 918, 492, 359           | 3 | 1054, 836, 427         |
| 5 | 3 | 1584, 1344, 439         | 5 | 1379, 1168, 940, 826, 541 |
| 6 | 4 | 1424, 1238, 1078, 413  | 1 | 541                    |
| 7 | 3 | 1078, 839, 705          | 3 | 627, 456, 313          |
| 8 | 4 | 142, 1131, 918, 519    | 4 | 1282, 1111, 997, 714   |
| 9 | 5 | 1398, 1078, 998, 839, 679, 541 |

Note: (1) *R. retusa*, (2) *V. tricolor*, (3) *D. anosmum* Gigantea Alba, (4) *D. anosmum* Gigantea, (5) *D. aphyllum*, (6) *D. fimbriatum*, (7) *D. moschatum*, (8) *D. crumenatum*, (9) *D. antennatum*

**Figure 1.a** Electrophoregram of nine orchids using RAPD OPA10

**Figure 1.b** Electrophoregram of nine orchids using RAPD OPA18

**Figure 2.** UPGMA dendrogram of OPA10 and OPA18

### 3.2. Discussion

OPA10 RAPD markers produce more than 2 fragments for all samples. The highest number of fragments (5 fragments) was produced by *R. retusa* (sample 1) while the least number of fragments (3
fragments) was produced by *D. anosmum* Gigantea (sample 4), *D. aphyllum* (sample 5), and *D. moschatum* (sample 7). Otherwise, OPA18 produce the highest number of fragments (7) by *R. retusa* (sample 1) and the least number of fragments was produced by *D. fimbriatum* (sample 6). The diversity of DNA migration patterns produced through amplification using RAPD is widely used for the identification of organisms based on plant genetic characters.

Several RAPD-based studies to analyse diversity and genetic characters have been carried out. The research on *Gosypium hirsutum* L. germplasms aimed at analysing diversity and genetic characters using OPA10 RAPD markers showed high polymorphism with a total of 44 fragments with fragments ranging in size from 500 - 2400 bp. Similar research was also conducted on *Oryza sativa* L. which showed 32 polymorphism fragments from 34 fragments [9]. The number and intensity of the DNA fragments produced are very dependent on the primer complementation component of the DNA template used [10]. DNA polymorphism is detected based on the appearance and absence of fragments. The absence of fragments can be caused by a primary failure in the process of annealing a site in some individuals due to differences in nucleotide sequences or due to insertion or deletion at the primary site [11].

*D. anosmum* Gigantea Alba (sample 3) and *D. anosmum* Gigantea (sample 4) are intraspecies orchids. The morphology of the two types of orchids has similar shapes, sizes, and colours of leaves, stems, roots. The difference between the two plants lies in the colour of the flower. Based on the results of the RAPD using the RAPD OPA10 and OPA18 the two plants showed different genetic characters but several DNA fragments showed the same size (i.e. fragments measuring 918 bp and 492 bp). In interspecies orchids (*D. fimbriatum* and *D. crumenatum*) show the same number of fragments (4 fragments) and have one fragment which is the same size (1424 bp). Based on the classification according to morphological characters, *D. anosmum*, *D. aphyllum*, *D. fimbriatum*, and *D. moschatum* in the same section (Dendrobium). Typical morphological characters in this section are the shape of the pseudo bulb with medium length. The leaves are in the upper 2/3 of the stem. The leaves change after a year. Have a leaf midrib. Flowers appear from the apical stem with a long or short flower arrangement during the dry season. The size of the flower is usually large and attracts attention [12].

The results of the relationship analysis based on the RAPD OPA10 and OPA18 markings contained in the UPGMA dendrogram indicate the genetic relationship of orchid plants based on the genetic similarity index (Figure 2). At the 75% similarity level, the genotype analysed is divided into seven clusters. Just one cluster (Cluster 5) showed genetic similarities in intraspecies orchids (*D. anosmum* Gigantea and *D. anosmum* Gigantea Alba) with level of similarity 92%. *D. moschatum* and *D. fimbriatum* showed genetic similarities in interspecies orchids with level 76%.

4. Conclusion

Nine orchid plants showed genetic diversity based on the RAPD OPA 10 and OPA 18 markers, each producing different number and size of fragments. The results of genetic analysis based on OPA10 and OPA18 RAPD markers showed that there was genetic diversity in 9 orchid plants. The similarity level of intraspecies orchids (*D. anosmum* Gigantea and *D. anosmum* Gigantea Alba) were higher than interspecies orchids (*D. aphyllum*, *D. fimbriatum*, *D. moschatum*, *D. crumenatum*, *D. antennatum*). The amplification results using RAPD OPA 10, OPA 18 markers, and a combination of the two markers, genotypically showing orchids *D. anosmum* Gigantea and *D. anosmum* Gigantea Alba is in the same group, OPA10 and OPA18 can be used as molecular markers for classification intraspesies orchid.

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