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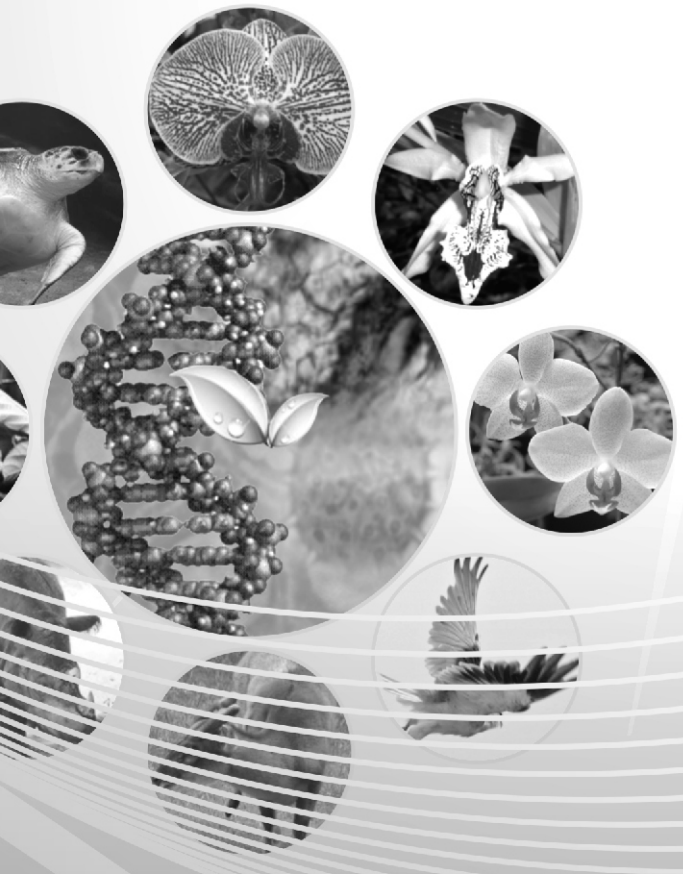

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O-MB08

STUDY ON GENETIC DIVERSITY AND CONSERVATION OF ORCHIDS IN WONOSADI FOREST, GUNUNGKIDUL BASED ON MOLECULAR ANALYSIS

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ABSTRACT

Wonosadi forest is located between Dusun Duren and Dusun Sidorejo, Beji village, Ngawen, Gunungkidul. The biodiversity inside Wonosadi is protected by people around Wonosadi using local wisdom. One of the endemic biological diversity in Wonosadi is natural orchid. Conservation which is related to biodiversity were needed to maintain the existence of natural orchid in Wonosadi sustainly. Study on natural orchid in Wonosadi can be used as the database for conservation programs. In this study, genetic variation was analysed using random amplified polymorphic DNA (RAPD), while viruses were detected using reverse transcript-polymerase chain reaction (RT-PCR).

The results show that there were genetic diversity in the populations of natural orchid in Wonosadi. It can be concluded that the population of natural orchid in Wonosadi can be adaptive to environmental change. Genetic diversity is required for populations to evolve to cope with environmental change. It can be used as a database to develop the potential of natural orchid in Wonosadi forest. The viruses found in population of natural orchid in Wonosadi were Cymbidium mosaic virus (CyMV) and Odontoglossum ringspot virus (ORSV). Viruses existance might be recognised from the physical symptom on plant and analysis of coat protein (CP) gene using polymerase chain reaction (PCR).

The research of diversity and conservation of natural orchid in Wonosadi forest may be used to support education for sustainable development (EfSD) concept in conservation of biological diversity. Exploration activity can be focused at area which is protected by local wisdom and involves local people. By doing this, local people may be actively included on protecting and developing biological diversity at their own comunity. Thus, it may result a sustain condition of natural resources. This research can be used in supporting and developing programs of natural orchid in Wonosadi forest in order to develop the conservation programs.

Keywords : genetic diversity, natural orchid, CyMV, ORSV, conservation

INTRODUCTION

Wonosadi forest is located between Dusun Duren and Dusun Sidorejo, Beji village, Ngawen, Gunungkidul. The biodiversity inside Wonosadi is protected by people around Wonosadi using local wisdom. The entire area of Wonosadi forest is about 25 Ha. About 15 Ha of the entire area is located in Dusun Duren, and the rest (10 Ha) is located in Dusun Sidorejo. Wonosadi forest consists of core zone and buffer zones. In the core zone, there was customary rules that forbid on taking anything in the forest. Whereas, the buffer zones were utilized by local people for plantation, especially woody plants.

Wonosadi forest is managed by local wisdom. It makes the biodiversity inside Wonosadi is totally protected by people around this forest. One of the endemic biological diversity in Wonosadi is natural orchid. Orchid in tropical forest can grow naturally and diverse, from epiphyte to terrestrial [2 & 3]. Orchids that natively grow in Wonosadi are terrestrial orchids that can be found in open area with high intensity of light. Conservation which is related to biodiversity were needed to maintain the existence of natural orchids in Wonosadi sustainly. It can be done through the study on diversity of natural orchid in Wonosadi [11 &14].

The maintenance of the natural orchid existence in Wonosadi forest can also be done by detection of disease that harm the orchid. Orchids in Wonosadi grow in the area which are not maintained by local people. There was great possibility that the orchid was attacked by viruses. Two viruses that commonly found in orchid are Cymbidium mosaic virus (CyMV) and Odontoglossum ringspot virus (ORSV) [9]. The study on genetic diversity and virus detection of natural orchid in Wonosadi forest has not been done. This research can be used as the database in supporting and developing programs of natural orchid in Wonosadi forest in order to develop the conservation programs. The people around Wonosadi can be involved to support education for sustainable development (EfSD) concept in conservation of biological diversity.

MATERIALS AND METHODS

Plant Materials

Young leaves approximately 50-100 mg of specimen will be used as the source of DNA and RNA extraction for PCR-RAPD analysis and viruses detection. Before used, the specimen will be kept in vinyl zipper bags with silica-gel until they were stored at -20° C in the laboratory [4]. Those samples were collected in May 2010 on the wet season, when terrestrial orchid in Wonosadi usually grown.

Genetic Diversity

Total DNA was extracted from young leaves collected using *Nucleon Phytopure* kit and adapted to Orchid as follows: 0,1 g young leaves extracted with Phytopure I reagent. Then, the Phytopure II reagent was added. The mixture was incubated at 65°C for 10 minute, followed by incubation at 4°C for 20 minute. Then Phytopure ressin was added, followed by extractions with isopropanol. Isopropanol was used to precipitate nucleic acids, and the pellet obtained was dissolved in Tris-EDTA (TE) buffer (10 mM Tris-HCl, pH = 8.0

and 1 mM EDTA, pH = 8.0). The total DNA was quantified by spectrophotometry [13]. DNA samples were stored at 4°C.

Six decamer oligonucleotides (Table 1) were used for polymerase chain reaction (PCR) amplification [10] following the procedures of Lim *et al.* (1998) with some modifications. Experiments were carried out with SuperHot *Master Mix* PCR *kit* which consists of 0,4 mM dNTPs of each, $MgCl_2$, Taq DNA Polymerase, 32 mM $(NH_4)_2SO_4$, and 130 mM TrisHCl, pH 8,8. The thermal cycler was programmed to have a cycling profile of 1 min denaturation at 95 °C, 2 min annealing at 35 °C and 2 min extension at 72 °C for a total of 45 cycles, using the fastest possible transitions between each temperature. A final extension at 72 °C for 10 min was included after the last cycle. The DNA fragments produced were visualized in a 1,5% agarose gel and stained with ethidium bromide. Replication of the RAPD reaction for every combination of template DNA and primer was carried out to ensure reproducibility. Only reproducible RAPD markers were included in the analysis.

The molecular sizes of the amplification products were estimated using 100 bp DNA ladder plus (Microzone, Ltd, UK). Bands on the photos were then scored. The RAPD bands were represented as '1' (present) and '0' (absent). The PCR was repeated at least twice in order to check reproducibility. The dendrogram following the NTSYS, UPGMA algorithm was generated with the Jaccard coefficient based on all the markers generated [12].

Table 1. Primers used in RAPD analysis.

Code	Sequence 5' to 3'
OPU3	CTATGCCGAC
OPU8	GGCGAAGGTT
OPU10	ACCTCGGCAC
OPU12	TCACCAGCCA
OPU13	GGCTGGTTCC
OPU16	CTGCGCTGGA

Virus detection

RNA virus was isolated from orchid's leaf samples by grinding 0,1 gram of leaf in 1 ml Redzol reagent, followed by chloroform extraction and ethanol treatment. RNA was separated from other contaminant by centrifugation of homogenate in SiMax™ *membrane spin column*. The yield of RNA was diluted in 50 µl DEPC water. cDNA synthesis was carried out using gene specific primer (reverse primer) with reverse trancription (RT) kit from Two step RT-PCR kit (SBS Genetech). PCR step was done directly after cDNA synthesis by

using the same kit. Specific primer which were used in RT-PCR were specific for amplifying the gene coat protein of CymMV and ORSV.

Table 2. Sequences of specific primer used in RT-PCR step [7]

Primer	Nucleotide sequence 5' – 3'
CymMV CP-F1	ATGGGAGAGYCCACTCCARCYCCAGC
CymMV CP-R1	TTCAGTAGGGGGTGCAGGCA
ORSV CP-F1	ATGTCTTACACTATTACAGACCCG
ORSV CP-R1	GGAAGAGGTCCAAGTAAGTCC

Amplification of cDNA was done by Thermocycler (Eppendorf) using time-design : Pre-denaturation at 94^0C for 5 minutes, denaturation at 94^0C for 1 minute, annealing 50^0C for 1 minute, elongation 72^0C for 2 minutes, and post-elongation 72^0C for 7 minutes. Cycle was programmed for 34 cycles. For further analysis, PCR products were analysed by electrophoresis in 2% agarose gel in TBE buffer. gel was stained with ethidium bromide (1 µg/10 ml aquades. The DNA bands on gel were examined under UV-transilluminator. DNA marker 100 bp was used to estimate the size of PCR products.

RESULTS AND DISCUSSION

The diversity of orchid in Wonosadi forest were analysed using RAPD method. The samples were taken from 3 populations : Pelataran Ngenuman (population 1), east buffer zone (population 2), and west buffer zone (population 3). From the observation, there were 3 species of natural orchid in Wonosadi forest, *Pecteilis sussanae*, *Liparis sp.*, dan *Spathoglottis sp.* RAPD results were shown in DNA fragments :

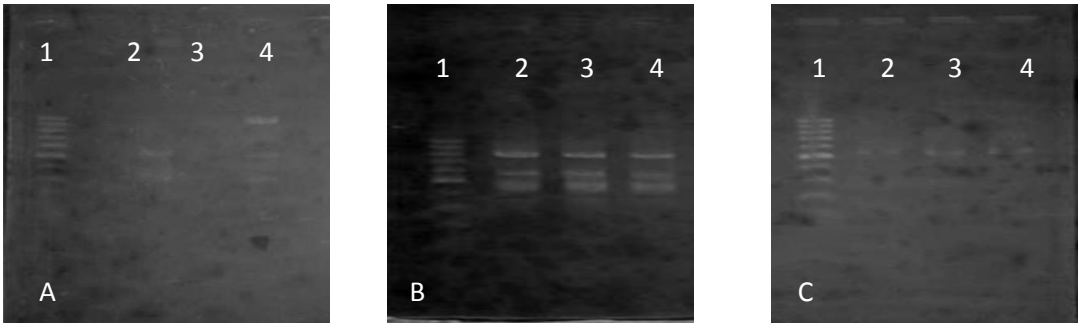

Figure 1. RAPD profiles of *Pecteilis susannae* using primer (A.) OPU 8, (B.) OPU 3, (C.) OPU 10, (1.) DNA ladder, (2.) Population 1 (3.) Population 2, and (3.) Population 3.

DNA profiles than analysed using NTSYS program to construct the dendrograms for each species :

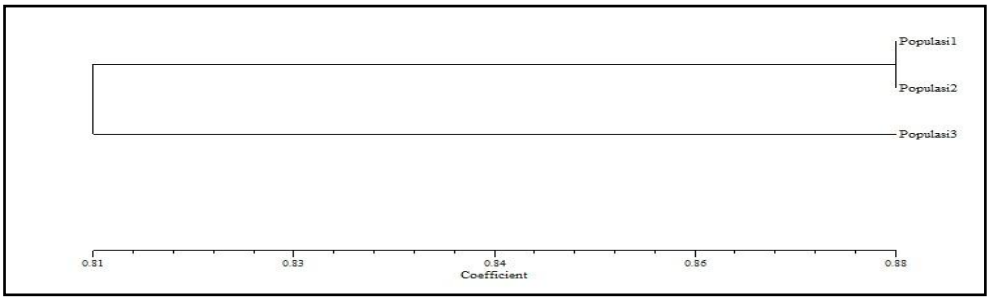

Figure 2. *Pecteilis susannae* dendrogram on 3 populations in Wonosadi forest.

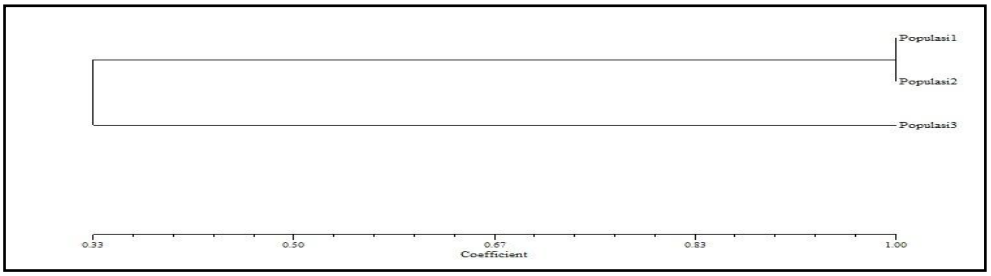

Figure 3. *Liparis sp.* dendrogram on 3 populations in Wonosadi forest.

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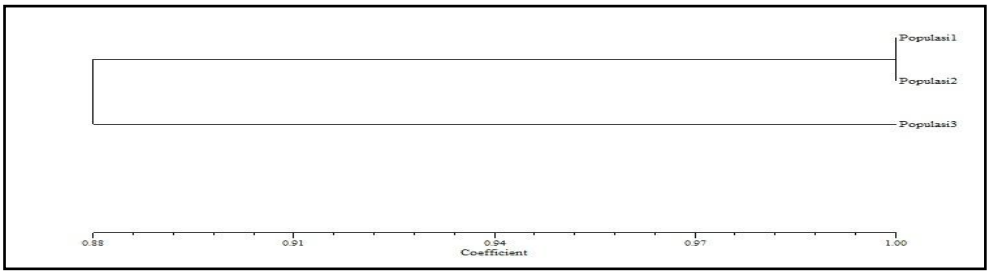

Figure 4. *Spathoglottis plicata* dendrogram on 3 populations in Wonosadi forest.

The results showed that 3 populations of orchid in Wonosadi were separated in 3 different branches. It revealed that there were genetic diversity in those orchid populations caused by adaptation in different habitat. Population 1 and 2 were located inside Wonosadi forest. Both were natural populations with minimal influence of human. Whereas, population 3 were located outside Wonosadi forest which is bordering with villages. Genetic diversity describes the evolutionary potential of population. Since evolution, at its most basic level, is a change in the genetic composition of a population, it only occurs when there is genetic diversity [6]. Genetic diversity allows populations to tolerate a wide range of environmental extremes. Loss of genetic diversity is often associated with inbreeding and reduction in

reproductive fittness and survival [1]. Genetic diversity in populations were required to respond the environmental change and avoid extinction [5].

Virus Detection

Based on electrophoresis result, it was known that Wonosadi orchid was infected by ORSV. DNA band at ±474 bp was appeared *Liparis* sp. sample (L2) and was the only positive sample for ORSV. Liparis was also assumed being infected by CymMV since there was faint band at ±669 bp. Compared to other Wonosadi orchids, Liparis has a thinner and smoother leaf which may lead to it's higher sensitivity to virus than other.

Other orchid that were not infected by virus (according to electrophresis result) may show similar symptom as virus-infected orchid since virus' symptoms is varied among orchid. The similar symptom may appear due to other pathogen attacks or extreme environmental factors.

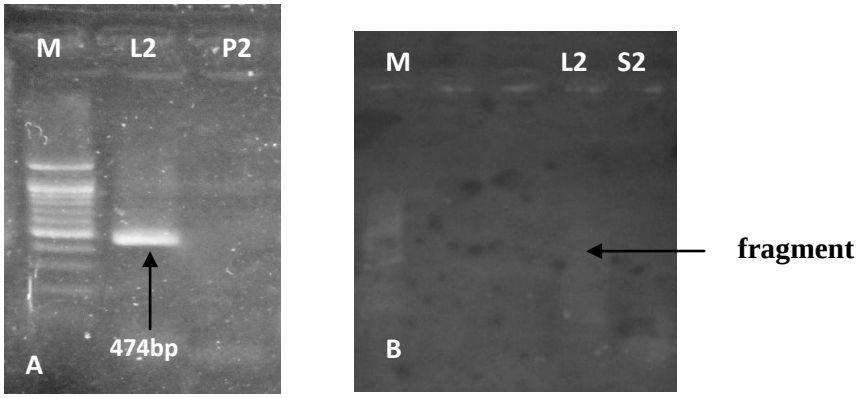

Figure 5. Electrophoresis result of PCR product for ORSV at 474 bp (left) and CymMV at 669 bp (right). M = marker; L2 = *Liparis* sp.

Activities Based on EfSD implementation

Research on genetic diversity and virus detection of natural orchid in Wonosadi forest has become potential effort to support Education for Sustainable Development (EfSD) concept. The activities may be foccused on biodiversity exploration around Wonosadi. Exploration activity can be focused at area which is protected by local wisdom and involves local people. By doing this, local people may be actively included on protecting and developing biological diversity at their own comunity. Thus, it may result a sustain condition of natural resources.

The implementations of EfSD concept were based on the results of the research. By understanding the genetic diversity of natural orchid, local people can be more familiar to orchid species inside Wonosadi, its potential, and how to maintain in an appropriate way.

Whereas, the research of virus detection in natural orchid can assists local people in understanding the symptoms and how to cope with the illness caused by viruses. The results of this research can be used to optimize the natural orchid conservation. The implementation of EfSD in this research was manifested by building of “Rumah Anggrek”. “Rumah Anggrek” was expected to be the center of ex-situ conservation of natural orchid which located near Wonosadi. Natural orchids from Wonosadi were taken and grown in ”Rumah Anggrek”. It also facilitates people who want to see the natural orchids in Wonosadi, without entering the forest. The making of “Rumah Anggrek” was conducted by cooperation with local people and students of KKN-PPM program from Gadjah Mada University. The development of natural orchid’s potential in Wonosadi Forest can also be used in ecotourism activities. It could support the economy condition of local people around Wonosadi.

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