Population Genetics of *Hoya multiflora* at Sukamantri of Gunung Salak, West Java, Indonesia Based on Isozyme Analysis

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Abstract

Hoya multiflora Blume is tropical epiphytic plant reported having some medicinal properties in the rural communities in Asiatic counties i.e India and Malaysia. In Europe and USA, this plant has been internationally traded as exotic ornamental plant. The traded plant was directly extracted from the wild habitat, which imply on the population equilibrium. Population genetic study on epiphytic *Hoya multiflora* plant has been conducted using the plant population at Sukamantri at gunung Salak, West Java Indonesia. The study was based on the isozyme analyses by using PER, EST, MDH, ADH, AAT. The results showed that analyses using POPGENE software indicated that the genetic differentiation was low, but the estimation on gene flow was high.

Keywords: Hoya multiflora*, isozyme, population genetic*

Introduction

Hoya multiflora Blume (*Apocynanceae:Asclepiadoideae*) is one of the economic important species as ornamental plant, and has been used traditionally as medicine, particularly to treat arthritis-rheumatism (Burkill, 2002) and stomach/intestinal ailments (Ambasta, 1986). The active compound of this plant is unrevealed yet, but it is predicted to be Indomethacine like compound. Indomethacine, a common non-steroidal anti-inflammatory drug (NSAID), has been used for more than 30 years to treat symptomatic pain of rheumatoid arthritis. Recently, this compound has been tested as a new class of anti HIV drug (Bourinbaiar & Lee-Huang, 1994) and it seems to be specific since no toxicity at clinical doses.

The distribution of *H. multiflora* ranges from India to New Guinea (Goyder, 2008) from 50 to1500 m above sea level (Rahayu *et al*., 2010). This species is characterized by its short (non vein) plant, leathery (non succulent) oblong leaves and arrow head form white corona with yellow tip of the corolla. Flowers is arranged in umbel appear at interpetiolar node. This plant produces white latex from all of its part (Rahayu 2006). In Europe and USA, this plant has been internationaly traded as exotic ornamental plant. The traded plant was directly extracted from the wild habitat, which imply on the population equilibrium.

All of *Hoya* plant species including *H. multiflora* are epiphyte. In the natural habitat, the presence of this plant depends on the presence of host plant, which defined as phorophyte (Benzing 2008). So this species faces problems in decreasing habitat due to the increase of deforestation. The decrease on the number of forest tree will imply on the population decrease of this species, which in turn will imply on the genetic erosion toward extinction. Little is known about population genetic of this species. The study on population genetic will provide data to support conservation strategies and development of this species as economic plant. Genetic markers generally have contributed to the study of plant biology by providing methods for detecting genetic differences among individuals. There are some important ecological topics which often use allozymes as powerful markers, especially to assess the genetic differentiation among populations

(Zeidler, 2000). This research was aimed to assess the genetic differentiation of *Hoya multiflora* populations at Sukamantri of gunung Salak, West Java Indonesia based on the isozyme analysis.

Materials and Methods

Sample source

A total 50 samples were obtained from three populations of *H. multiflora* from Sukamantri. All populations were located at 700 – 800 m above sea level. Population 1 was from Buper Sukamantri with the total sample of 16, population 2 was from Tapos with the total sample of 16 and population of 3 were from Bobojong with the total sample of 18. All of sample were collected as stem cutting and planted at the greenhouse at Bogor Botanical Gardens as pot plants. After 2 months of acclimation, the plant produced new shoot and young leaves. Young leaves with the size less than 3 cm long were used as enzyme source.

Isozyme extraction

The method of enzyme extraction followed Soltis and Soltis (1989). Leaves were processed at the same day they were collected from the plants. Approximately 400 mg of samples were placed in a mortar, extract buffer were added than it was grinded well. The liquid of extract was adsorbed by a 0.5 x 0.5 cm filter paper and ready to placed in to the prepared gel.

Electrophoreses

Starch was prepared as follows: equal amounts of the two starch types (16.5 + 16.5 g) were mixed well and fully suspended in 100 mL the appropriate gel buffer in a flask. The remaining 200 mL of this buffer were brought to a boil before being added to the suspension. The buffer systems used depended on the enzyme activities to be assayed, and included the following: AAT (Aminoacid transferase), ADH (Alcohol dehidrogenase), EST (Esterase), PER (Peroksidase), MDH (Malate dehidrogenase), ACP (Acid phosphatase). The gel were mold to become firm. The wells (0.5 x 0.5 cm) were prepared and fill with the samples and indicator (bromphenol blue). The electrophoresis was run in a refrigerator at 4oC for 3 h at 100 V at the beginning and increase to 200V.

Staining and visualization

After electrophoresis the gels were sliced producing three replicas that were placed in different enzyme staining solutions. Gels were placed on a white light transilluminator to improve the identification of bands colocalizing with the specific enzyme activities.

Data Analysis

The polymorphic bands were transformed into numeric data as co-dominant data, and run by using POPGENE software (Yeh *et all*, 1999). The genetic variation intra and inter population were measure with assumption population at Hardy–Weinberg's equilibrium, $p2+2pq+q2 = 1$. Genetic variation (Ht) in a population was measure by Nei's (1978), while genetic variation inter population (genetic diferentiation=Gst) was measure as fixation index (Fst) of (Wright 1978); $Gst = Fst = (Ht-Hs)/Ht$.

Results and Discussion

There were four alleles found with the various distributions. The common two allele was A and B which express at the all isozymes. Four alleles were found at the EST, three alleles at the ADH, ACP, MDH, and two alleles at PER and AAT (Table 1). Among the four alleles, A was the

most frequent in EST, ADH, and ACP, and express more than 50% (dominant). The allele C was only found at EST with the lowest frequency (0.06) and categorized as a rare allele. Allele O express in EST, ADH, ACP and MDH in low frequency (below 0,3).

Table 1. Allele frequency from total populations

Allele \ Locus	EST	PER	ADH	ACP	MDH	AAT
Allele A	0.5600	0.4400	0.8400	0.5600	0.3000	0.3800
Allele B	0.1400	0.5600	0.1600	0.1600	0.6600	0.3800
Allele C	0.0600	-	-	-	-	-
Allele O	0.2400	-	0.2800	0.0400	0.2400	-

Allele heterozygocity as shown at Table 2, was range from 0.2303 to 0.5631 with the mean was 0.4197 (under 50%). The highest was at EST (0.5631), and the lowest was at ADH (0.2303). The result express the low heterozygocity wich mean its low genetic diversity.

Table 2. Allele heterozygocity

Locus	Sample size	Obs-Hom	Obs-het	Exp-hom*	Exp Het*	Nei**	Ave Het
EST	50	0.6000	0.4000	0.3820	0.6180	0.6056	0.5631
PER	50	0.7600	0.2400	0.4971	0.5029	0.4928	0.3307
ADH	50	0.6800	0.3200	0.7257	0.2743	0.2688	0.2303
ACP	50	0.8400	0.1600	0.4057	0.5943	0.5824	0.4220
MDH	50	0.4800	0.5200	0.5176	0.4824	0.4728	0.4641
AAT	50	0.7200	0.2800	0.3331	0.6669	0.6536	0.5083
Mean	50	0.6800	0.3200	0.4769	0.5231	0.5127	0.4197
St. Dev		0.1265	0.1265	0.1405	0.1405	0.1377	0.1218

* Homozygocity and heterozygocity estimation (Levene ,1949)

**Number of polymorphic loci = 6; Proportion of polymorphic loci = 100.00 %

The genetic differentiation (Gst = Fst) as shown at Table 3, was very large, which performed by the Fst = 1.1485. A very large genetic differentiation was performed by Fst more than 0.25.

Table 3. F statistic and gene flow estimation

Locus	Sample size	Fis	Fit	Fst	Nm*
EST	50	0.2847	0.3339	0.0688	3.3863
PER	50	0.2581	0.5015	0.3280	0.5121
ADH	50	-0.4273	-0.1967	0.1616	1.2970
ACP	50	0.6161	0.7165	0.2617	0.7053
MDH	50	-0.1472	-0.1293	0.0156	15.7817
AAT	50	0.4444	0.5663	0.2194	0.8896
Mean	50	0.2268	0.3650	0.1788	1.1485

*Nm = Gene flow estimated from Fst = 0.25(1 - Fst)/Fst.

According to Wright (1978), genetic differentiation classified as follows:

Fst = 0 – 0,05 indicated low genetic diffrentiation
Fst = 0,05 – 0,15 indicated moderate genetic differentiation
Fst = 0,15 – 0,25 indicated large genetic differentiation
Fst > 0,25 indicated very large genetic differentiation

Gene flow (Nm) was estimated from genetic differentiation (Gst) as follow:

Nm = 0.5(1 - Gst)/Gst

A very large genetic differentiation, mean there were very large differentiation between (inter) populations, and indicated low genetic diversity at intra populations. This is also indicated the very low of gene flow. At this condition, conservation strategy was need to conserve of all population, and sampling was need from representative of all population.

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-- back to Table of Content --