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Diversity of *Sonneratia alba* in coastal area of Central Java based on isozymic patterns of esterase and peroxidase

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Abstract. *Setyawan AD. 2009. Diversity of Sonneratia alba in coastal area of Central Java based on isozymic patterns of esterase and peroxidase. Nusantara Bioscience 1: 92-103.* The study was intended to observe the diversity and the relationship of *Sonneratia alba* in southern and northern coast of Central Java Province based on isozymic patterns of esterase and peroxidase. This research was conducted in July until December 2003, at six sites, i.e. Wulan (WUL), Juwana (JUW), Pasar Bangi (PAS), in the northern coast, and Bogowonto (BOG), Motean (MOT), and Muara Dua (MUA) in the southern coast. The laboratory assay was conducted in Central Laboratory of Mathematics and Natural Sciences, Sebelas Maret University (UNS) Surakarta. The seedling plant samples that were collected from enam mangrove habitats transplanted in green house in the laboratory. There were 20 individual samples each sites. The result indicated that the individual of *S. alba* of the same or near location has the same genetic diversity in common, because the genetic exchange on a same population was higher than on a different population. Therefore, the populations of *S. alba* from northern coast had higher similarity each others than southern coast one, on the other way the populations from southern coast had higher similarity each others than northern coast one.

Key word: *Sonneratia alba*, isozyme, esterase, peroxidase, Central Java.

Abstrak. *Setyawan AD. 2009. Keanekaragaman Sonneratia alba di pesisir Pantai Jawa Tengah berdasarkan pola pita isozim esterase dan peroksidase. Nusantara Bioscience 1: 92-103.* Penelitian ini bertujuan untuk mengetahui keragaman dan hubungan kekerabatan *Sonneratia alba* di pantai selatan dan utara Propinsi Jawa Tengah berdasarkan pola isozim esterase dan peroksidase. Pengambilan sampel dilakukan pada Juli-Desember 2003, di enam lokasi, yaitu Wulan (WUL), Juwana (JUW), Pasar Bangi (PAS), di pantai utara, serta Bogowonto (BOG), Motean (MOT), dan Muara Dua (Mua) di pantai selatan. Uji laboratorium dilakukan di Laboratorium Pusat Matematika dan Ilmu Pengetahuan Alam, Universitas Sebelas Maret (UNS) Surakarta. Sampel bibit tanaman yang dikumpulkan dari enam habitat mangrove ditanam di rumah kaca di laboratorium. Ada 20 individu sampel pada setiap lokasi. Hasil penelitian menunjukkan bahwa individu *S. alba* dari lokasi yang sama atau berdekatan biasanya memiliki keanekaragaman genetik yang dekat, karena pertukaran genetik pada populasi yang sama lebih tinggi dari pada populasi yang berbeda. Oleh karena itu, populasi-populasi *S. alba* dari pantai utara memiliki kesamaan lebih tinggi satu sama lain daripada dengan populasi dari pantai selatan; sebaliknya populasi-populasi dari pantai selatan memiliki kesamaan yang lebih tinggi satu sama lain daripada dengan populasi dari pantai utara.

Kata kunci: *Sonneratia alba*, isozim, esterase, peroxidase, Jawa Tengah.

INTRODUCTION

Mangrove habitat on the north coast and south coast of Central Java has undergone long-term geographic isolation. Natural movement of propagules or other genetic materials (pollen) from the south coast to north coast or vice versa is almost impossible due to the natural barrier, namely the land of Java. On the south coast itself, the movement of propagules from one river estuary to other river estuary is also difficult to occur considering the large waves of Indian Ocean (South Sea), while on the north shore, the movement of propagules between the beaches is easier because of weak waves of the Java Sea. The mangrove ecosystem on the north coast and south coast of Central Java has different environmental conditions; in addition, these regions are directly adjacent to the area of community activities. Then, it is estimated that the condition of mangroves is also influenced by human activities as well as the condition of non-biotic and biotic environment. There will be possible that every location has different diversity of mangrove plants, both at the level of genetic, species, and ecosystems.

Nowadays, the mangrove ecosystems in Central Java are generally fragmented into small groups and are more isolated. Small and isolated populations are more sensitive to environmental disturbance (Shields 1993; Caro and Laurenson 1994; Caughley 1994). Degradation and fragmentation of habitat is the major cause of species extinction (Falk 1992), because it can reduce the size of the population and attract the arrival of invasively non native species (Opdam et al. 1994). Fragmentation also quickly changes the interaction between populations within a species, interactions intra species and the interaction between species with the a biotic environment (Cooperrider 1991; Thompson 1996), and also increases the inbreeding

and genetic drift (Barrett and Kohn 1991; Ellstrand and Elam 1993; Young et al. 1996). Genetic diversity is associated with the level of isolation and fragmentation of plant species (Treuren et al. 1991; Godt and Hamrick 1993). Natural selection can cause the occurrence of isozyme differentiation among populations (Aitken and Libby 1994), which also affects the morphological characteristics (Antonovics 1971). Genetic differences may reflect the phylogenetic relationship between the plant which is a synergy of the equilibrium of gene flow, genetic drift and natural selection (Max et al. 1999). The diversity and the distribution of plants are influenced by the origin, population history, evolution, and environmental factors such as geographic distribution (biogeography), reproductive ecology and mechanism of dispersal (Armbruster and Schwaegerle 1996; Purps and Kadereit 1998).

One of the mangroves which is very widely distributed is *Sonneratia alba*. In Java, there is also *S. ovata*, and *S. caseolaris* as their close relatives (Backer and Bakhuizen van den Brink 1968; Whitten et al. 2000). All three can do the hybridization and produce fertile offspring, so it is often difficult to do identification morphologically; but this is interesting to be studied (Tomlison 1986). Natural Hybridization often occurs in many groups of flowering plants (Angiospermae) (Knobloch 1972; Ellstrand et al. 1996). Hybridization is a pretty annoying problem for the concept of types based on the reproductive isolation, and can blur the distinction between ecological units (Mayr 1992; Gornall 1997).

Morphological differences that are commonly used to distinguish the three species of *Sonneratia* are flowers, fruit, leaves and stems. *S. caseolaris* flowers has petals and filaments which are almost entirely red, while *S. alba* has petals and filaments which are almost entirely white, although their bases sometimes are red, whereas *S. ovata* has petals and filaments which are entirely white (Backer and Bakhuizen van den Brink 1968; Ng and Sivasothi 2001). The Fruit of *S. caseolaris* is generally flat with petals opening flat direction and its fruit base is prominent, while the fruits of *S. alba* is generally round, with prominent fruit base and flipping petals. In *S. ovata* the fruit is round with a non-prominent fruit base and the calyx covering fruit (Whitten et al. 2000). The leaves of *S. alba* are generally round with the tip curving inward, while the leaves of *S. ovata* are round with rounded ends, while the leaves of *S. caseolaris* are generally more slender with pointed tip, but the diversity of leaf shapes can also be found in *S. alba* (Tomlison 1986). The rod shape of *S. alba* is usually stronger, larger, and erect with smoother and whiter bark than the other two species (Ng and Sivasothi 2001).

In Central Java, *S. alba* is able to grow on relic mangrove habitat, which other mangrove species do not grow, especially on the south coast which are sandy, so this plant is potential for restoration (rehabilitation) degraded mangrove (Setyawan et al. 2002). The failure of transplant experimental of *S. alba* with seedlings taken from Segara Anakan, Cilacap to Bogowonto river estuary by Djohan in 1997, is estimated due to the effect of inundation, while the local seedlings that grow naturally are still survive (Tjut S. Djohan 2001, personal communication). This indicates the existence of genetic diversity, in response to the environmental conditions, although the morphologic evidence is difficult to use to distinguish. In dry season, the estuary of the Bogowonto River experiences flooding approximately for 4-6 weeks, because the formation of dunes/sand dikes that dams the river estuary. This dam will be broken by itself in the rainy season, when the volume of water overflows. The plant of *S. alba* mentioned above can last for two years until 1999. In 1997-1998 there was no significant inundation because of the low level of rainfall and of river water supply, but during the dry season in 1999, there was an inundation over the estuary for 6 weeks, just like the earlier normal years, so that almost all populations of *S. alba* from Segara Anakan, Cilacap is submerged and died. According to McPhaden (1999), the El Nino South Oscillation (ENSO) in 1997-1998 is the largest storm in history, sea surface temperature rose by 1-4°C and causes severe droughts and fires, including in Indonesia.

The genetic diversity in a population can be traced using genetic markers based on electrophoretic separation of proteins/isozymes and DNA (McDonald and McDermont 1993). Isozyme and DNA data are very useful when the morphologic distinguishing characteristic overlaps each other (Karp et al. 1996). Isozymes are enzymes which have active molecules and although having different chemical structures but catalyzing the same chemical reaction (Tanksely 1983, Beer et al. 1993; Murphy and Phillips 1993). Isozymes which are produced in the cytosol, organelles, or in both; generally derive from vegetative parts (Weeden and Wendel 1989). The term of isozymes or isoenzymes was first introduced by the Market and Moller (1959, in Acquaah 1992). Isozymes can be separated by starch gel electrophoresis or polyacrylamide gel electrophoresis (PAGE), the result is zymogram band-pattern obtained after histochemical staining. Zymogram as a result of electrophoresis has a typical pattern, so it can be used as a feature to reflect genetic differences (Winston et al. 2002). This method is very useful to study low-level taxa, such as species, subspecies, and populations (Crawford 1983, 1989; Riesenberg et al. 1988; Brown 1990), including the identification of varieties and hybrids (Pierce and Brewbaker 1973; Beer et al. 1993 ; Murphy and Phillips 1993), so it is very important in biology studies of plant population (Tanksely 1983, Wendel and Weeden 1989; Comps et al. 2001).

Isozyme is usually governed by single genes and is codominant in characteristic heredity (Arulsekar and Parfitt 1986). Isozyme can detect three genetic and biochemical conditions that are different, namely: (i) multiple alleles at a single locus, (ii) single or multiple alleles at multiple loci and (iii) secondary isozyme (Rothe 1994; Micales and Bonde 1995). These bands are encoded by more than one structural gene locus, when encoded by alleles that segregated at a single locus called allozyme (Brown 1990). Alleles is a unit of genetic diversity, some of the types are unit of ecological diversity (Butlin and Tregenza 1998). Isozyme is widely used because the method is easy, cheap,

fast, polymorphisms obtained from a single molecule (Auler et al. 2002). According Suranto (1991), the use of isozyme data for population study is better using multiple enzymes than only one. This study aims to identify the diversity and relationship of *Sonneratia alba* populations in the north coast and south coast of Central Java based on band pattern of isozyme esterase (EST) and peroxides (PER, PRX).

MATERIALS AND METHODS

Time and research area

This research was conducted from July until December 2003. Field research was conducted at six locations. Three sites were located on the northern coast of Central Java, namely Wulan (WUL), Juwana (JUW), Pasar Banggi (PAS), and the remaining three locations were on the south coast of Central Java, namely Bogowonto (BOG), Motean (MOT), and Muara Dua (MUA). The selection of the six locations was based on the availability of seedlings of *S. alba*. Based on the preliminary survey in March till May 2002, and October till November 2002 each of these locations had at least 20 individual of *S. alba*. Laboratory studies were conducted in Sub-Lab of Biology, Central Laboratory of Mathematics and Natural Sciences, Sebelas Maret University (UNS) Surakarta. The planting of seedlings samples was conducted in greenhouse facilities at the laboratory.

Procedures

The study began with collecting seedlings of *S. alba* which were then planted in the greenhouse, followed by the electrophoresis procedure that includes the collection of leaves, the making of buffer, the making of stock solution, the gel manufacture, the leaves extraction, the implementation of electrophoresis and the manufacture of isozyme dyes (esterase and peroxidase). The electrophoresis procedure refers to Weeden and Wendel (1989), Crawford (1990), and Suranto (1991).

Collecting and planting the seedlings in the greenhouse. On six predetermined locations, 20 individual seedlings of *S. alba* were taken to be tested their isozime pattern diversity. Seedlings were planted in a greenhouse with soil composition of the origional soil of mangrove environment and humus (1: 1), until a new shoot grew.

Leaves collecting. The leaves were taken from the third leaf from branch tip which grew optimal with relatively equal in appearance, age and size. Leaf collection were stored in a refrigerator with temperature of 4°C. The leaves could survive in storage for 14 days, after that isozyme would not active any longer. Leaves should be used within seven days after cutting. The leaves storage in the form of extract at refrigerator with temperature of 4°C will enable the isozime to survive for 30 days.

Making of buffer. The required buffer includes *tank buffer, extraction buffer,* and *the running buffer*. (i) Tank buffer was made by dissolving: 14.4 g of borax acid and 31.5 of g borax in a distilled water until it reached the volume of 2 liters. (ii) Sample buffer was prepared by dissolving 0.018 g of cysteine, 0.021 g of ascorbic acid, and 5 g of sucrose (PA) in 20 mL of borax buffer with pH of 8.4. The comparison of extraction buffer with the sample of leaves was 3 to 1, in units of µl for extraction buffer and of µg for leaf samples. (iii) Running buffer used was TAE (Tris-Acetic Acid-EDTA), 50x diluted to 1x concentration.

Making of stock solutions. To prepare the acrylamide gel, first, the researchers made stock solution that is: a solution of "L", "M", "N", and loading dye (color marker). (i) Solution "L": 27.2 g of Tris and 0.6 g of SDS were dissolved in 120 mL of aquabides. The pH was adjusted to 8.8 by adding HCl, then they were added with aquabides until the volume was 150 mL. (ii) Solution "M": 9.08 g of Tris and 0.6 g of SDS was dissolved in 140 mL of aquabides. The pH was adjusted to 6.8 till 7.0 by adding HCl, then they were added with aquabides until the volume was 150 mL. (iii) Solution "N": 175.2 g of acrylamide and 4.8 g of bisacrylamide were dissolved in 400 mL of aquabides and the volume was made to be 600 mL. (iv) Loading dye: 250 µl of glycerol was added with 50 µl of bromphenol blue which was distilled in 200 µl of aquades.

Making of gel. The preparation of gel mold began with assembling the gel mold, namely a glass mold which had glass spacer (separator) was placed behind another smaller glass mold. The glass mold was mounted on a casting frame, then it was mounted on a casting stand. To create a discontinuous gel 12.5%, the mixed materials were: (i) Gel separator: 3.15 mL of solution "L", 5.25 mL of solution "N", 4.15 l of H_2O, 5 µl of TEMED, and finally they were mixed with 10 µl of 10% concentrated of APS (new). Separating gel was poured onto the mold, then was added with saturated iso-butanol. After the gel was formed within approximately 45 minutes, then, the saturated iso-butanol was absorbed by tissue paper and then, it was discarded. The gel was then rinsed with water and the remaining water was absorbed with tissue paper. After that, the materials for manufacturing stacking gel were prepared. (ii) Stacking gel: 1.9 mL of solution "M", 1.15 mL of solution "N", 4.5 mL of H_2O, 5 µ l of TEMED, and finally they were all mixed with 10% concentrated APS (new). After the stacking gel was poured onto the top of separation gel, a comb was placed on it. If the gel has been formed, the comb was removed from the mold. The formed gel was transferred to the clamping frame and inserted into the buffer tank, filled with running buffer until submerged.

Extraction of leaves. The fresh leaf tissues were put into in the extraction buffer, with a ratio of 1: 3 (w /v), i.e. 68 µg (0,068 g) of leaf sample was crushed in 204 µ l (0,204 mL) of extraction buffer. Then they were crushed in a porcelain cup that was placed on pieces of ice crystals, in order to be in cool condition (4°C). The would be used samples were centrifuged with speed of 8500 rpm for 20 minutes at 4°C, then they were submerged in ice crystal shavings. The formed supernatant was immediately inserted to the slot of electrophoresis gel.

Implementation of electrophoresis. Supernatant of leaves samples were taken using micropipette for as much as 10 µl and they were added with loading dye and, assisted with loading guide, the sample was placed on a gel that has been molded. Then, the samples were

electrophoresed, for the first time, using voltage of 200 volts, 60 mA for 5 minutes until the samples entered the separating gel. Then the samples were electrophoresed for the second time with constant voltage of 150 V, 400 mA, for 90-120 minutes. The electrophoresis was ended when the color marker bromphenol blue has reached the length of about 56 mm from the slot toward the anode. After that, the gel was transferred into plastic trays and colored with dyes enzyme.

Manufacturing of dyes. The isozyme band pattern was detected using two enzyme systems, namely esterase and peroxides. The steps of manufacturing were as follows: (i) *Esterase*: 0.0125 g of α-naphthyl acetate was put into the Erlenmeyer and added with 50 mL of 0.2 M phosphate buffer pH 6.5 and 0.0125 g of fast blue BB salt. Then, the electrophoresed gel was put into the dye solution and incubated for 10 minutes, whilst it was shook slowly every 2 minutes. After the band pattern appeared, the dye solution was removed and rinsed with distilled water. Gel images were recorded with a camera or scanner. (ii) *Peroxides*: a total of 0.0125 g of o-Dianisidine was taken into the Erlenmeyer and dissolved with 2.5 mL of acetone, and then it was added with 50 mL of 0.2 M acetate buffer pH 4.5 and then was added with 2 drops of H_2O_2. The electrophoresed gel was submerged in dye solution for more than 10 minutes as it was shook gently every 2 minutes. After the band pattern appeared, dye was removed and rinsed with distilled water. Gel images were recorded with a camera or scanner.

Data analysis

The level of genetic similarity of *S. alba* of the six sites was determined by Jaccard similarity index, based on the presence of isozyme band. Dendrogram of relationship associations was made with a coefficient of association, as for groupings using the UPGMA (Unweighted Pair Group Method using Averages) (Sneath and Sokal 1973).

RESULTS AND DISCUSSION

Results

The tests of genetic diversity with esterase isozyme totally raised 9 bands, namely the Rf value (retardation factor) of 0.04, 0.11, 0.16, 0.21, 0.29, 0.33, 0.37, 0, 41, and 0.45. The nine bands formed 12 variations of isozyme band pattern (genotype) (Figure 3A), whereas the peroxides isozyme emerged 6 bands, namely the Rf value of 0.09, 0.20, 0.29, 0.33, 0.37, and 0.47. The six bands form 11 variations of genotype (Figure 3B). Esterase band pattern interpretation was presented in Figure 1, whereas the peroxides band pattern interpretation was presented in Figure 2.

Relationship dendrogram based on esterase band pattern showed 13 groups. Each represented a different genotype. The 13th group was an individual of *S. alba* which emerged no esterase bands. Except for the 13th group, all individuals could join together at similarity level of 67% (Figure 4A). Whereas, the dendrogram based on relationship peroxides band pattern indicated the existence of 12 groups. Each represented a different genotype. The 12th group was a collection of individual *S. alba* which emerged no band peroxides; except for the 12th group, all individuals were collected at similarity level of 66% (Figure 4B).

The selection of populations of *S. alba* to represent the mangrove plant species which tested their genetic diversity was based on the fact that *S. alba* was one of the most widely spread species, which was found at 15 locations out of 20 research locations, with relatively evenly spread both north and south coast of Central Java (Setyawan et al. 2005a), so they were potential for restoration of damaged mangrove ecosystems. In addition, morphologically, *S. alba* and two close relatives *S. caseolaris* and *S. ovata* had a very similar morphology, with overlapping characters, so it often made the identification confusing. Genetically (reproductively), these three species could mate and produce fertile offspring. This seemed to be increasingly difficult to identify morphologically, because the formation of the intermediate characters. As in ecology, the experimental planting of *S. alba* seedlings taking from the Segara Anakan lagoon in Bogowonto estuary generally failed because of the failure of seed in adapting with abundant water and finally died, while the local seed from Bogowonto itself could grow well.

Esterase and peroxidase are widely used in studies of plant genetic diversity because of its spread is very wide. Peroxides are a heme-containing enzyme that uses hydrogen peroxide in the one-electron oxidation of organic substrates (Howes et al. 1999). Peroxides affect various physiological processes in plants, such as cell growth, biosynthesis of lignin and suberin, auxin metabolism, resistance to disease and wound healing (Gross 1977; Gaspar 1982; Espelie et al. 1986; Kennedy et al. 1996). Esterase and peroxidase enzymes tend resistant to SDS-PAGE gel used in electrophoresis. Enzyme that breaks down by the SDS can immediately return to the original shape, so that activities can still be detected in electrophoresis (Rothe 1994).

Esterase banding pattern

Based on the esterase banding pattern (genotype), the population of *S. alba* with the highest genetic diversity were found in samples from Motean (6 genotypes), followed by Muara Dua and Juwana (each 5), Wulan (4), and the Pasar Banggi and Bogowonto (respectively 3). In addition, there were also samples which showed no activity of esterase isozyme bands, namely Pasar Banggi (3 individuals) and Bogowonto (1). The spread of genotypes between sites varied greatly. In this study there was no genotype found in all locations. The most frequently encountered genotype was **a** and **c** (each 5 locations), followed by genotype f (4), genotype **j** (3), genotype **b** and g (2 for each), and genotype **d, e, h, i , k,** and **l** (1 for each).

Genotype **a** was found in all locations except in Bogowonto. Genotype **c** was found in all locations except in Pasar Banggi. Genotype **f** was found in all locations except Wulan and Bogowonto. Genotype **j** was found in Bogowonto (10 individuals), Motean (1) and Muara Dua (5). Genotype **b** was found in Wulan (1) and Motean (2). The unique genotypes was found only in one location, as

follows: genotype **d** was only found in Wulan (3), genotype E was only found in Juwana (1), genotype **i** was only found on Pasar Banggi (10), genotype **h** was only found in Juwana (2), genotype **k** was only found in Bogowonto (2), and genotype **l** was only found in Motean (1).

The existence of genotypes that were found in many locations, such as genotype **a** and **c** (each 5 locations), and genotype f (4), showed the distribution of certain genetic material was even on *S. alba*. This is understandable because they belong to the same species. According to Wang et al. (2000), isozyme pattern in most enzyme systems are not affected by differences in environment and many of them are stable in ontogeny. The genotype j is only found on the south coast only, indicating the existence of genetic exchange between the two sites which locations are relatively close, although there are big waves of south sea. While, the some genotypes found only in one location, Pasar Banggi, namely six genotypes (d, e, h, i, k, l) shows the variation of high local environmental conditions. This genetically is responded in the form of adaptation to local conditions. One of the causes of genetic diversity at least in this study probably is that the population size of *S. alba* left at each location was relatively in small number. According to Sokal and Oden (1978), in small populations with limited area there will be increased inbreeding and reduced gene flow, but on the other hand the level of differentiation through selection or genetic drift is higher in response to local conditions. According to Fitzpatrick (2002) isolation can increase the genetic diversity among populations.

The limited extent of habitat led to relatively small growing populations, so they are more susceptible to environmental changes (Menges 1991; Holsinger 2000). In addition, the limitation of population size will reduce the success of pollination (Groom 1998; Richards et al 1999; Lennartsson 2002). Seedlings from small population size have decreased the ability of germination and survival (Newman and Pilson 1997). In the long term sustainability of these populations can be threatened because of lowered quality of habitat as a result of human pressure and declining soil fertility (Kleijn and Verbeek 2000; Garbutt and Sparks, 2002; Blomqvist et al. 2003).

Relationship relationship dendrogram based on esterase banding pattern classifies the 120 individual of *S. alba* from six sites into 13 groups, where group XIII is a group of species that do not raise bands with esterase staining. Group I consists of 38 individuals which dominate Wulan, Juwana, Motean, and Muara Dua with a balanced composition. Group II consists of five individuals from Wulan, Motean, and Muara Dua. Group III consists of 16 individuals who dominated from Bogowonto and Muara Dua. Group IV consists of three individuals from Wulan. Group V contains two individuals from Juwana. Group VI contains 16 individuals from Juwana, Pasar Banggi, Motean, and Muara Dua. Group VII consists of four individuals from Juwana and Motean. Group VIII consists of three individuals from Juwana and Motean. IX group consists of 10 individuals from Banggi Market. X group consists of 17 individuals who dominated from Juwana. Group XI consists of two individuals from Bogowonto. Group XII consists of one individual from Motean. XIII group consists of three individuals from the Pasar Banggi (Figure 4A).

Jaccard similarity index calculations shows that group I, II, III, IV, V, VI, VII, VIII, X are in the same value of 0.89, meaning that these groups have high levels of genetic similarity with up to 89% esterase isozyme. Then the combined group one with group XII is in the same value of 0.78, meaning that these groups have high levels of genetic similarity to 78%. On the other hand, groups IX and XI stay together in the value of 0.78, meaning that these groups have high levels of genetic similarity to 78%. Furthermore, both the combined group stays in the value of 0.67, meaning that these groups have high levels of genetic similarity to 67%. Group XIII stays by itself because its members do not have a band esterase. The high level of genetic similarity in this study is reasonable, considering the same species generally has a high diversity of about 60% or more.

The above results indicate that individuals from the same location generally have the same genetic diversity that tends to be the same, as shown in group IV, V, IX, XI, and XIII. This is reasonable considering genetic exchange within a population is generally more intensive than with other populations. Nevertheless, there is generally more than one genotype composition in each location, there are even genotypes that spread very widely, for example in group I, III, and VI.

Group I, whose members come from locations that are relatively more diverse than the other group's shows that among the populations of *S. alba* there are individuals with similar genetic composition spread widely. This happens because they are still one species, thus they tend to share similar traits. Nevertheless there are also individuals whose spread is limited, such as group IV, V, IX, XI, XII, and XIII. Such individuals may be formed as a result of adaptation to local environment. This genetic uniqueness is needed in the process of evolution to maintain genetic diversity within populations. Individuals with a specific genetic composition are required by living things to withstand environmental changes (adaptation).

No detection of esterase isozyme bands in group XIII also proves the existence of genetic uniqueness, there are individuals who can survive without synthesize esterase enzyme, although this enzyme is very broad distributed and is required in the metabolism of the body such as the degradation of fat. But it is possible that the unavailability of the band is caused by the imperfectness of the laboratory techniques implementation.

Peroxides banding pattern

Based on the peroxides banding pattern (genotype), the population of *S. alba* with the highest genetic diversity is found in samples from Pasar Banggi (8 genotypes). While, on other locations it can only be found three genotypes (Wulan and Juwana) or only 2 genotypes (Bogowonto, Motean, and Muara Dua). In addition, there are also samples which showed no activity of peroxides isozyme

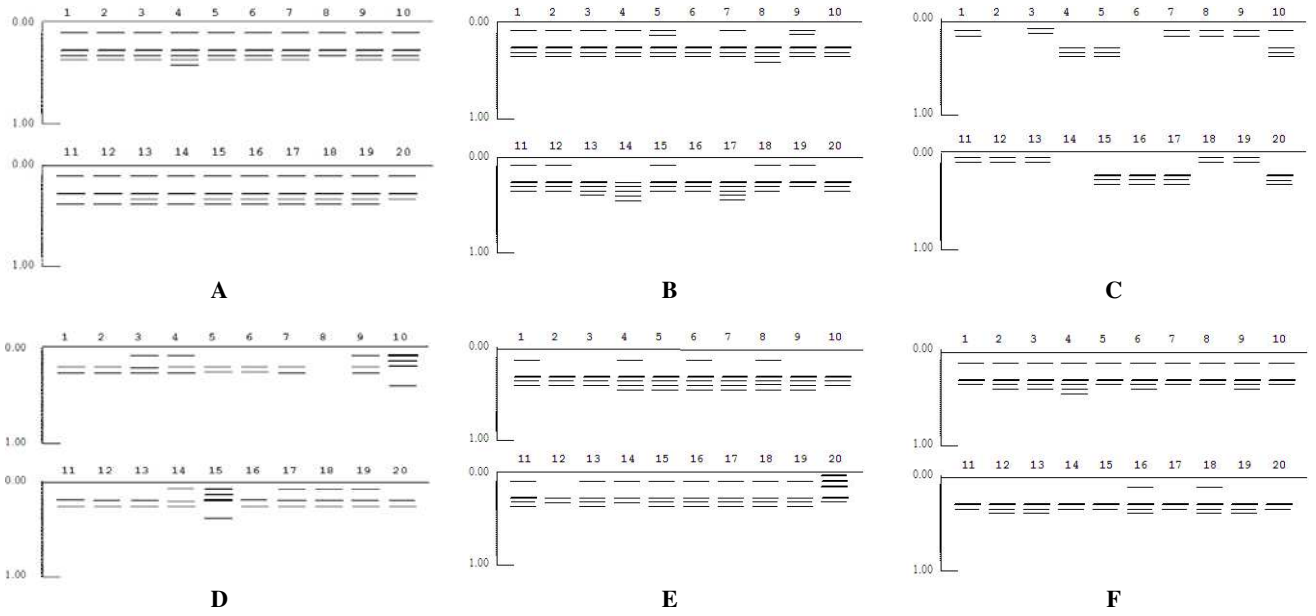

Figure 1. Esterase isozyme band of *S. alba* from the north and the south coast of Central Java. Note: A = Wulan (WUL), B = Juwana (JUW), C = Pasar Banggi (PAS), D = Bogowonto (Bog), E = Motean (MOT), F = Muara Dua (MUA).

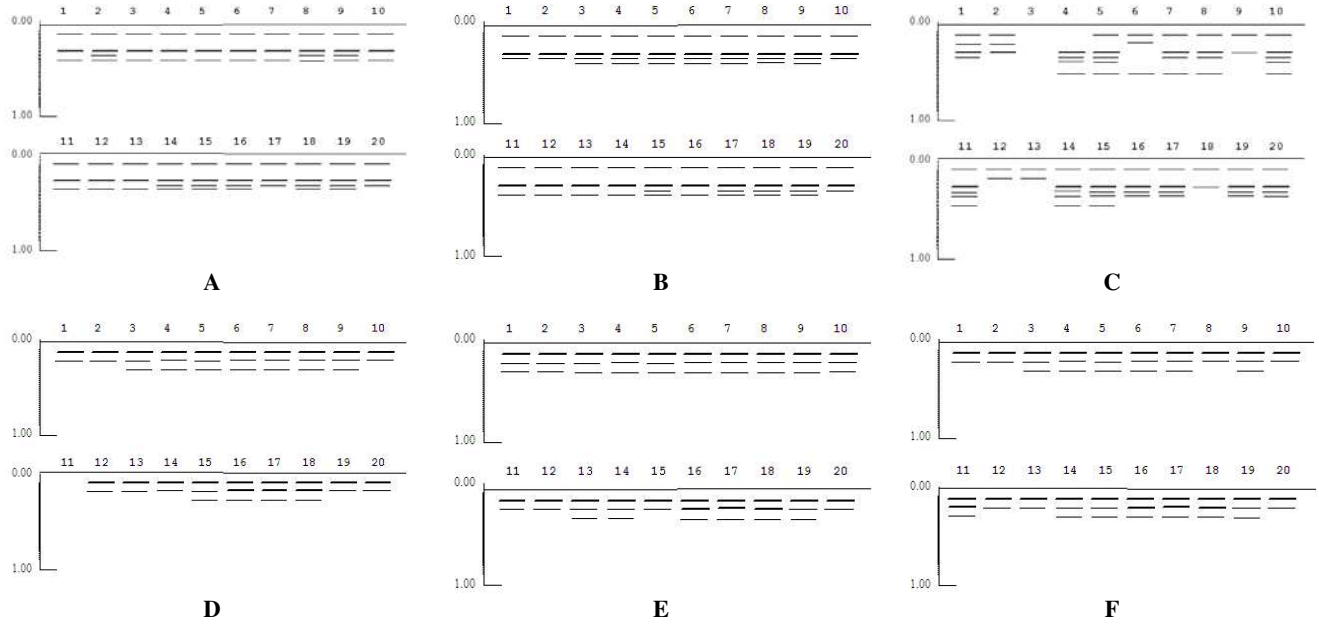

Figure 2. Peroxides of isozyme band of *S. alba* from the north and the south coast of Central Java. Description: A = Wulan (WUL), B = Juwana (JUW), C = Pasar Banggi (PAS), D = Bogowonto (Bog), E = Motean (MOT), F = Muara Dua (MUA).

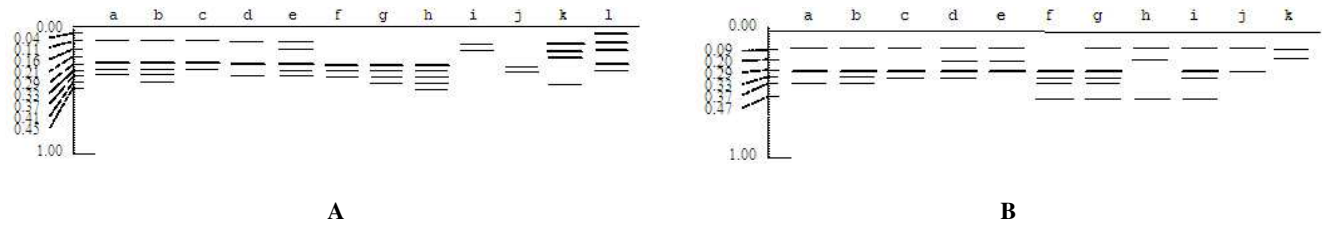

Figure 3. Genotype variations of *S. alba* on the north coast and south coast of Central Java: A. based on esterase isozyme bands; B. based on peroxides isozyme bands.

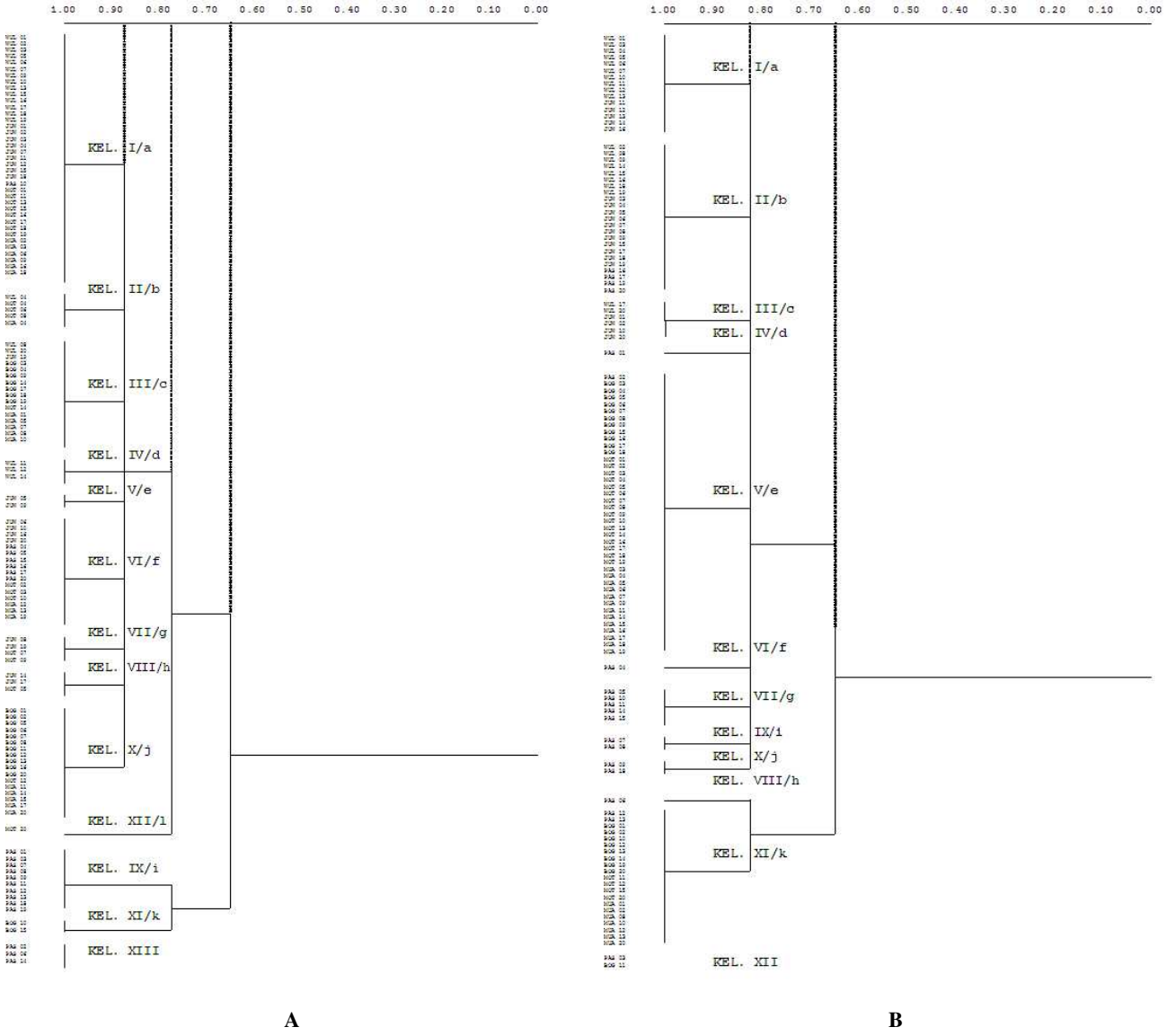

Figure 4. Dendogram relationship of *S. alba* from the north and the south coast of Central Java: A. based on esterase isozyme bands; B. based on peroxides isozyme bands. Description: WUL = Wulan, JUW = Juwana, PAS = Pasar Banggi, Bog = Bogowonto, MOT = Motehan, MUA = Muara Dua. In group VII = Isozyme bands did not appear.

bands, namely Pasar Banggi and Bogowonto (each 1 individual). The spread of genotypes between sites varies greatly. In this study there are no genotypes that are found at all locations.

Genotype **e** and **k** were found in the same four locations, namely Pasar Banggi (each is 1 and 2 individuals), Bogowonto (11, 8), Motean (16, 4) and Muara Dua (13, 7). Genotype **b** was found in three locations, Wulan (8 individuals), Juwana (11), and Pasar Banggi (4). Genotype **a** was found in two locations, namely Wulan (14) and Juwana (9). Genotype **c** was found in two locations, namely Wulan (2) and Juwana (3). There was specific genotype which was only being found in one location, Pasar Banggi, namely: genotype **d, f, g, h, i, j,** each is 1, 1, 3, 1, 2, 2 individuals.

In this study, genotypes from the north coast generally have in common with their peers, as well as genotypes from the southern coast generally has a resemblance with each other, except for some genotypes of Pasar Banggi that can be found on the coast south and north. Genotype **a, b, c** was only found on the north coast either on two or three locations. Whereas, genotype **e** and **k** were only found in three locations on the southern coast, except for a small area in Pasar Banggi. Each genotype was successively represented by 1 and 2 individuals. This shows that the intensity of exchange of genetic material (propagules) among populations from the north coast, and among populations of the south coast is relatively high, whereas genetic exchange between populations of the north coast with the south coast is relatively limited. This is the inevitable result of natural barriers that cause isolation geography.

The high number of specific genotypes in Pasar Banggi (6 genotypes) are probably due to the location which is

situated in the easternmost part of mangrove habitat on the northern coast of Java. Before contacting to mangrove habitat in delta Bengawan Solo which is separated by the long karst beach of Tuban, Lamongan, and Gresik, therefore, it becomes the first recipient of propagules from the east. In addition, as an open managed location, there is possibility of deliberately planting *S. alba* from other locations with different genetic material, as well as the possibility of hybridization with their close relatives *S. caseolaris* and *S. ovata*. In this study, it is found at Pasar Banggi, the existence of *S. caseolaris*, beside the existence of *S. alba*, while in Lasem which is located not far away on the east side was found *S. ovata*, the only sample that was recorded from 20 locations in northern and southern coast of Central Java (Setyawan et al. 2005a).

The test with peroxides isozyme shows 12 groups. Group XII consists of two individuals who do not produce the pattern of peroxides isozyme bands. Group I consists of 16 individuals from Wulan and Juwana. Group II includes 23 individuals from Wulan, Juwana, and Pasar Banggi. Group III consists of 6 individuals from Wulan and Juwana. Group IV includes one individual from Pasar Banggi. Group V consists of 41 individuals who generally come from the south coast, namely Bogowonto, Motean, and Muara Dua. Group VI consists of a single individual from Pasar Banggi. Group VII consists of five individuals from Pasar Banggi. Group VIII includes one individual from Pasar Banggi. Group IX consists of two individuals from Pasar Banggi. Group X consists of two individuals from Pasar Banggi. Group XI consists of 21 individuals, mainly from Bogowonto, Motean, and Muara Dua. Group XII consists of two individuals from the Pasar Banggi and Bogowonto (Figure 4B).

Jaccard similarity index calculations shows that group I, II, III, IV, V, VI, VII, IX, and X fuse to the value of 0.83, meaning that these groups have high levels of genetic similarity with peroxides isozyme to 83%. On the other hand, groups VIII and XI also separately fuse to the value of 0.83, meaning that these groups have high levels of genetic similarity to 83%. Furthermore, both join in the value of 0.66, meaning that these groups have high levels of genetic similarity to 66%. Group XII stays alone since its members do not have a band peroxides.

Similar to esterase isozyme, the dendrogram based on the relationship of this peroxides banding patterns of individuals from the same location or nearby locations tends to have similar genetic composition, as group I, II, and III are dominated by individuals from the north coast, and group V and XI is dominated by individuals from the south coast. This is reasonable considering the population of the same or neighboring locations has the possibility of exchanging genetic material more intensively.

Nevertheless there are also a number of individuals from a remote location, but united in one group, for example a small number of individuals from the northern coast that blends with the group V and XI which are dominated by individuals from the south coast. This shows that individuals of *S. alba* still share a common genotype, although the nature of the growth is far from its location. This is reasonable considering they still belong to one species. In this peroxides isozyme banding pattern, the genetic uniqueness is contributed by groups IV, VI, and VII, each of which consists of only one individual, and group IX, X, and XIII, each of which consists of only two individuals.

The fact that the experimental planting of seedlings of *S. alba* from Segara Anakan to Bogowonto finally ends up with a failure is not detected in this study. This could be because the genes that encode individual defense against inundation are metabolically less or not related to the synthesis of esterase and peroxides, so, with these two enzymes, it is difficult to distinguish the difference in genetic composition between species of Segara Anakan and Bogowonto. On testing with esterase Isozyme, there was a evidence that the individuals of Bogowonto generally joined individuals from Motean and Muara Dua, as shown in group III and X. Similarly, in testing with peroxidase isozymes, the individuals of Bogowonto generally joined with the individuals of Motean and Muara Dua, as shown in group V and XI. This indicates the persistence of exchange of genetic resources between the two locations, since they are located on the same coastline, although there are large waves.

Genetic structure and habitat restoration

Population genetic structure

In this study, it appears that *S. alba* has genetic diversity, both assessed on the basis of esterase and peroxidase isozymes. In the hypothesis of biodiversity alpha stated that a high diversity will lead to ecosystem stability, productivity and storage because of high nutrients (Tilman 1999). Populations with high genetic diversity can further affect the ecosystem (Heywood 1995). On the south coast and north, *Sonneratia* spp. along with *Avicennia* spp., and *Rhizophora* spp. always has the highest importance value (Setyawan et al. 2005b). The main factor influencing plant diversity is geographic distribution (32%), forms of life (25%), and mating systems (17%) (Hamrick et al. 1979, 1991). It is observed from the differences in genetic diversity of individuals who grew up in north and south coast, namely specimens from the north coast generally has a higher uniformity compared with specimens from the south coast, and vice versa. The dispersal ability is one of the main characteristics in response to habitat fragmentation (Bring et al. 1991; Vankat et al. 1991; Fore et al. 1992). Dispersal is a key process that affects the ecology, genetics, and geographic distribution of species (Van der Pijl 1969; Sauer 1988; Dingle 1996). The condition of coastal ocean waves which is relatively quiet in north coast causes an exchange of genetic resources among mangrove habitats relatively higher than in the south coast.

The tendency of the genetic diversity of *S. alba* is probably due to the high fragmentation of habitat where the mangrove ecosystem separated in small groups. One form of changes in natural landscapes by human activity is the habitat fragmentation into small groups, scattered, and isolated (Barrett and Kohn 1991; Fenster and Dudash 1994; Fore and Guttman 1996, 1999). The loss of natural habitat due to human activities is the main factors that cause the

extinction of species (Sih et al. 2000). Fragmentation causes the farther distance among populations; thereby it inhibits migration among neighboring populations, and increases the incidence of isolation (Fahrig and Merriam 1994).

Habitat fragmentation and geographical isolation of *S. alba* on the north and south coast results the increasing of genetic uniformity within each fragment habitat, on the contrary it increases the genetic diversity between fragments. This occurs because of the restrictiveness of gene flow from outside populations will increase genetic differentiation between populations (Dickinson and Antonovics 1973; Fore and Guttman 1992; Rhodes and Chesser 1994; Husband and Barrett 1996). Genetic differentiation between populations reduces the possibility of restoration of the natural genetic diversity of the surrounding population (Ellstrand 1992; Newman and Tallmon 2001; Hewitt and Kellman 2002). Genetic diversity is essential to be species survival for a long time, because it affects the ability of species to be adaptive with environmental changes (e.g. Westemeier et al 1998; Knapp and Connors 1999; Keller and Waller 2002). Therefore, restoration of mangrove habitat often requires local seeds, the introduction of new seedlings from other sites sometimes fail to grow despite of the same type.

Restoration and population genetic structure

Genetic structure within and among populations is important in the restoration of damaged ecosystems (Brown 1989; Ceska et al. 1997). The absence of knowledge of the genetic structure of native populations before disturbance is a serious problem in the restoration of ecosystems, as well as to maintain the diversity and reduce post-disturbance genetic changes (Young et al. 1996; Fore and Guttmann 1999). The prediction of genetic diversity can be incorporated in the design of restoration management, for example in the choice of seedlings to improve the success of restoration (Hamrick and Godt. 1989; Brown and Briggs 1991; Holsinger and Gottlieb 1991). The successful restoration of mangrove ecosystems on the north coast of Rembang is apparently because of the selection of local seed as a source of explants (Setyawan and Winarno 2006).

Factors that influence the level of ecological diversity also affect genetic diversity at the molecular level, such as: fragmentation, weeding, decomposition of nutrients, soil poisoning, changes in global temperature, and aspects of forestry (Linhart and Grant 1996; Tilman 1999). Information about genetic diversity at the level of species within ecosystems helps to determine breeding strategies, management and genetic conservation (Auler et al. 2002). In a restored population, long-term durability can be determined through the selection of local or non local seed, single or multiple source, natural or cultivated varieties, as well as adaptability and competitiveness of seedlings in the new environment. Marriage among individuals from the same location may decrease the resistance ability due to inbreeding pressure; on the contrary, marriage among different populations may decrease the resistance ability because of the out breeding pressure (Fenster and Dudash 1994; Gustafson et al 2001). A better understanding of the interaction between durability, genetic equality and diversity, as well as the competitiveness of a plant species will be useful for maintaining ecosystems that still exist and restoration of disturbed ecosystems (Gustafson et al. 2002).

Competitiveness differences between populations can influence the genetic structure of populations (Aarssen and Turkington 1985; Turkington 1994). In a restoration project, plant propagule should come from an environment similar to the restored habitat conditions, because the introduction of seedlings with a stronger competitiveness may urge the remnants of native vegetation (Etterson and Shaw 2001). If the population of introductions seedlings and native seedlings is genetically different, out breeding pressures may happened, this may affect the success of restoration. The populations probably take several generations to heal themselves from these interbred (Emlen 1991). But on rare species, it is sometimes needed the outbreeding pressure for conservation and restoration of these populations (Gerard et al 1995, Byers 1998). Out breeding pressure that causes interbreeding is very likely to occur if the two populations from different locations amassed in one place (Montalvo and Ellstrand 2001).

The election *S. alba* in mangrove restoration program needs to be done carefully, in order to tug and pull between inbreeding and out breeding pressure produces no negative resultant, given the existence of genetic diversity in this population. Selection of explants from the local site can lead to inbreeding pressure due to inbreeding. This can cause very high homogeneity of the population so that its adaptation to environmental changes decreases; on the other hand the high homogeneity is a specific form of adaptation to environmental conditions for so long. Selection of explants from other locations can lead to out breeding pressure due to interbreeding, which can cause changes in genetic structure of populations. It is necessary to supply the new gene so that the explants may adapt to the environmental changes, on the other hand these changes in genetic structure so far can cause adapting failure to local environmental conditions.

Conservation management

Knowledge of the genetic structure of *S. alba* is needed in mangrove restoration and conservation programs of this plant, namely (i) identifying and evaluating threats endangering its continuity, and (ii) determining the design of restoration programs (Vrijenhoek 1994). On the restoration program, knowledge about the distribution of genetic diversity is guidance in the management of important genetic resources (Barrett and Kohn 1991). Genetic diversity is a container for adaptation and evolution, so that the maintenance of genetic diversity is very important in the conservation of biodiversity (Thomas et al. 1999). Presuming the level and the distribution of genetic diversity in plant species can be used as guidance in the management of species with restricted range (Hamrick et al 1991). Plants with limited distribution tend to have less diversity of isozyme compared with broad distribution of species (Hamrick and Godt 1989; Hamrick et al. 1992),

though; the geographic spread does not always affect the genetic structure of a species (Soltis and Soltis 1991; Lewis and Crawford 1995.)

The election of local *S. alba* as a source of explants in management needs to be given priority, given the main goal of conservation is to preserve biodiversity by maintaining the existence of native species in the ecosystem as long as possible (Harrison et al. 1984; Falk 1992). the resistance decrease of species in habitats that have been degraded and fragmented requires action to protect habitats from damage, to lessen habitat loss and fragmentation, as well as to conserve and to enhance biodiversity (Noss et al. 1997). Genetic conservation can be done in situ and ex situ. In situ conservation will restore the potential evolution of a population including the enrichment and the establishment of new populations, along with ecological management. Ex situ conservation includes the formation of collections that contain the highest genetic diversity. This can only be done if the genetic structure of species and populations have been known (Machon et al. 2001).

CONCLUSION

The genetic diversity tests of *S. alba* with esterase isozyme totally show 9 bands, with 12 variations in genotype, whereas the peroxides isozyme emerges 6 bands, with 11 variations in genotype. Relationship dendrogram based on esterase banding pattern showed 13 group, which fused at the similarity level of 67% (except the 13th group which brings up no band esterase). The dendrogram based on the peroxides banding pattern indicates the existence of 12 groups, which also fused at the similarity level of 66% (except for the 12th group which brings up no band peroxides). The individuals of *S. alba* from the same or adjacent locations generally have the same genetic diversity, given the genetic exchange within a population or between adjacent populations is generally higher than the exchange with other populations, so that the population of *S. alba* from the north coast has a higher genetic similarity among themselves than the population of the south coast, and vice versa, the population of the south coast have a higher genetic similarity among themselves than the population of the north coast.

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