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

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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 was 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 morphological purposes. The sample was taken from 5 places with different heights in which five 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 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 means that genetic variation on the white grubs population at salak pondoh agroecosystem in Mount Merapi's slope was found. The environmental condition also influenced the isozyme banding pattern's appearance because each location had a different state.

Keywords: 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 centers, 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 a cream or white body, reddish-brown head with cutters mouth type, three pairs of the leg right at the back of the head. The body length ranges from 2-6 cm; body diameter ranges 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, resulting in crop damage of salak pondoh widespread.

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 trees in the soil so that it can cause the 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 Texas and other sub-tropical regions, the white grub of *Phyllophaga crinita* species is 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 and insects larvae of family Melolonthidae, order Coleoptera.

So far, the methods of white grub pest control have been made through various physical and chemical approaches, but the results are not satisfactory. 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, which are often influenced by environmental factors. But the main disadvantage of this morphological approach, according to Delluchi et al. (1989) and Suskendriyati et al. (2000), is character recognition at the level of subspecies, 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 a 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 based on 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 to make them stay alive until the isozyme banding pattern analysis is done. Environmental factors include 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 reached 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 Suranto (1991) method 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. It was added by 30 mL of ammonium persulphate (APS) for gel polymerization.

Extraction and sample preparation. The digestive organ was extracted using an extraction buffer with a ratio of 1:3, in μg for samples and μl for buffer extraction. Organs are crushed using mortar on top of ice crystals flake. Samples that had been destroyed were then centrifuged at 8500 rpm for 3 minutes. The supernatant is taken as many as seven μL for peroxidase staining and 15 μL for esterase staining. Electrophoresis device used to analyze isozyme banding pattern is a BIO-RAD Mini Protean 3 Cell vertical type, made in the 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-dianisidine was 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 α-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 a zymogram. Data is obtained by calculating Rf's value, 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 the thin band formed.

RESULTS AND DISCUSSION

Environmental factors

All environmental parameters measured can affect the white grub's physiological processes (metabolism). 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. (ºC)	Soil temp. (ºC)	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 decreases as the height increases (from the station I to V). Soil temperature plays a key role in the soil environment. Insects have a certain temperature range where they can live. The insects will die from the cold or heat outside the temperature range. The 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, i.e., 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. The soil water content will affect soil moisture. The higher soil water content, soil moisture will also be higher. Soil organic matter is a food source (energy source) for the major white grub, so its availability is needed.

By considering the vegetation that makes up each research station, station I is salak pondoh agrotourism, so the plants that dominate this place is the salak pondoh. Station II is a salak pondoh garden treated with water treatment in anticipation of white grub pests; therefore, the soil moisture content is high enough. Besides salak pondoh plants, cassava and bananas crops are planted nearby, as intercropping plants to distract the white grub from eating the plant roots of salak pondoh. Station III is a pure salak garden and is not planted by the other plants. Station IV represents salak pondoh garden planted with crops intercropping and station II. Other vegetation found in this station is cassava, banana, coconut, distance, mahogany, and weed was pretty much seen. Station V had the most different field conditions among other observation stations because the soil tends to be dry and sandy.

White grub (Melolonthidae: Coleoptera)

Among the insect group, beetles (Coleoptera) are the largest group since they set about 40% of all insect species and contain fewer than 250 thousand species (Pracaya 1999). White grub is included in the Melolonthidae family of the Coleoptera order (Chu 1992). Borror (1992) also consists of a white grub into a Scarabaeidae family. Larvae (*uret*) included in this family often damage 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. The white larva lives in the soil and takes ± seven months before it becomes a cocoon.

Of the five study sites, i.e., the stations I to V determined based on the gradation of heights, a white grub with similar morphological features can be found. The morphological features include; a body shape like the letter 'C,' head reddish-brown, three pairs of legs just behind the head, and three segments of the thorax (the first segment is spiracles). Spiracles serve as the exit point of O2 and CO2; they also evaporate H2O (Jumar 2000). The abdomen has ten segments, eight segments have spiracles on the lateral body, while the last two segments do not have spiracles 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 in 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*. It has the species name of *Phyllophaga javana* Brsk, and the other name is *Holotrichia javana* Brsk (Pracaya 1999). The local name for this insect species is *ampal*. *P. javana* larvae are multiphytophagous; if soil organic matter content is high, this larva is more saprophagous, but if the soil conditions are in shortages of organic material, the larvae eat the roots of plants, so it causes crop damage.

Isozyme banding pattern

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

Peroxidase isozyme

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

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

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

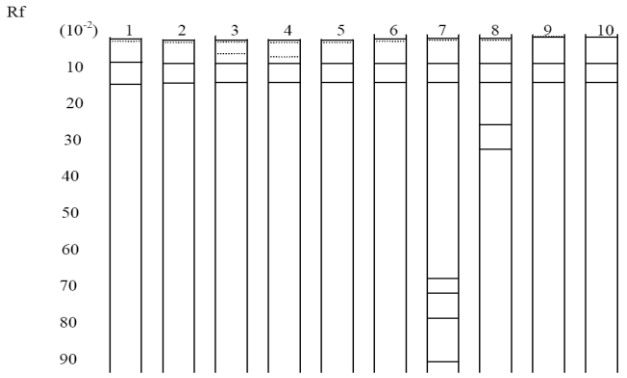

Figure 1. Zymogram peroxidase isozyme electrophoresis results of white grub in agroecosystem salak pondoh on the slopes of Mount Merapi. Description: 1.2 = sample station I; 3.4 = sample station II; 5.6 = sample station III; 7.8 = sample station IV; 9.10 V = sample station

From the zymogram of peroxidase isozyme electrophoresis results, it is known that the isozyme peroxidase produces 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, suggesting a 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 have a band not present in other individuals of the five stations. The band is located at 3.5 mm (Rf 0.060) and 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, absent in individuals from other stations, also offers 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 patterns, it can be assumed that there are genetic differences that encode the enzyme. According to Cahyarini (2004), the difference in migration distance bands manifests differences in the content and form of the enzyme molecule. Rahayu et al. (2006) add that the enzyme or protein can be used to show variation both qualitatively and quantitatively. These variations 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, stations II and IV are prepared by salak pondoh plants and other plants that make up the ecosystem. While stations I, III, and V are pure salak pondoh plantations, in other words, there is no other plant that dominates. Differences in vegetation may affect the variation of the 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 vaguely indicates that the isozyme content or concentration is small.

Esterase isozyme

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

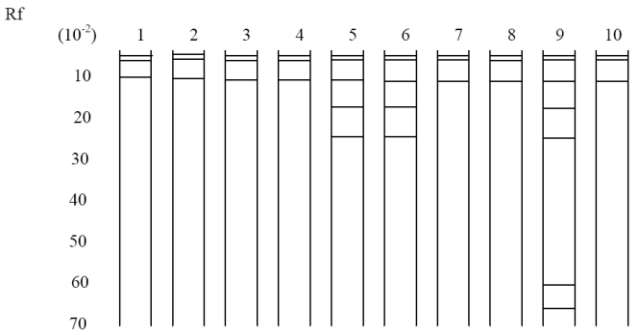

Figure 2. Zymogram esterase isozyme electrophoresis results of white grub in agroecosystem salak pondoh on the slopes of Mount Merapi. Description: 1.2 = sample station I; 3.4 = sample station II; 5.6 = sample station III; 7.8 = sample station IV; 9.10 V = sample station

From Figure 2, it is known that esterase isozymes in white grub produce seven bands. The first, second, and third bands appear on all individuals from the five stations, with each migration distance being 1.5, 2, and 6 mm from the slot or at Rf 0.026, 0.034, and 0.103. These three bands are unique because they are always found in all individuals from the five stations, showing uniformity in a banding pattern. The specialties are in individuals from stations III and V because besides 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, bands at a length 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 seems that individuals from the station I, II, and IV have the same number of bands and the same banding pattern. Meanwhile, individuals from stations III and V show the variation of the esterase isozyme banding pattern. The gel's

band esterase isozyme appears brown with almost the same intensity.

Based on electrophoresis results of either peroxidase staining or esterase staining, the diversity of isozyme banding pattern is more likely to belong to the qualitative variety, namely the presence or absence of bands on the gel. The thickness of the tape, which is quantitative, is mostly the same. According to Setianto (2001), qualitative properties are preferred because they relate to the presence of a particular band in a specific 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 the 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). 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 a variety of environmental conditions. Salisbury and Ross (1992) state that if environmental factors change, the most active isozyme in those environments would carry out its functions and help the organism 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 a 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.

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