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Characterization of taro (*Colocasia esculenta*) based on morphological and isozymic patterns markers

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Abstract. *Trimanto, Sajidan, Sugiyarto. 2011. Characterization of taro (*Colocasia esculenta*) based on morphological and isozymic patterns markers. Nusantara Bioscience: 7-14.* The aims of this research were to find out: (i) the variety of *Colocasia esculenta* based on the morphological characteristics; (ii) the variety of *C. esculenta* based on the isozymic banding pattern; and (iii) the correlation of genetic distance based on the morphological characteristics and isozymic banding pattern. Survey research conducted in the Karanganyar district, which include high, medium and low altitude. The sample was taken using random purposive sampling technique, including 9 sampling points. The morphological data was elaborated descriptively and then made dendrogram. The data on isozymic banding pattern was analyzed quantitatively based on the presence or absence of bands appeared on the gel, and then made dendrogram. The correlation based on the morphological characteristics and isozymic banding pattern were analyzed based on the product-moment correlation coefficient with goodness of fit criterion. The result showed : (i) in Karanganyar was founded 10 variety of *C. esculenta*; (ii) morphological characteristics are not affected by altitude; (iii) isozymic banding pattern of peroxides forms 14 banding patterns, esterase forms 11 banding patterns and shikimic dehydrogenase forms 15 banding patterns; (iv) the correlation of morphological data and the isozymic banding pattern of peroxidase has good correlation (0.893542288) while esterase and shikimic dehydrogenase isozymes have very good correlation (0.917557716 and 0.9121985446); (v) isozymic banding pattern of data supports the morphological character data.

Keywords: taro, *Colocasia esculenta*, morphology, isozyme.

Abstrak. *Trimanto, Sajidan, Sugiyarto. 2011. Karakterisasi talas (*Colocasia esculenta*) berdasarkan penanda morfologi dan pola pita isozim. Nusantara Bioscience: 7-14.* Tujuan penelitian ini adalah untuk mengetahui: (i) keragaman *Colocasia esculenta* berdasarkan karakter morfologi; (ii) keragaman *C. esculenta* berdasarkan pola pita isozim, dan (iii) hubungan jarak genetik berdasarkan karakter morfologi dan pola pita isozim. Survei penelitian dilakukan di Kabupaten Karanganyar, di ketinggian tinggi, sedang dan rendah. Sampel diambil menggunakan teknik random sampling purposif, mencakup 9 titik cuplikan. Data morfologi diuraikan secara deskriptif dan kemudian dibuat dendogram kekerabatan. Data pola pita isozim dianalisis secara kuantitatif berdasarkan ada atau tidaknya pita di gel, kemudian dibuat dendogramnya. Korelasi berdasarkan karakter morfologi dan pola pita isozim dianalisis berdasarkan korelasi koefisien momen-produk kriteria *goodness of fit*. Hasil penelitian menunjukkan: (i) di Karanganyar terdapat 10 varietas *C. esculenta*; (ii) karakter morfologi tidak terpengaruh oleh ketinggian; (iii) peroksidase membentuk 14 pola pita isozim, esterse membentuk 11 pola pita dan shikimate dehidrogenase membentuk 15 pola pita; (iv) data morfologi dengan isozim peroksidase memiliki korelasi yang baik (0,893542288), sementara data morfologi dengan isozim esterse dan shikimate dehidrogenase memiliki korelasi yang sangat baik (0,917557716 dan 0,9121985446); (v) data pola pita isozim mendukung data karakter morfologi.

Kata kunci: talas, *Colocasia esculenta*, morfologi, isozim

INTRODUCTION

The diversity of food crops in Indonesia can be developed to overcome the food problem. Types of tubers that can be utilized more optimally as a staple food rice substitutes include cassava, sweet potato, taro, purse, arrowroot, and canna. These tubers have a lot of the pre-eminent, among them having a high content of carbohydrates as energy sources (Liu et al. 2006), not containing gluten (Rekha and Padmaja 2002), containing angiotensin (Lee et al. 2003), antioxidative (Nagai et al. 2006),which can be applied to various purposes (Aprianita 2009), and produce more energy per hectare than rice and wheat. Tubers can be grown on marginal areas (Louwagie et al. 2006), where other plants can not grow and can be stored in the form of flour and starch (Aboubakar et al. 2008).

Taro has a good variety of morphological characters such as tubers, leaves, and flowers as well as chemicals such as flavor, aroma, and others (Xu et al. 2001). Characterization of taro plants now has started to be developed through two approaches. The diversity among the varieties can be distinguished based on morphological and molecular markers. Diversity based on morphological marker has a weakness, because the morphological characteristics do not necessarily indicate genetic diversity. Morphological diversity is influenced by the environment, because every environment has different conditions, so the plants adapt to their home range.

Molecular marker is an effective technique in genetic analysis of a plant variety. Molecular markers have been

applied widely in plant breeding programs. Molecular marker that is often used to distinguish plant diversity is a marker of isozyme and DNA (Asains et al. 1995; Setyo 2001). Isozyme is a direct product of genes and relatively free from environmental factors. Isozyme can be used as a genetic trait to study and identify the diversity of individuals or a cultivar. Isozymes were enzymes that have active molecules and different chemical structure, but catalyze the same chemical reaction. Different forms of an enzyme molecule can be used as the basis of chemical separation, by electrophoresis method will result in banding patterns produced by different distances (Purwanto et al. 2002).

Information about the genetic diversity of taro (*Colocasia esculenta* L.) is needed for plant breeding and improvement for the offsprings to obtain superior varieties. Based on the background, the research was conducted on taro plants in different areas in a region that had high altitude, medium and low that included morphological characters and isozyme banding patter pita on different varieties of taro plants in Karanganyar, Central Java.

MATERIALS AND METHODS

The experiment was conducted from March 2009 to August 2009. Taro plants (*Colocasia esculenta* L.) were collected from Karanganyar District, Central Java differentiated by differences in altitude, namely: (i) the highlands (> 1000 m asl), (ii) plain medium (500-1000 m asl), and (iii) Lowland (<500 m asl.). Location of the study covers nine districts in the district Karanganyar (Table 1). Characterization of isozyme of taro plants was conducted at the Faculty of Forestry Gadjah Mada University, Yogyakarta, using three enzyme systems namely esterase (EST), peroxidase (POD) and shikimate dehydrogenase (ShDH).

Characterization of morphology

Characterization of morphology includes: range of plants, plant's height, stolon's number, stolon's length, leaf shape of basalt, the dominant position of leaves, leaf edges, leaf color, leaf blade edge color, pattern Petiole junction, crossing the color, the color of the liquid at the tip of the leaf blade, the main color of the leaves of bone, bone leaf pattern, the ratio petiole length/leaf blade length, color Petiole upper third, middle third Petiole color, lower third Petiole color, color Petiole lines, color Petiole ring bottom, bottom Petiole transverse incision, midrib length ratio/display total Petiole, leaf midrib color, waxy coating on leaves. Manifestation cormus, cormus length, branch cormus, cormus shape, weight, cortex color, and the flesh color the middle, the color of the meat fibers, cormus skin surface, skin thickness cormus, cormus fiber levels, and color shoots.

Isozyme analysis

The third leaf from the top it was extracted with a mortal, by adding a solution of extract buffer ± 1 mL. Once crushed and homogenized, the sample was inserted into the Eppendorf, then played with the speed of 15,000 rpm for 20 minutes. Making the gel: Gel Polyacrylamide consists of two parts, i.e., running a gel that lies at the bottom with a concentration of 7.5% and spacer gel located on top of running gel with a concentration of 3.75%. Electrophoresis: electrophoresis tanks were filled with a solution of electrode buffer tanks as high as ± 2 cm. Mounted on gel electrophoresis, supernatant solution was filled into the hole 5 mL samples using injection equipment (stepper). Electrophoresis process carried out by an electric current ± 100 mA for 180-200 min. Staining performed after gel electrophoresis, namely by putting that has been removed from the glass electrophoresis into a plastic tray, then soaked in dye solution of dye esterase (EST), peroxidase (POD) and shikimate dehydrogenase (ShDH). Observations gel performed after fixation with seeing a pattern emerging bands, and copy it in the form zymogram.

Data analysis

Taro plant morphology data were described by descriptive method that covers all the observed variables in accordance with Kusumo et al. (2002). On isozyme data, the tape that emerged was given a value of 1 while the ones that did not arise given the value 0. Dendrogram analysis performed with the method of grouping the Average Linkage Cluster Method with DICE coefficient (Rohlf 2005). The grouping was done by UPGMA (Unweighted Pair Group with Arithmetic Mean) is calculated by SHAN on NTSYS program (Numerical Taxonomy and Multivariate Analysis System) version of 2:02, while the dendrogram analysis using the statistical program Minitab 14 Average linkage method with Euclidean distance measurement. The result made dendrogram based on isozyme relationship. Results were analyzed by distance dendrogram relationship more than 60% similarity (Cahyarini 2004). The correlation between genetic distance based on morphological characteristics and genetic similarity based on isozyme banding pattern was analyzed based on product-moment correlation coefficient with the criteria of goodness of fit based on the correlation according to Rohlf (2005).

RESULTS AND DISCUSSION

Characterization of morphology of *C. esculenta*

The results of characterization of taro plants performed on three different plains in the district Karanganyar obtained 11 variants showed that the plant *C. esculenta* were scattered in several districts, namely: Benthul, Lompongan, Laos, Mberek, Kladi, Plompong, Sarangan, Kladitem, Jabon, Japan, and Linjik. In this study 18 samples taken taro with research sites with environmental factors as listed in Table 1. The diversity is seen in the type of plant, leaf, and cormus (bulb). The characterization results show that there is a difference between 11 variants of taro. Description of the morphology of the leaf, midrib, and cormus (bulb) in each of the varieties of taro were as Table 2.

Table 1. Environmental conditions where the growth of taro in Karanganyar.

Locations/ Subdistricts	Environmental factors						
	Type of taro	Altitude (meter)	Temp. (°C)	Type of soil	Shade	Rainfall (mm/y)	Cultivation
Lowland							
Gondangrejo	Benthul	150	29	Grumosol	-	1537	√
	Mberek	150	29	Grumosol	-	1537	-
Jaten	Kladi	98	29	Aluvial	-	1680	√
	Linjik	98	29	Aluvial	√	1680	√
Karanganyar	Lompongan	320	30	Mediteran	-	2012	-
Kebakkramat	Plompong	95	29	Mediteran	-	2012	√
Medium plain							
Karangpandan	Benthul	650	28	Mediteran	-	2818	√
	Lompongan	600	28	Mediteran	√	2818	-
	Sarangan	650	28	Mediteran	-	2818	√
Matesih	Jabon	700	28	Litosol	-	2480	√
	Laos	750	27	Litosol	-	2480	√
	Linjik	700	28	Litosol	√	2480	-
Plateau							
Tawangmangu	Kladitem	1500	23	Andosol	√	3299	√
	Benthul	1700	22	Andosol	-	3299	√
	Lompongan	1500	23	Andosol	√	3299	-
Ngargoyoso	Laos	1000	26	Andosol	√	3182	√
Jatiyoso	Sarangan	1300	26	Andosol	-	3098	√
	Jepang	1200	26	Andosol	-	3098	√

In the dendrogram similarity coefficient of 60% was used to analyze the phylogenetic relationship of the 18 samples found in different locations with 11 different varieties. According Cahyarini (2004) said the similarity distance away if less than 0.60 or 60%, so that separate groups at a distance of less than 0.60 still has a close resemblance. In this dendrogram analysis, the number 1 or 100% indicates that the group members have a perfect resemblance, while getting closer to the number 0 means the similarity distance farther.

Benthul

Dendrogram analysis results showed that the Benthul taro of different height have the same morphological characteristics and have a high relationship. This is evident in the coefficient of 0.60 which was still in one group. But there was a tendency that Benthul of different heights shows different sizes, ranging from leaf size, plant height, stem, and tuber. Benthul is commonly grown as a crop population between the rice fields and gardens, and allowed to grow without special treatment. Environmental factors such as temperature at any altitude, soil, and availability of different light and water, thought to cause the size of the plants to experience the difference. According to and Djukri (2006), each deal with environmental stress of plants continues to adapt, including changes in morphological characteristics and physiology.

Benthul that grows in the highlands appear higher with habitus width, leaf midrib and stalk thin and big. This was observed in taro grown in more than 1500 m asl with high 22°C, and high rainfall reaches 2299 mm /±humidity, low temperature year. According to Basri (2002) plant growth is influenced by environmental factors. Altitude above 1500 m causes gas and water vapor content (humidity) and the number of clouds blocking sunlight to the plants, so plants were capturing light by raising levels of chlorophyll and surface area. Taro plants tend to have broad leaves because of the availability of adequate water due to high rainfall in the area still support the optimum process in photosynthesis.±Low temperature 22°C

Benthul that grows in the lowlands tends to have narrower leaves and smaller and lighter bulbs. According to Menzel (1980), the temperature is too high may cause leaves to hinder the development of broad and narrow leaf photosynthetic rate high as a result of reducing the weight of tuber. But when the temperature is too low to reach less than 10°C, the plant tissue can be damaged and an interruption of growth, so the plants tend to be stunted.

Lompongan

Dendrogram Lompongan relationship found in three different heights showed only the size difference. Broadly speaking taro from the highlands, medium and low still have the same morphological characteristics. Lompongan plants grow wildly around the edge of rice fields and waterways. Lompongan plants from the highlands have differences with the lowlands, such as: green leaf color is more concentrated, browner midrib color, and the size is larger. Unlike Lompongan plants in the highlands that were often found on the outskirts of the river with shade trees around it, the ones in the lowlands were found in around the edges of fields full of water. Environmental factors in the form of light, temperature, and humidity cause the plants to have different adaptations. According to Taiz and Zeiger (1991), leaf surface area increased because of the shade, and color changes due to the increased levels of chlorophyll a and b.

In the circumstances shaded light spectrum that is active in the process of photosynthesis (wavelength 400-700 nm) get decreased. Plants will make adjustments to streamline the capture of light energy that is by increasing leaf area, plant height and chlorophyll a and b (Lambers et al. 1998).

Altitude causes humidity, light, temperature, and moisture content to vary. According to Fitter and Hay (1998), environmental factors were related to one another so that the plant held a response to the environment. High water levels in the soil cause leaf's cell turgor to increase which in turns causes leaf's expansion. Reduced light causes the leaves to add the proportion of mesophyll tissue. Temperatures that were too high (> 40 ° C) cause defective enzyme and respiration is rapid, so the plants have stunted growth. The temperature is too low (<1°C) causes decreased enzyme activity cause plant tissue damage and death. The optimum temperature for photosynthesis is 20-30°C.

Table 2. 18 samples of *C. esculenta* in Karanganyar district with characteristics

Characteristics	Varieties																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Type of plant																		
1. Plant range																		
Narrow	-	-	-	-	-	√	√	√	√	-	-	-	-	-	√	-	-	-
Medium	-	√	√	√	√	-	-	-	-	√	√	√	√	-	-	-	-	√
Width	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	√	√	-
2. Plant height																		
Dwarf (< 50 cm)	-	-	-	-	-	√	√	√	-	-	-	-	-	-	√	-	-	-
Medium (< 50 cm)	-	√	√	√	√	-	-	-	√	√	√	√	√	√	-	-	-	-
Height (< 50 cm)	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	√	√	√
3. Number of stolons																		
1-5 buah	-	-	-	-	-	-	-	-	√	√	-	-	-	√	-	√	√	√
6- 10 buah	√	√	√	-	-	-	-	-	-	-	√	√	√	-	-	-	-	-
11-20 buah	-	-	-	√	√	√	√	√	-	-	-	-	-	-	√	-	-	-
4. Length of stolon																		
Short (<15 cm)	√	√	√	-	-	√	√	√	√	√	√	√	√	-	√	√	-	√
Length (>15 cm)	-	-	-	√	√	-	-	-	-	-	-	-	-	√	-	-	√	-
Cormus (tuber)																		
1.Cormus manifestations																		
Yes	√	√	√	-	-	-	√	√	√	-	-	√	-	√	√	√	-	√
None	-	-	-	√	√	√	-	-	-	√	√	-	√	-	-	-	√	-
2. Length of Cormus																		
Short (± 8 cm)	-	-	√	-	-	-	√	√	-	-	-	-	-	-	-	-	√	-
Medium (± 12 cm)	√	√	-	-	√	√	-	-	-	-	-	-	√	√	√	√	-	-
Length (± 18 cm)	-	-	-	√	-	-	-	-	√	√	√	√	-	-	-	-	-	√
3. Cormus branch																		
Branching	-	-	-	-	-	-	√	√	-	-	-	-	-	√	√	-	-	-
No branching	√	√	√	√	√	√	-	-	√	√	√	√	√	-	-	√	√	√
4. Form of cormus																		
Cone	√	√	√	-	-	-	-	-	-	-	-	-	√	-	-	√	√	-
Rounded	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	√
Cylindrical	-	-	-	√	√	√	-	-	-	-	-	-	-	-	-	-	-	-
Elongated	-	-	-	-	-	-	-	-	√	√	√	√	-	-	-	-	-	-
Flat and open	-	-	-	-	-	-	√	√	-	-	-	-	-	√	√	-	-	-
5. Cormus weight																		
Light (± 0.5 kg)	-	-	-	-	-	-	-	√	√	-	-	-	-	√	-	-	-	√
Medium (± 2 kg)	-	-	√	√	√	√	-	-	√	√	-	-	-	-	√	√	√	-
Weight (± 4 kg)	√	√	-	-	-	-	-	-	-	-	√	√	-	-	-	-	-	√
6. Color of cormus corte																		
White	-	-	-	-	-	-	-	√	√	√	√	-	-	-	√	√	√	-	√
Yellow-orange	√	√	√	√	√	√	-	-	-	-	√	√	√	-	-	-	√	-
7. Middle meat color																		
White	-	-	-	√	√	√	√	√	√	√	√	√	-	√	√	√	√	√
Yellow	√	√	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Orange	-	-	-	-	-	-	-	-	-	-	-	-	√	-	-	-	-	-
8. Color of meat fiber																		
White	-	-	-	√	√	√	-	-	-	-	-	-	-	√	-	-	√	√
Light yellow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	√	-	-
Yellow-orange	-	-	-	-	-	-	-	-	-	-	√	√	√	-	√	-	-	-
Red	√	√	√	-	-	-	√	√	√	√	-	-	-	-	-	-	-	-
9. Surface of cormus ski																		
Stringy	-	-	-	√	√	√	-	-	-	-	√	√	-	-	-	-	√	-
Scaly	-	-	-	-	-	-	-	√	√	√	√	-	-	√	-	-	√	-	√
Stringy and scaly	√	√	√	-	-	-	-	-	-	-	-	-	-	-	√	√	-	-	-
10. Skin thickness																		
Thick	√	√	√	-	-	-	-	-	√	√	-	-	√	√	√	-	-	-
Thin	-	-	-	√	√	√	√	√	-	-	√	√	-	-	-	√	√	√
11. Fiber level																		
Little	-	-	-	√	√	√	√	√	-	-	-	-	-	-	-	-	√	-
Much	√	√	√	-	-	-	-	-	√	√	√	√	√	√	√	√	-	√
12. Color of shoots																		
Yellow green	-	-	-	-	-	-	-	√	√	-	-	√	√	-	√	√	√	-
Pink	√	√	√	√	√	√	-	-	√	√	-	-	-	-	-	-	-	√
Purple	-	-	-	-	-	-	-	-	-	-	-	-	√	-	-	-	-	-
Leaves																		
1. Position of dominant le																		
Flat	-	-	-	√	√	√	-	-	-	-	-	-	-	-	-	-	-	-
Bowl	√	√	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	√
Upright up	-	-	-	-	-	-	-	√	√	-	-	-	-	-	√	√	-	-
Upright down	-	-	-	-	-	-	-	-	-	√	√	√	√	√	-	-	√	√
2. Edge of leaves																		
Entire	-	-	-	-	-	-	-	-	-	-	-	-	-	-	√	√	-	-
Wavy	-	-	-	√	√	√	-	-	√	√	-	-	√	-	-	√	√	-
Squiggling	√	√	√	-	-	-	√	√	-	-	√	√	-	-	-	-	-	√
3. Leaf strands																		
Green	-	-	-	√	√	√	√	√	√	√	√	√	-	√	√	-	-	-
Dark green	√	√	√	-	-	-	-	-	-	-	-	-	-	-	-	√	√	√
Purple	-	-	-	-	-	-	-	-	-	-	-	-	√	-	-	-	-	-
4. Color of leaf blade edg																		
Whitish	-	-	-	-	-	-	-	√	√	-	-	√	√	-	-	-	√	-
Green	-	-	-	-	-	-	-	-	-	-	-	-	-	√	-	-	√	-
Pink	-	-	-	-	√	√	-	-	-	-	-	-	-	-	-	-	-	-
Purple	√	√	√	-	-	-	-	-	√	√	-	-	√	-	√	-	-	√
5. Leaf tip liquid color																		
Whitish	-	-	-	√	√	√	√	√	-	-	√	√	-	-	√	√	√	-
Yellow	-	-	-	-	-	-	-	-	-	-	-	-	-	√				√
Pink	√	√	√	-	-	-	-	-	√	√	-	-	-	-	-	-	-	-
Dark red	-	-	-	-	-	-	-	-	-	-	-	-	√	-	-	-	-	-
6. Main color of vena																		
Yellow	-	-	-	-	-	-	-	√	√	-	-	-	-	-	√	√	-	-
Green	-	-	-	-	-	-	-	-	√	√	√	√	-	√	-	-	√	√
Pink	√	√	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Purple	-	-	-	√	√	√	-	-	-	-	-	√	-	-	-	-	-	-
7. Main pattern of vena																		
Y form	√	√	√	√	√	√	√	√	-	-	√	√	-	√	√	√	√	√
Y form extends	-	-	-	-	-	-	-	-	√	√	-	-	√	-	-	-	-	-
8. Color of petiole																		
Top third																		
Yellow	-	-	-	-	-	-	-	√	√	-	-	-	-	-	√	√	√	-	-
Light green	-	-	-	-	-	-	-	-	-	-	-	√	√	-	-	-	-	√	-
Brown	√	√	√	-	-	-	-	√	√	-	-	-	-	-	-	-	-	-	-
Purple	-	-	-	√	√	√	-	-	-	-	-	-	√	-	-	-	-	√	
Bottom third																		
Yellow	-	-	-	-	-	-	-	-	-	-	-	-	-	√	-	-	-	-	
Light green	-	-	-	-	-	-	-	√	√	-	-	√	√	-	-	√	√	√	
Brown	√	√	√	√	√	√	-	-	√	√	-	-	-	-	-	-	-	-	√
Purple	-	-	-	-	-	-	-	-	-	-	-	-	√	-	-	-	-	-	
9. Color of petiole line																		
Green	-	-	-	-	-	-	-	√	√	-	-	√	√	-	√	√	-	√	-
Purple	√	√	√	√	√	√	-	-	√	√	-	-	√	-	-	√	-	√	
10. Bottom slices																		
Open	√	√	√	√	√	√	-	-	√	√	√	√	√	√	√	√	-	√	
Closed	-	-	-	-	-	-	-	√	√	-	-	-	-	-	-	-	√	-	
11. Color of petiole ring																		
White	-	-	-	√	√	√	-	-	-	-	√	√	-	-	-	-	-	-	
Yellowish green	-	-	-	-	-	-	-	-	-	-	-	-	-	-	√	√	√	√	-
Pink	√	√	√	-	-	-	√	√	√	√	-	-	-	-	-	-	-	-	√
Purple	-	-	-	-	-	-	-	-	-	-	-	-	√	-	-	-	-	-	
12. Color of leaf midril																		
Whitish	-	-	-	-	-	-	-	√	√	-	-	-	-	-	-	-	-	-	
Light green	-	-	-	-	-	-	-	-	-	-	√	√	-	√	√	√	√		
Purplish red	√	√	√	√	√	√	-	-	√	√	-	-	√	-	-	-	-	√	
13. Wax coating																		
None	-	-	-	-	-	-	-	-	-	√	√	-	-	√	-	-	-	-	
Low	√	√	√	-	-	-	√	√	-	-	√	√	-	-	-	-	√	-	
Medium	-	-	-	√	√	√	-	-	-	-	-	-	-	-	√	√	-	-	
High	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	√	-	√

Note: 1. Benthul (plateau), 2. Benthul (plain medium), 3.Benthul (lowlands), 4. Lompongan (plateau), 5.Lompongan (plain medium), 6. Lompongan (lowlands), 7. Laos (plateau), 8. Laos (plain medium), 9. Linjik (plain medium), 10. Linjik (lowland), 11. Sarangan (plateau), 12. Sarangan (plain medium), 13. Kladitem (plateau), 14. Plompong (lowland), 15. Kladi (lowland), 16. jabon (plain medium), 17. Mberek (lowland), 18. Japan (plateau).

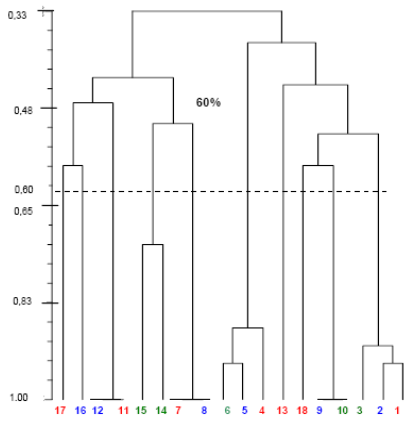

Figure 1. Dendrogram relationship 18 samples of *C. esculenta* from three different heights based on morphological characters. Description: No. 1-18 same as Table 2.

Characterization of isozyme taro

Peroxidase

Results with the dye peroxidase isozyme analysis, shikimate dehydrogenase and esterase can be seen in Figure 2. Peroxidase in 18 samples of *C. esculenta* tested to form 14 different banding pattern. Banding pattern I with migration distance (Rf) 0586, 0630, 0717, 0761 and 0804 being owned by the sample 1. Banding pattern II with Rf 0586, 0717, 0761 and 0804 were owned by sample 2 and 3. Banding pattern III is owned by sample 5 with Rf 0630, 0739, 0782 and 0826. Banding pattern IV with Rf 0630, 0739, 0782 owned by samples 4 and 6. Banding pattern V with Rf 0652, 0739, 0782, and 0874 were owned by sample 7 and 8. Rf banding pattern VI 0652, 0739, 0782 and 0869 were owned by the sample 9 and 10. Banding pattern VII with Rf 0565, 0717 0739 and 0847 held by the sample 11. Banding pattern VIII with Rf 0565, 0717 0739 owned by sample 12. IX banding pattern with a distance of 0630 and 0739 held by sample 13. Banding pattern X with Rf 0630 and 0739 held by sample 14. XI banding pattern with a distance of 0630, 0739, and 0804 is owned by the sample 15. XII banding pattern with a distance of 0630, 0739, and 0826 is owned by the sample 16. XIII banding pattern with a distance of 0630, 0717 and 0761 held by the sample 17. Banding pattern XIV with Rf 0607, 0652 and 0.761 were owned by the sample 18.

Shikimate dehydrogenase

Isozyme analysis results with dye shikimate dehydrogenase (ShDH) on 18 samples of *C. esculenta* tested to form 15 different banding pattern. Banding pattern I with Rf 0523, 0568, and 0863 is owned by sample 1. Banding pattern II with Rf 0523, 0568, 0614 and 0863 were owned by sample 2 and 3. Banding pattern III with Rf 0523, 0568, 0614 and 0840 were owned by the sample 4. Banding pattern IV with Rf 0500, 0523, 0568, 0614 and 0840 were owned by samples 5 and 6. Banding pattern V with Rf 0568 and 0840 was owned by sample 7 and 8. Banding pattern VI with Rf 0523 and 0840 held by sample 9. Banding pattern VII with Rf 0500, 0523 and 0840 were owned by the sample 10. Banding pattern VIII with Rf 0523 and 0818 held by sample 11. Banding pattern IX with Rf 0500, 0523 and 0818 were held by the sample 12. Banding pattern X with Rf 0416, 0432, 0523, 0795 owned by sample 13. Banding pattern of Rf 0500 XI, 0523, 0727 and 0750 was owned by the sample 14. XII banding pattern of Rf 0523, 0546, 0581 and 0818 held by the sample 15. Banding pattern XIII with Rf 0523, 0546, 0568 and 0795 held by the sample 16. Banding pattern XIV with Rf 0500, 0546, and 0795 was owned by the sample 17. Banding pattern XV with Rf 0546, 0568 and 0795 were held by the sample 18.

Esterase

Results with the dye esterase isozyme analysis on 18 samples of *C. esculenta* were tested forming 11 different banding patterns. Banding pattern I with Rf the same but having different shapes, and shown at Rf 0.22, 12:26 and 12:32 were owned by the sample 1, 2 and 3 (quantitative and qualitative). Banding pattern II with Rf 0.20, 0:28, 0:32 and 0.36 were owned by the sample 4, 5 and 6 (quantitative and qualitative). Banding pattern III with Rf 0:30, 0:34, 0:38, 0:40 was owned by sample 7 and 8 (quantitative and qualitative). Banding pattern IV with Rf 0.20, 0:30, 0:34, 0:38 and 0:44 is owned by the sample 9 and 10. Banding pattern V with Rf 0.20, 12:26 and 12:38 were owned by sample 11 and 12 (quantitative and qualitative). VI banding pattern was owned by the sample 13 with Rf 0.20, 0:28, 0:30, 0:46, 0:48. Banding pattern VII owned by sample 14 with Rf 0.20, 0.26, 0:30, 0:34. VIII banding pattern VIII was owned by the sample 15 with Rf 0.20, 0.26, 0:30, 0:36. Banding pattern IX was owned by the sample 16 with Rf 0.20, 0:22, 0:26, 0:32. Banding pattern X was owned by the sample 17 with Rf 0.20, 0.24, 0:32 and banding pattern XI with Rf 0.20, 12:28 and 12:32 were owned by the sample 18.

Similarity on taro genetics based on isozyme markers

Genetic similarity between samples can be tested using cluster analysis (average group analysis), which results in the form dendrogram or tree diagram. The result is a dendrogram of relationship were tested by three different enzymes (peroxidase, shikimate dehydrogenase, and esterase) (Figure 3).

Election peroxidase has advantages including a broad spectrum and has a very important role in the process of plant physiology. This enzyme can be isolated and scattered in the cell or plant tissue, especially in plant tissues that had been developed (Butt 1980; Hartati 2001). Shikimate dehydrogenase (ShDH) is an enzyme which spread to most living things. Shikimate dehydrogenase involved in oxidoreductase that catalyzes NADP + shikimate into three main products dehydroshikimate + $NADPH+H^+$. At the plant, esterase is a hydrolytic enzyme that functions to withhold simple esters in organic acids, inorganic acids and phenols and alcohols have low molecular weight and easily soluble.

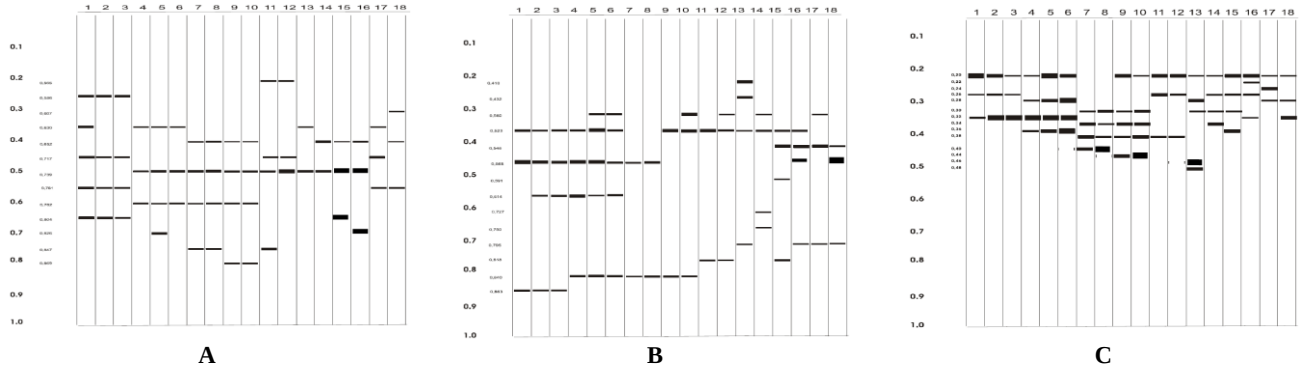

Figure 2. The variation of 18 isozyme banding pattern of sample *C. esculenta* from three different heights. Description: a. Banding pattern of peroxidase, b. Shikimate dehydrogenase banding pattern, c. Esterase banding pattern. No. 1-18 same as Table 2.

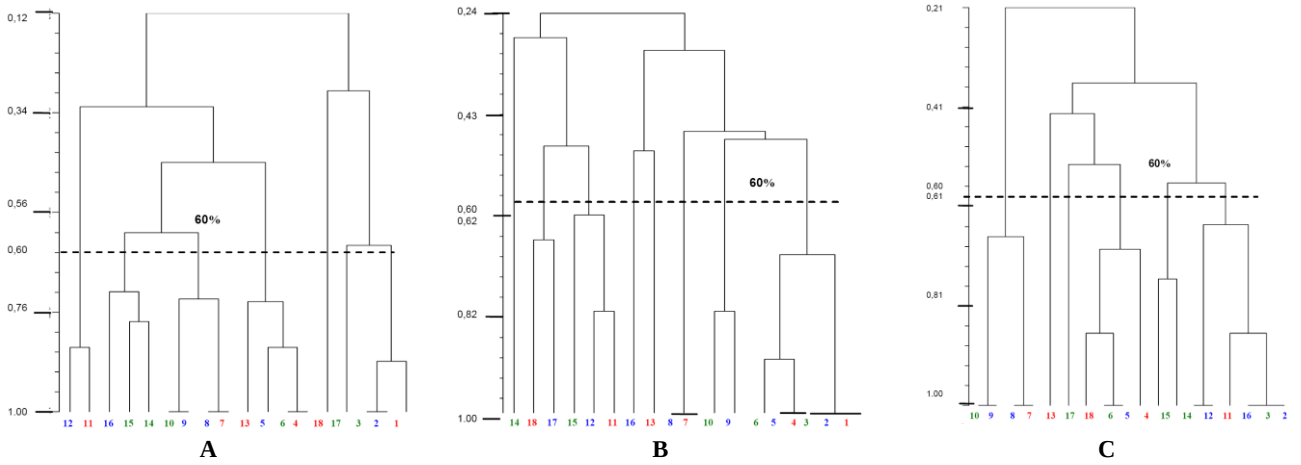

Figure 3. Relationship dendrogram 18 samples of *C. esculenta* from three different heights based on isozyme banding pattern. A. peroxidase, B. shikimate dehydrogenase, c. Esterase. No. 1-18 same as Table 2.

Results dendrogram relationship between the use of peroxidase enzymes, shikimate dehydrogenase, and esterase showed generally taro of the same variety have the same banding pattern, although from different height locations, so that enzymatically still have a high relationship, since it is estimated the same parent. On different taro varieties tend to have a different banding pattern. Formation of the group between the use of esterase, peroxidase and shikimate dehydrogenase gave different relationship relations, but in one variety is generally joined in one group at a distance of more than 60% similarity, although originating from different locations' height.

Esterase formed seven groups which were of more than 60% similarity between one another, where there was taro who joined another group. Jabon formed a group with Plompong which were of 0.80 similarities. Kladi formed a group with Plompong at a distance of 0.75 similarities. Lompongan joined with Japan at a distance of 0.70 similarities. Laos and Linjik form one group at a distance of 0.67 similarities. Even when they are different taro varieties but when they form one group, they still have a high genetic relationship.

Peroxidase also formed seven groups. In general, a variety of taro is still present in one group even though planted in different locations altitude place. Peroxidase formed a different group variation taro with esterase. In peroxidase, Laos, and Linjik in one group that was of 0.75 similarities. Plompong and Kladi form one group, but Jabon joined at a distance of 0.70 similarities. Lompongan and Kladitem form one group at a distance of 0.75 similarities. Peroxidase added information that was not the presence of new groups formed on the use of esterase.

Shikimate dehydrogenase provided the formation of different groups of taro with esterase and peroxidase. In shikimate dehydrogenase, formed three groups originating from different varieties, but it formed one group that was of more than 60% similarity. Lompongan and Benthul joined at 0.65 similarity distance. Kladi joined Sarangan at a distance of 0.62 similarities. Mberek and Japan formed one group that was of 0.67 similarities.

The use of different enzymes gave results in different groups, although there is formation of the same group with a different enzyme's use. The use of different enzyme will complement the formation of groups of different taro varieties. The genetic pattern of bands that formed in the

use of enzymes is the expression of taro varieties in question. With a specific enzyme that cannot afford some taro that expresses band patterns, but with other enzymes can express ribbon patterns. So that more types of enzymes used then it will complete the formation of groups on varieties of taro.

Results dendrogram through morphological markers and isozyme banding pattern shows the difference. From the morphological marker of the 11 varieties, obtained taro formed 10 groups at a distance of 0.60 similarities. Different taro varieties, most will form a separate group means morphologically different taro varieties have different morphological characteristics. Talas who formed a group on the analysis of relationship is Kladi and Plompong.

When the isozyme was used, more groups were formed, this means that between the different taro varieties there is still a high relationship. If the different varieties of taro belong to one group with a distance of close to 1 it is possible that the similarity comes from the older of taro. Environmental factors affect plant morphology, if the environmental factor is more dominant than genetic factors, the plant will experience a change in morphology (Suranto 1999, 2001). In the long term, it is possible to crop genetic trait changes in her body. Plants that were stressed environment would be possible to have mutations, so that in the long term can happen speciation.

New types were also possible as a result of hybridization, so having a close relationship with both of the parent species. The property of taro which has a close relationship is what can be used to search for superior taro through crossbreeding. Some taros found in Karanganyar were wild taro. Wild Taro and of likely no benefit is possible to have genetic traits that superior, so that the hybridization process to obtain high yielding varieties can be applied.

Generative breeding of taro is naturally difficult to occur because the male and female flowers get mature at different times and a new flowering occurs after more than 6 months of age. Many plants are not considered going through a flowering because the flowering process is too long. Many cultivated plants are harvested before adulthood, so many plants are difficult to perform in generative breeding.

Characterization of taro plants through morphological marker is more easily done, by observing external nature, taro plants can be assumed to have superior properties. But genetic markers also play an important role because it is more fundamental and is not influenced environment. Data morphology and isozyme banding pattern on taro plants in Karanganyar can be used in addition to the identification of the food plant breeding efforts.

Characterization relations of morphology and isozyme

The correlation between genetic distance based on morphological markers and similarity based on isozyme banding pattern were analyzed based on product-moment correlation coefficient with the criteria of goodness of fit according to Rohlf (1993). Result of calculation correlation between genetic distance based on morphological markers and genetic similarity based on isozyme banding pattern showed that between morphology and isozyme has a good correlation and a very good (Table 4). Correlation between morphological data and isozyme banding pattern of peroxidase, esterase, and shikimate dehydrogenase, respectively, also were on the value of 0.893542288, 0.917557716, 0.9121985446. This shows the characterization of taro-based on morphological markers consistent with isozyme banding pattern, so that the isozyme data support the morphological data.

Diversity is difficult to observe the morphological marker would be more accurate if you have the genetic markers such as isozymes. Morphological characters that were equipped with the character of isozyme banding pattern adds accuracy of the data to identify plant diversity. Isozyme has advantages because it requires little sample of the plant, were not inhibited during plant dormancy, can be used to perform characterization of the plant in very much.

Table 4. Relationships and morphological characterization results based on isozyme banding pattern

Characters that correlated	Level	Criteria
Morphology and POD	0.893542288	Good
Morphology and EST	0.917557716	Very good
Morphology and ShDH	0.9121985446	Very good

The relationships of taro plants obtained from places of different heights can be made into a dendrogram between morphology and marker pattern of the isozyme's band. Dendrogram based on morphological markers and isozyme banding pattern of peroxidase, shikimate dehydrogenase, and esterase showed that taro with the same type from a different altitude did not show any difference at a distance of 60% similarity. Of the eighteen samples were divided into 10 groups. Each taro with the same type, although located in different places still reflect the height of high relationship. This proved that taro plants of the same type belonged to a single group.

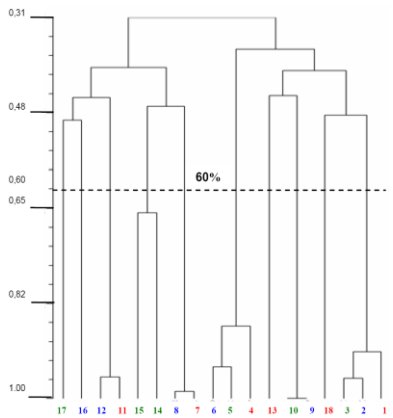

Figure 4. Dendrogram relationship 18 samples of *C. esculenta* from three different heights based on morphological markers and isozyme banding pattern of peroxidase, esterase, and shikimate dehydrogenase. Description: No. 1-18 same as Table 2.

Taro varieties which become one group is based on morphological markers and isozyme banding pattern, where the isozyme banding pattern supports the morphological data. This is evident in samples 1, 2, 3, ie Bentul from three different height locations which join one group. Other evidence was sample 4, 5 and 6, which were from three different altitude sites that also formed one group. This indicated that the isozyme data support the morphological data, so as to identify the plant in addition to morphological data, isozyme data is also needed to increase the accuracy of the data. There were varieties of taro which have a close relationship that are Kladi and Plompong that have a high relationship when viewed from the merger with its isozyme morphological characteristics, both were at the coefficient of 0.68. Allegedly the two taro plants have elders who have a high relationship, because almost the same its relation of morphology and isozyme almost the same. From the characterization results obtained that has a relationship Kladi and Plompong highest compared with other varieties of taro. Taro with different varieties formed their own groups at a distance of 60% similarity. This means that at a distance of 60% of all varieties of taro had different characters.

CONCLUSION

There is a diversity of morphological characters in 18 samples of taro plants (*Colocasia esculenta* L.) that grow in Karanganyar. Taro is still in one variety that is at different height diversity appears only on the size of the vegetative plant. The results showed isozyme banding pattern of the variability in isozyme banding pattern of peroxidase, esterase and shikimate dehydrogenase in taro varieties found in different locations. Characterization of taro-based on morphological markers is consistent with the characterization based on isozymes. Isozyme data support the morphological character data.

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