

# Characterization of white grubs (Melolonthidae: Coleoptera) at salak pondoh agroecosystem in Mount Merapi based on isozymic banding patterns

SRI WARDANI<sup>1,✉</sup>, SUGIYARTO<sup>1,2</sup>

<sup>1</sup>Department of Biology, Faculty of Mathematic and Natural Sciences, Sebelas Maret University, Jl. Ir. Sutami 36a Surakarta 57126, Central Java, Indonesia. Tel./Fax.: +92-271-663375. ✉email: noor\_handajani@yahoo.com.

<sup>2</sup>Bioscience Program, School of Graduates, Sebelas Maret University, Surakarta 57126, Central Java, Indonesia

Manuscript received: 5 March 2008. Revision accepted: 24 May 2008.

**Abstract.** Wardani S, Sugiyarto. 2009. *Characterization of white grubs (Melolonthidae: Coleoptera) at salak pondoh agroecosystem in Mount Merapi based on isozymic banding patterns. Nusantara Bioscience 1: 38-42.* The aim of this research is to know the characteristics of white grubs (Melolonthidae: Coleoptera) based on isozyme banding patterns. This research was conducted at Sleman, Yogyakarta and Magelang-Central Java for the morphological purposes. The sample was taken from 5 places with different height in wich 5 samples were taken from each location. The method used in this research was polyacrylamide gel electrophoresis (PAGE) using the vertical type. The enzyme system used in this research were peroxidase and esterase to detect the isozyme banding patterns. The results showed that there was a variation in isozyme banding patterns of white grubs (Melolonthidae: Coleoptera) at salak pondoh agroecosystem in Mount Merapi's slope (peroxidase in station II and IV while esterase in station III and V). It's mean that genetic variation on white grubs population at salak pondoh agroecosystem in Mount Merapi's slope was found. The environmental condition also contributed to the influence of the appear of isozyme banding pattern's variation because each location had a different condition.

**Key words:** white grub, isozyme banding patterns, electrophoresis, Mount Merapi, salak pondoh.

**Abstrak.** Wardani S, Sugiyarto. 2009. *Karakterisasi lundi putih (Melolonthidae: Coleoptera) pada agroekosistem salak pondoh di Gunung Merapi berdasarkan pola pita isozim. Nusantara Bioscience 1: 38-42.* Tujuan penelitian ini adalah mengetahui karakteristik lundi putih (Melolonthidae: Coleoptera) didasarkan pada pola pita isozim. Penelitian morfologi dilakukan di Sleman, Yogyakarta dan Magelang, Jawa Tengah. Sampel diambil dari lima tempat dengan ketinggian yang berbeda dimana lima sampel diambil dari setiap lokasi. Metode yang digunakan dalam penelitian ini adalah elektroforesis gel poliakrilamida (PAGE) menggunakan jenis vertikal. Sistem enzim yang digunakan adalah peroksidase dan esterase untuk mendeteksi pola pita isozim. Hasil penelitian menunjukkan bahwa terdapat variasi pola pita isozim lundi putih (Melolonthidae: Coleoptera) pada agroekosistem salak pondoh di lereng Gunung Merapi (peroksidase di stasiun II dan IV sedangkan esterase di stasiun III dan V). Hal ini menunjukkan bahwa terdapat variasi genetik pada populasi lundi putih pada agroekosistem salak pondoh di lereng Gunung Merapi. Kondisi lingkungan juga berpengaruh terhadap munculnya variasi pola pita isozim karena setiap lokasi memiliki kondisi lingkungan yang berbeda.

**Kata kunci:** lundi putih, pola pita isozim, elektroforesis, Gunung Merapi, salak pondoh.

## INTRODUCTION

Salak pondoh (*Salacca zalacca* (Gaert) Voss) is one type of fruit that is loved by the people of Indonesia. The area around the slope of Mount Merapi, particularly in Sleman district, Yogyakarta and Magelang regency, Central Java is one of the salak cropping center, especially salak pondoh (Kusumo et al. 1995; Suskendriyati et al. 2000). The pests often attack salak pondoh plants in Sleman, Yogyakarta is the white grub. White grub is the name of a group of insect larvae (Family Melolonthidae, order Coleoptera) that have a body shape like the letter "C" or "scarabaeid". It has cream or white body, reddish brown head with cutters mouth type, three pairs of leg right at the back of the head. The body length ranges from 2-6 cm, body diameter ranged from 0.5 to 1.5 cm. In some areas of Java, it is also known by the name "uret" or "embug"

(Sugiyarto 2000; Sugiyarto et al. 2002; Kompas 4/10/2003). These pests attack sporadically that resulted in crop damage of salak pondoh widedly.

Pracaya (1999) states that at the beginning, these pests only eat humus and other debris, but after a little bigger, they eat the roots of plants that are still alive, sometimes even eat salak tree in the soil so that it can cause plant to die. In the adult phase, in the form of beetles, these pests eat the leaves of plants but the damage is not so visible. In area of Texas and other sub-tropical regions, the white grub of *Phyllophaga crinita* species is known as the pest of grassland and various kinds of ornamental plants with huge losses (Crocker et al. 1999; Drees and Jackman 1999). White grub is also the pest of main auxiliary crops that attack the area of *sengon*-based agroforestry in Jatirejo, Kediri District and we have not found how to control them (Sugiyarto 2004).



**Figure 1.** Morphology of white grub an insect larvae of family Melolonthidae, order Coleoptera.

So far, the white grub pest control efforts have been made through various approaches such as physical and chemical approaches, but the results are not satisfactory. In order to develop a biological control, the key to success is the presence of complete information on the characteristics of the specimen. Until now there has been no complete information about the characteristics of white grub, and therefore the pest needs to be characterized. Characterization of the morphological approach has several weaknesses, including the appearance of the characters are often influenced by environmental factors. But the main weakness of this morphological approach, according to Delluchi et al. (1989) and Suskendriyati et al (2000), is character recognition at the level of sub-species, especially with the presence of twin species or sibling species.

One alternative way that can be used to characterize the white grub is through isozyme. Isozymes are some enzymes that have different chemical structures but catalyze the same reaction. Isozyme has several advantages, including: can be used to identify the properties that are not visible in morphology (Mariani 2002), can be applied to determine the genetic structure of intra-and inter-population (Fitriyah 2002), and many samples can be analyzed in relatively short time (Hadiati and Sukmadjaja 2002). The purpose of this study is to determine the characteristics of white grub (Melolonthidae: Coleoptera) on salak pondoh agroecosystem on the slopes of Mount Merapi on the basis of isozyme banding pattern.

## MATERIALS AND METHODS

### Field research

White grub (Melolonthidae: Coleoptera) were taken from the agroecosystem of salak pondoh on the slopes of Mount Merapi, precisely in Sleman regency, Yogyakarta and Magelang regency, Central Java. Samples were taken at 5 stations with different altitude, namely: station I: 484 m asl (Turi, Sleman), station II: 545 m asl (Srumbung, Magelang), station III: 620 m asl (Srumbung, Magelang), station IV: 751 m asl (Turi, Sleman), and station V: 820 m asl (Pakem, Sleman). Five samples were taken from each station and are well-treated in order to make them stay alive until the isozyme banding pattern analysis is done. The measurement of environmental factors includes air temperature, soil temperature, soil pH, soil moisture and Soil Organic Matter (SOM).

### Isozyme analysis

Isozyme banding pattern analysis was performed by polyacrylamide gel electrophoresis (PAGE). The preparation of buffers and stock solutions follows the method of Suranto (1991, 2001, 2002).

**Making a buffer.** Tank buffer (borax buffer) was made by dissolving the borax acid of 14.4 g and 31.5 g of borax in distilled water until it reaches the volume of 2 liters. Extraction buffer is made by dissolving 0.018 g of cysteine, 0.021 g of ascorbic acid, and 5 g of sucrose in 20 mL of buffer tank with pH 8.4.

**Preparation of stock solutions.** A stock solution was prepared by dissolving 4.5 g of tris and 0.51 g of citric acid in 500 mL aquabidest. B stock solution was prepared by dissolving 30 g of acrylamide, combined with 0.80 g of N,N'-methylene-bis-Acrylamide (bisacrylamide) into 100 mL aquabidest.

**Preparation of gel.** Gels prepared according to the method of Suranto (1991) with modification, namely by mixing 2.5 mL of stock solution B and 5 mL of stock solution A, then added with 0.02 mL of N,N,N',N'-tetramethyl-ethylenediamine (TEMED) and mixed them carefully. For gel polymerization, it was added by 30 mL of ammonium persulphate (APS).

**Extraction and sample preparation.** Digestive organ was extracted using extraction buffer with a ratio of 1:3, in  $\mu\text{g}$  for samples and  $\mu\text{l}$  for buffer extraction. Organs are crushed using a mortar on top of ice crystals flake. Samples that have been destroyed then centrifuged at 8500 rpm for 3 minutes. Supernatant is taken as many as 7  $\mu\text{L}$  for peroxidase staining and 15  $\mu\text{L}$  for esterase staining. Electrophoresis device used for the analysis of isozyme banding pattern is a BIO-RAD Mini Protean 3 Cell vertical type, made in USA.

**Staining.** The staining in this study used two enzyme systems, namely peroxidase and esterase. For peroxidase staining, a total of 0.0125 g of O-dianisidin dissolved in 2.5 mL of acetone and then added by 50 mL of acetate buffer pH 4.5 and 2 drops of hydrogen peroxide. While for esterase staining a total of 0.0125 g of  $\alpha$ -naphthyl acetate was dissolved in 2.5 mL of acetone, then added with 50 mL

of 0.2 M phosphate buffer pH 6.5 and 0.0125 g of fast Blue BB salt.

### Data analysis

Band formed was drawn in the shape of zimogram. Data obtained by calculating the value of Rf, which is the ratio of migration distance of band to the migration distance of loading dye. Data were analyzed based on whether the tape appeared on the gel and the thickness of thin band formed.

## RESULTS AND DISCUSSION

### Environmental factors

All environmental parameters measured can affect physiological processes (metabolism) of white grub. There are variations on the five environmental parameters measured at five observation stations (Table 1).

**Table 1.** Environmental factors of the research site.

Station	Air temp. (°C)	Soil temp. (°C)	Soil pH	GWT (%)	SOM (%)
I: 484 m asl	30.9	27.3	6.72	12.77	5.52
II: 545 m asl	29.7	25.8	5.44	19.24	3.61
III: 620 m asl	32.3	27.2	6.76	20.16	5.72
IV: 751 m asl	27.8	24.4	6.98	5.14	3.45
V: 820 m asl	26.8	29.3	7	7.95	6.14

Note: I&IV: Turi, Sleman, II&III: Srumbung, Magelang, V: Pakem, Sleman, SOM: Soil Organic Matter, GWT: Ground Water Levels.

The temperature tends to decrease as the height increases (from station I to V). Soil temperature plays a key role in soil environment. Insects have a certain temperature range where he can live. Outside the temperature range, the insects will die from the cold or heat. Temperature effect is clearly visible in the process of insect physiology. At a certain temperature, insect activity is high, but at other temperatures, it will be reduced (down). In general, the minimum temperature is 15°C, the optimum temperature is 25°C and 45°C for maximum temperature (Jumar 2000). The five stations showed a normal pH range, ie close to pH 7, except for station II, which has the lowest soil acidity (pH 5.44). For land animals, soil pH also influences. Living things can run these processes with a good life if in the range of their optimum pH. The existence of extreme pH can affect the survival of the organism. The soil also contained water needed by plant roots and soil organisms to survive. Soil water content will affect the soil moisture. The higher soil water content, soil moisture will also be higher. Soil organic matter is a source of food (energy source) for the major white grub so that its availability is needed.

By considering the vegetation that makes up each research station, the station I is salak pondoh agrotourism, so the plants that dominate this place is the salak pondoh. Station II is salak pondoh garden that has been treated with water treatment in anticipation of a white grub pests, therefore the soil moisture content is high enough. Beside

salak pondoh plants, cassava and bananas crops are planted nearby, as intercropping plants to distract the white grub not to eat the plant roots of salak pondoh. Station III is pure salak garden and is not planted by the other plants. Station IV represents salak pondoh garden planted with crops intercropping, as well as station II. Other vegetation found in this station is a cassava, banana, coconut, distance, mahogany, and weed is pretty much found. Station V had the most different field conditions among other observation stations because the soil tends to dry and sandy.

### White grub (Melolonthidae: Coleoptera)

Among the group of insects, beetles (Coleoptera) are the largest group since they set about 40% of all insect species and consist of not less than 250 thousand species (Pracaya 1999). White grub is included in Melolonthidae family of Coleoptera order (Chu 1992). Borror (1992) also include a white grub into a Scarabaeidae family. Larvae (*uret*) included in this family often damages the roots of plants and when they grow up, the adults will eat the leaves, but the damage is not as bad as its larvae stage. White larva lives in the soil and takes ± 7 months before it becomes a cocoon.

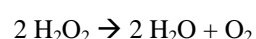
Of the five study sites, i.e. the stations I to V which are determined based on the gradation of heights, it can be found a white grub that has similar morphological features. The morphological features including: a body shape like the letter 'C', head reddish brown, 3 pairs of legs just behind the head, and 3 segments of thorax (the first segment is spiracles). Spiracles serve as the exit point of O<sub>2</sub> and CO<sub>2</sub>, it also is as the evaporation of H<sub>2</sub>O (Jumar 2000). The abdomen has 10 segments, 8 segments have spiracles on the lateral body, while the last 2 segments do not have spiracle and serve as a place to store the rest of digestion so the color is darker (black) and there is an anus. The body is yellowish-white color, the body length ranges from 2-6 cm, the diameter ranges from 0.5 to 1.5 cm. Based on morphology, white grub found in the study area is included in the genus of *Phyllophaga*, and has species name of *Phyllophaga javana* Brsk and the other name is *Holotrichia javana* Brsk (Pracaya 1999). Local name for this insect species is *ampal*. *P. javana* larvae is multiphagous, if soil organic matter content is high, this larvae is more as saprophagous, but if the soil conditions is in shortages of organic material, the larvae eats the roots of plants so it causes crop damage.

### Isozyme banding pattern

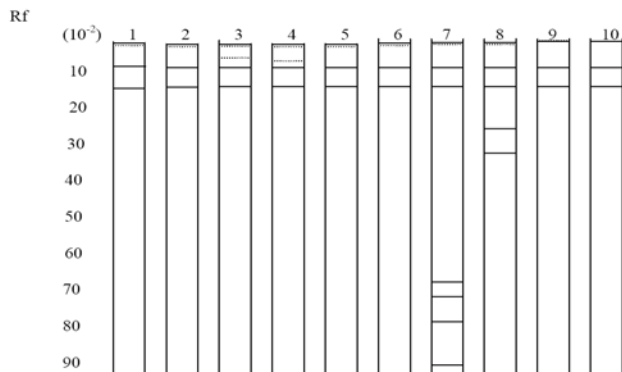
Isozyme is the enzyme which has different chemical structures but catalyze the same reaction. The difference form of an enzyme molecule can serve as the basis of chemical separation, such as by electrophoresis, which produces tapes with a range of different migration.

#### *Peroxidase isozyme*

Peroxidase (PER) enzyme is categorized in the group of oxydo-reductase. The reactions that occur in the peroxidase staining were:



Peroxidase catalyzes the  $H_2O_2$  into  $H_2O$  and  $O_2$ . The existence of peroxidase can be easily detected because of high activity and stability and it can use a number of substrates as hydrogen donors (Cahyarini 2004).



**Figure 1.** Zimogram peroxidase isozyme electrophoresis results of white grub in agroecosystem salak pondoh on the slopes of Mount Merapi. Description: 1.2 = sample station I; 3.4 = sample station II; 5.6 = sample station III; 7.8 = sample station IV; 9.10 V = sample station

From zimogram of peroxidase isozyme electrophoresis results, it is known that the isozyme peroxidase produce 12 bands based on the relative motion of the enzyme (Rf). Of the twelve bands, four bands always appear or are found in all individuals from station I to station V. The four bands are located at a distance migration 1, 1.5, 5 and 9 mm from the slot or at Rf 0.017, 0.026, 0.086 and 0.155. The second tape is faintly visible or thin, which suggests that small molecular weight enzyme. The four bands can be used as a characteristic pattern of peroxidase isozyme bands on white grub. Besides the four major bands that appear, the individuals from station II has a band that is not present in other individuals of the five stations. The band is located at 3.5 mm (Rf 0.060) and at 4 mm from the slot (Rf 0.069). Figure 1 shows the band of individual No. 3 and No. 4. Quantitatively, the two bands are thin. Besides the individuals of station II, individuals from station IV also shows the anomaly with the advent of tape on the migration distance of 15 and 18 mm or at Rf 0.259 and 0.310 (in Figure 1, it is shown by individual No. 8), and 38, 41, 45 and 52 mm or at Rf 0.655, 0.707, 0.776 and 0.896 (in Figure 1 is shown by individual No. 7). The emergence of bands in individuals from station IV which is absent in individuals from other stations also shows a variation pattern of peroxidase isozyme bands on white grub.

Based on the results above, it can be explained that the individuals from stations I, III and V have the same banding pattern. While individuals from stations II and IV show the diversity of isozyme banding pattern so that it can be assumed that there are genetic differences that encode the enzyme. According Cahyarini (2004), the difference in migration distance bands is a manifestation of differences in content and form of the enzyme molecule. Rahayu et al. (2006) adds that the enzyme or protein can be used to show variation both qualitatively and quantitatively. These variations are result from the role of the gene that directs

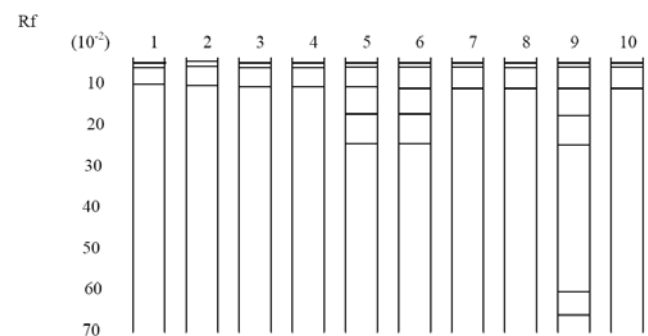
the formation of the enzyme in question, therefore the variation of enzyme can describe the gene variation.

In terms of vegetation, station II and IV are prepared not only by salak pondoh plants, but there are other plants that make up the ecosystem. While the stations I, III, and V is pure salak pondoh plantations, in other word, there is no other plant that dominates. It is possible that differences in vegetation gives the effect of the variation of isozyme banding pattern of white grub, considering that every living being will try to maintain its survival in case of environmental changes.

Band thickness can basically be divided into two, namely a thick band and a thin band. A thin band or vaguely indicates that the isozyme content or concentration is small.

#### Esterase isozyme

Esterase enzyme included in the reaction hidrolase class specific chemical bond is determined by adding the element of water (Salisbury and Ross 1992). Esterase is a hydrolytic enzyme that functions to withhold simple esters in organic acids, inorganic acids and alcohols and phenols that have a low molecular weight and soluble (Subronto 1989 in Setianto 2001).



**Figure 2.** Zimogram esterase isozyme electrophoresis results of white grub in agroecosystem salak pondoh on the slopes of Mount Merapi. Description: 1.2 = sample station I; 3.4 = sample station II; 5.6 = sample station III; 7.8 = sample station IV; 9.10 V = sample station

From Figure 2, it is known that esterase isozymes in white grub produce 7 bands. The first band, the second band and the third band appear on all individuals from the five stations, with each migration distance is 1.5, 2 and 6 mm from the slot or at Rf 0.026, 0.034 and 0.103. The three of these bands are unique because they are always found in all individuals from the five stations that show uniformity in banding pattern. The specialties are in individuals from station III and V, because beside having a third band mentioned above, they also have a band on the migration distances of 10 and 14 mm (Rf 0.172 and 0.241). In addition, band at a distance of 35 and 38 mm (Rf 0.603 and 0.655) are found only in individuals from station V. Based on the comparison of bands that appear at the five stations, it appears that individuals from station I, II, and IV have the same number of bands and the same banding pattern. Meanwhile, individuals from station III and V

shows the variation of esterase isozyme banding pattern. Band esterase isozyme in the gel appears to be brown with almost the same intensity.

Based on the results of electrophoresis of either peroxidase staining or esterase staining, the diversity of isozyme banding pattern is more likely to belong to the qualitative diversity, namely the presence or absence of bands on the gel. The thickness of the tape which is a quantitative nature is mostly the same. According to Setianto (2001), qualitative properties are preferred because they relate to the presence of a particular band at a particular distance migration that reflects the presence or absence of amino acids making up the enzyme that is a product of the gene itself.

From those facts, it can be explained that the peroxidase and esterase isozyme can show the variation of isozyme banding pattern on a white grub. The variation of isozyme banding pattern shown by the distance of migration of different bands indicates different forms or different chemical structures (conformation), and it can be presumed that the genes that encode enzymes are not the same. In addition, environmental factors also affect the appearance of this isozyme banding pattern variation considering that the sampling sites have variation of environmental conditions. Salisbury and Ross (1992) states that if there are environmental factors that change, the most active isozyme in those environments would carry out its functions and help the organism to survive.

### CONCLUSION

Based on research results obtained, it can be concluded that the white grub (Melolonthidae: Coleoptera) on salak pondoh agroecosystem on the slopes of Mount Merapi showed good variation of isozyme banding pattern of peroxidase isozyme (in individuals from station II and IV) and esterase (in individuals from station III and V). Thus there is genetic variation among populations of white grub (Melolonthidae: Coleoptera) in agroecosystem salak pondoh on the slopes of Mount Merapi. Environmental factors also affect the appearance of this isozyme banding pattern variation considering the sampling sites have environmental conditions variation.

### REFERENCES

Borrer DJ, Triplehorn CA, Johnson NF. 1992. Pengenalan pelajaran serangga. Gadjah Mada University Press. Yogyakarta. [Indonesia]

- Cahyarini RD. 2004. Identifikasi keragaman genetik beberapa varietas lokal kedelai di Jawa berdasarkan analisis isozim. Tesis. Program Pascasarjana Universitas Sebelas Maret, Surakarta.
- Chu HF. 1992. How to know immature insects. WMC Brown. Dubuque.
- Crocker RL, Nailon WT, Matis JH, Woodruff RE. 1999. Temporal pattern of ovipositional readiness in spring species of *Phyllophaga* (Coleoptera: Scarabaeidae) in North Central Texas. *Ann Entomol Soc Am* 92 (1): 47-52.
- Delluchi VD, Rosen D, Schlinger EI. 1989. Relation of systematic and biological control. In: Huffaker CB, Messenger PS (eds) *Theory and practice of biological control*. University of Indonesia Press. Jakarta.
- Drees BM, Jackman J. 1999. *Field guide to Texas insect*. Gulf Publishing. Texas.
- Fitriyah F. 2002. Comparative study of isozyme banding pattern and morphology of green leafhoppers (*Nephotettix virescens*) from West Java, Central Java and East Java. [Thesis S1]. Faculty of Agriculture, University Eleven March. Surakarta. [Indonesia]
- Hadiati S, Sukmadjaja D. 2002. The diversity of some accessions of pineapple based on isozyme banding pattern analysis. *J Bioteknologi Pertanian* 7 (20): 62-70. [Indonesia]
- Jumar. 2000. *Agricultural entomology*. Rineka Cipta, Jakarta. [Indonesia]
- Kompas. 2003. Salak pondoh plant threatened by white grubs pests in Sleman. 10 April 2003. [Indonesia]
- Kusumo S, Farid AB, Sulihanti S, Yusri K, Suhardjo, Sudaryono T. 1995. Salak production technology. Research and Development Center for Horticulture. Research and Development Agency, Department of Agriculture. Jakarta. [Indonesia]
- Mariani Y. 2002. Study on isozyme variation of a few colonies of green leafhoppers (*Nephotettix virescens*) as a vector of rice tungro disease. [Thesis S1]. Faculty of Agriculture, Sebelas Maret University. Surakarta. [Indonesia]
- Pracaya. 1999. *Pests and plant diseases*. Penebar Swadaya. Jakarta. [Indonesia]
- Rahayu S, Sumitro SB, Susilawati T, Soemarno. 2006. Analysis of isoenzymes to study the genetic variation of Bali cattle in Bali Province. *Berk Penel Hayati* 12: 1-5. [Indonesia]
- Salisbury FB, Ross CW. 1992. *Plant physiology*. Vol. 2. Penerbit ITB. Bandung. [Indonesia]
- Setianto A. 2001. Characterization of a large orange (*Citrus grandis* (L.) Obsbeck) in Jepon and Jiken Subdistrict, Blora District based on isozyme and morphological of fruit. [Thesis S1]. Faculty of Agriculture, Sebelas Maret University. Surakarta. [Indonesia]
- Subronto 1989. Isolation and properties of isozymes of palm leaf Deli Dura. [Thesis]. Bogor Agricultural University. Bogor. [Indonesia]
- Sugiyarto, Sugito Y, Handayanto E, Agustina L. 2002. Effect of land use systems on soil macroinvertebrate diversity in RPH Jatirejo, Kediri, East Java. *BioSMART* 4 (2): 66-69. [Indonesia]
- Sugiyarto. 2000. Diversity of soil macrofauna in different sengon age stands at RPH Jatirejo, Kediri District. *Biodiversity* 1 (2): 47-54. [Indonesia]
- Sugiyarto. 2004. Macroinvertebrate diversity between the soil and crop productivity in sengon agroforestry systems. [Dissertation]. Postgraduate Program, Brawijaya University. Malang. [Indonesia]
- Suranto. 1991. Studies of population variation in species of *Ranunculus*. [Thesis]. Department of Plant Science, University of Tasmania. Hobart, TAS.
- Suranto. 2001. Isozyme studies on the morphological variation of *Ranunculus nanus* populations. *Agrivita* 23 (2): 139-146.
- Suranto. 2002. Cluster analysis of *Ranunculus* species. *Biodiversitas* 3 (1): 201-206.
- Suskendriyati HA, Wijayati, Nurhidayah, Cahyuningdari D. 2000. Morphological studies and phylogenetic relationship of salak pondoh varieties (*Salacca zalacca* (Gaert.) Voss.) in the highlands of Sleman. *Biodiversitas* 1 (1): 26-31. [Indonesia]