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Variation of morphological and protein pattern of cassava (*Manihot esculenta*) varieties of Adira1 and Cabak makao in Ngawi, East Java

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Abstract. *Tribadi, Suranto, Sajidan. 2009. Variation of morphological and protein pattern of cassava (*Manihot esculenta*) varieties of Adira1 and Cabak makao in Ngawi, East Java. Nusantara Bioscience 2: 14-22.* This research is intended to find out the morphological and anatomical variation as well as the protein band pattern of cassava (*Manihot esculenta* Crantz) widely spread in three different areas of height. The sample collecting is done using simple random sampling in the three different areas of height that is 50, 300, 1000 meters asl in Ngawi District, East Java while the analysis of protein band pattern is done using SDS-PAGE. The result of the reseach of morphology and anatomy is analyzed descriptively and presented in the form of tabels, histograms and figures. The analysis of protein band pattern is done using quantitative and qualitative analysis that is based on the appearance or not the gel band pattern by counting the molecular weights based on code marker S 8445 and qualitative method based on the quality of the band formed. The band pattern formed is istimated and presented in the form of zimogram. The result of the research shows that the height of the cultivating site very much influences toward variations of root, stem and leaf morphology. The longest root is at 50 meter heights asl (Cabak makao local variety, the widest stem diameter is at 50 meters asl (Cabak makao local variety) the longest leaf and branch is at 300 meters asl (Cabak makao local variety) and 1000 meters asl (Cabak makao local variety). There is no difference of anatomy in the root, stem and leaf and no difference of protein band pattern either in Adira1 or Cabak makao local variety.

Key words: *Manihot esculenta*, morphologic variation, anatomy, protein band pattern.

Abstrak. *Tribadi, Suranto, Sajidan. 2009. Variasi morfologi dan pola pita protein uni kayu (*Manihot esculenta*) varietas Adira1 dan Cabak makao di Ngawi, Jawa Timur. Nusantara Bioscience 2: 14-22.* Penelitian ini bertujuan untuk mengetahui variasi morfologi dan anatomi serta pola pita protein ubi kayu (*Manihot esculenta* Crantz) yang tumbuh pada tiga daerah ketinggian berbeda. Pengambilan sampel dilakukan dengan metode sampel acak sederhana (simple random sampling) pada tiga ketinggian tempat yang berbeda yaitu 50,300,1000 m dpl di Kabupaten Ngawi, Jawa Timur serta analisis pola pita protein dilakukan dengan metode SDS-PAGE. Hasil penelitian morfologi dan anatomi diuraikan secara deskriptip dan disajikan dalam bentuk tabel, histogram dan gambar. Analisis pola pita protein dilakukan dengan menggunakan analisis kuantitatif dan kualitatif yaitu berdasarkan muncul tidaknya pola pita protein pada gel dengan menghitung berat molekul berdasarkan marker kode S 8445 dan metode kualitatif berdasarkan kualitas pita yang terbentuk.Pola pita yang terbentuk diestimasikan dan disajikan dalam bentuk zimogram. Hasil penelitian menunjukan bahwa ketinggian habitat berpengaruh terhadap variasi morfologi akar, batang, dan daun. Umbi akar terpanjang pada ketinggian 50 m dpl (Cabak makao), diameter batang terlebar pada ketinggian 50 m dpl (Cabak makao), panjang daun dan tangkai terpanjang pada ketinggian 300 m (Cabak makao) dan 1000 m dpl (Cabak makao).Tidak ada perbedaan anatomi pada akar, batang dan daun serta tidak ada perbedaan pola pita protein baik pada varietas Adira-1 maupun Cabak makao.

Kata kunci: *Manihot esculenta*, variasi morfologi, anatomi, pola pita protein.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is not a plant native to Indonesia, but has become very popular in Indonesia. Cassava belongs to the family of shrubs plant that is originally from the American continents, specifically from Brazil and Central America (Mexico, Bolivia, Peru, Venezuela, Guyana, and Suriname) (Nassar 1978 1992; Olsen and Schaal 1999; Nassar et al. 1996, 2008; Allem 1994). The spread of cassava has been almost to the entire world, including Africa, Madagascar, India and China. These plants came into Indonesia in 1852. Cassava is grown in agricultural areas. Cassava plant is widespread throughout Indonesia, which has many local names, such as *katela, kentila, ubi kayee* (Aceh), *ubi parancik* (Minangkabau), *ubi singkong* (Jakarta), *batata kayu* (Manado), *bistungkel* (Ambon), *buari deur, vori jendral, kasapen, sampeu, ubi kayu* (Sunda), *balet kasame, kaspa, kaspe, ketela buding, katela jendral, katela kaspe, kaspa, kaspe, katela budin,katela mantra, katila marikan, katela menyok, katela paung, katela prasman, katela sabekan, katela sarmunah, katela tapah, katela cengkol, ubi kayu, tela pohong* (Jawa), *blandong, manggala menyok, pohung, pahoung, sambrang balada, same, katela balada, tengsak* (Madura), *kesame, ketal kayu, sabrang same* (Bali), *kasubi* (Gorontalo), *bare, padu, lame kayu* (Makasar), *lame ayu*

(Bugis Majene), and *kasibi* (Ternate, Tidore) (Heyne 1987; Balitkabi 2009).

Cassava has been a staple food as well as comodity. It has been the major source of food in food in South Africa and certain areas in Indonesia. Cassava is a source of carbohydrates for an estimated 800 million people around the world (CIAT 1993; Nweke 1996). In Indonesia, this plant stays in the third place after rice and maize as the main source of carbohydrates. As a commodity Cassava can be processed to produce dried cassava, tapioca, ethanol, liquid sugar, sorbitol, monosodium glutamate, and modified cassava flour (mocaf) (Harnowo et al. 2006; Wargiono 2006). Cassava can be also an alternative source of energy. This is in accordance with the Presidential Regulation No. 5 of 2006 which says that the increased production of cassava can be used as bio-ethanol fuel that is mixed with 10% premium (premium mix E10).

There are three subspecies of Cassava. Cultivated subspecies are all included *M. esculenta* subsp. *esculenta*, which are closely related with the wild subspecies namely *M. esculenta* subsp. *peruviana* that grows in Peru and Brazil and wild species of *M. esculenta* subsp. flabellifolia that grows in Brazil and Venezuela (Allem 1994, 2002). This variety cassava (*M. esculenta* subsp. *esculenta*) which consists of 7200 cultivars has been released. The varieties of superior cassava that are commonly grown today include: Adira-1, Adira 2, Adira 4, Darul Hidayah, Malang 1, Malang 2, Malang 4, Malang 6, 3 and UJ 5 (Subandi 2007). Cultivar Adira 4, Malang 6, UJ 3 and 5 has a superior characteristic in accordance with the criteria for the raw material of bioethanol (high starch content) (Balitkabi 2009).

The purpose of this study are to identify the (i) morphological, (ii) anatomical, and (iii) protein banding pattern variation of cassava that exists in areas with three different level of heights (50 m, 300 m, 1000 m) above the sea level in the Ngawi District, East Java.

MATERIALS AND METHODS

The experiment was conducted from June 2008 to June 2009. Research morphology and the leaves sample of *M. esculenta* done in several sub-centers of cultivation and production of cassava in Ngawi District, East Java, namely: (i) Northern Ngawi (+ 50 m asl), covering sub-district Karangjati, Bringin and Karanganyar; (ii) Central Ngawi (+ 300 m asl), covering sub-district Kwadungan, Paron and Mantingan; (iii) Southern Ngawi (+ 1000 m asl), covering sub-district Jogorogo, Ngrambe and Sine (Figure 1).

Ngawi District represents the northern lowlands, with the height of 50 m above the sea level, so too the central part of Ngawi, with the height of about 300 m above the sea level. The area has the air temperature of 26-380ºC, the

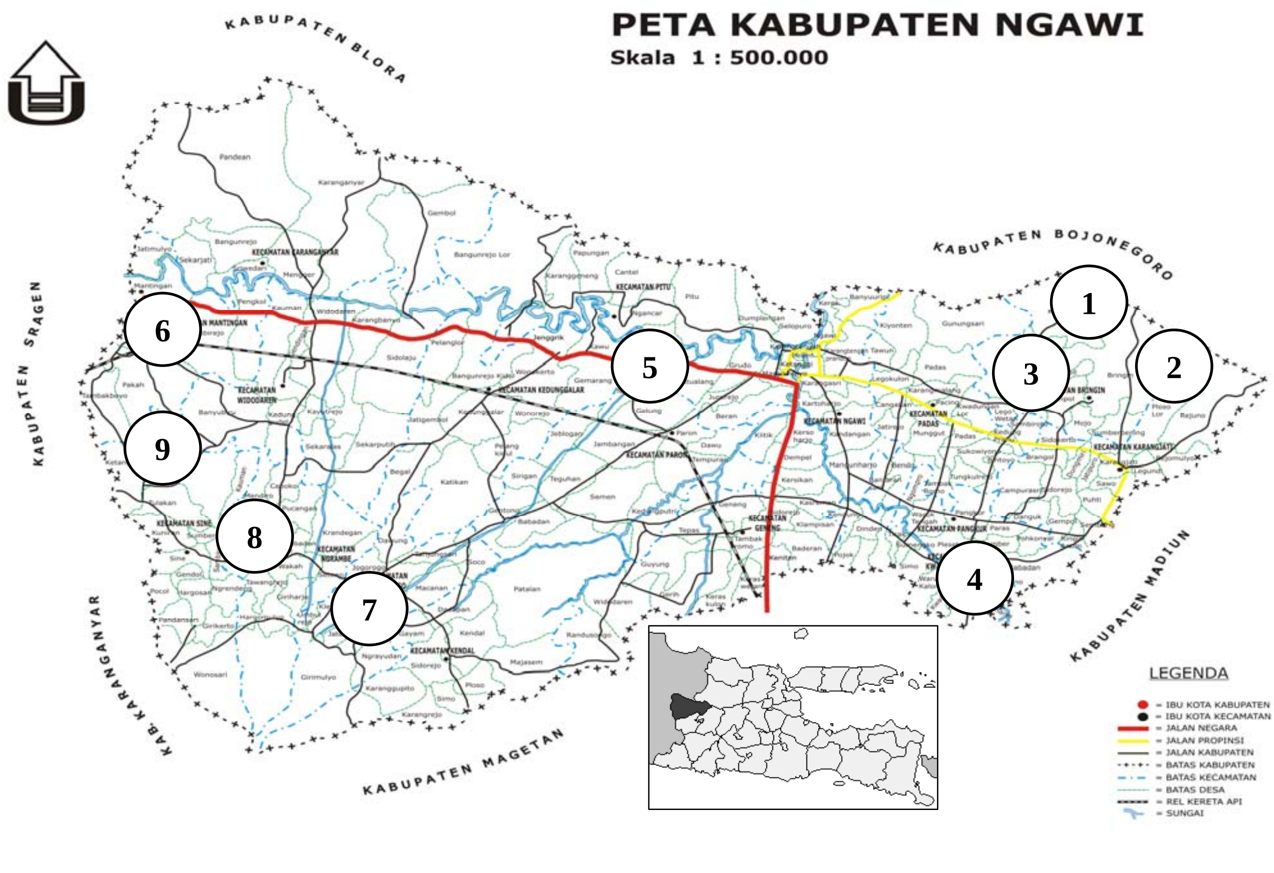

Figure 1. Research sites of cassava in the Ngawi District. The north: 1. Bringin, 2. Karangjati, 3. Karanganyar; the center: 4. Kwadungan, 5. Paron, 6. Mantingan; and the south: 7. Jogorogo, 8. Ngrambe and 9. Sine.

rainfall of 1800 mm/year, its soil type is clay that becomes hard when it is dry. The south part of Ngawi is a plateau with the average of about 1000 m above sea level, with the peak in Mount Lawu (3265 m asl). The nort part of Ngawi is dominated by several plantations, cassava, tobacco, teak, soybeans, corn and a little rice. The central part of Ngawi is dominated by plantations, rice, cassava, tobacco, soybeans, corn, sugarcane. The southern part Ngawi is dominated by plantations, rambutan, tea, coffee, cassava, soybean, corn, cocoa, and zalacca fruits (Office of Agriculture, Plantation and Horticulture, Ngawi District 2009).

Material

The materials used in the research is *M. esculenta* from three different altitudes in Ngawi District. The entire plant is used for morphological study and to test the protein banding pattern the third leaf from top of the pant is used. The pattern of protein bands revealed by SDS-PAGE method with protein dye system is Comassie blue, premises marker code S 8445 (Sigma, Germany). Samples were taken by simple random sampling method.

Procedures

Morphology and anatomy. Observations of morphology include cassava's roots (skin color, tuber color and flavor), stems (distance segment and color), and leaves (shape, color and stems). Observation of cross-sectional anatomy covers the roots, stems and leaves.

Protein band patterns. Protein banding pattern analysis was conducted using SDS-PAGE (Schägger et al. 1988; Artama 1991; Tarkka 2000). The third leaf from the top part of cassava plant (two varieties, three locations in Ngawi) is washed with a mortar and pestle mixed extract buffer 500 uL. Once crushed and homogenized it is put into in a tube with ependorf. Centrifugation is prepared and when it has been more or less cold (with temperature of ± 0ºC) then the tube is inserted to be centrifuged with the speed of 12,000 rpm for 5 minutes. Thus, the sample solution is divided into two parts. The top of the colored nodes (supernatant) will be used in the process of electrophoresis, which is then stored in a place with the temperature of 4ºC, while the bottom solid forms (pellets) are removed. Supernatant is boiled for two minutes with so that the protein can open.

Polyacrylamide gel consists of 2 parts, ie separating gel that lies at the bottom with a concentration of 12% and stacking gel which is located at the top with a concentration of 3%. Separating gel is made by mixing ± 10 ml of stock SDS PAGE 12%, plus 7 uL Temed and 80 uL APS 10%. While the 3% stacking gel is made by mixing 5 ml of stock 3% stacking gel plus 3.5 uL Temed and 50 uL APS 10%. Polyacrylamide gel solution is mixed. After it is homogeneous the separating gel electrophoresis is put into in the glass, after somewhat thickened some saturated isobutene is added. After that the saturated isobutene is removed and the stacking gel electrophoresis is included in the glass just above the staking gel. After that the sample comb is mounted on the stacking gel and released after it gets solid, and until some holes are formed that will be filled with the supernatant. The samples of Supernatant are loaded into the hole as much as 10 uL. Before the installation of the plate glass on the vessel electrophoresis, it must be ensured that circulator temperature is less than 4ºC. After that the clip tube clamps and shied from the glass plates are removed and then the glass plates are set face to face to each other on the vessel electrophoresis, with the notched glass plate is put inside. At the time of installation there should be no air bubbles between the glass plates, then tighten the bar holder. The running buffer solution is added into the plate glass tanks that have been installed face to face to each other so that it is just right below the notch. After that the electrode buffer is filled again until it is full and bathtub's lid is put back again. The power supply is turned on again to run the electrophoresis process with electric current at 125 volts for 90 minutes or until the supernatant reaches the lowest limit. After the electrophoresis process is complete, the gel is taken and continued to get colored. By putting the gel on the the plastic tray, then blue comassie is poured onto it and let it there overnight. After that the gel is rinsed with the destaining until clear. When the gel is clear, then the washing is stopped by replacing the destaining with 10% glacial acetic acid.

Data analysis

All data are described in descriptive method. Observation of morphology, including roots (tuber), stems and leaves are tested by doing analysis of the variance followed by Duncan test to know the difference; then presented in the form of tables, images and histograms. In observation of the anatomy of the roots, stems, and leaves, the preparats are microscopically photographed, and then presented in the form of images and the results are compared descriptively based on of the heights of the area the plants are grown and the varieties. Data analysis performed by the pattern of protein bands in quantitative and qualitative method is based on gel banding pattern appears or not by calculating the molecular weight marker code based on the S 8445 (Sigma, Germany) and qualitative methods based on the quality of banding pattern formation.

RESULTS AND DISCUSSION

Morphology

The result of morphological study of cassava varieties with research samples of Adira-1 and Cabak macao in the areas with the height of 50 m, 300 m, 1000 m above sea level in Ngawi District showed the presence of variations. The results of morphological observation of cassava, the varieties of Adira-1 and Cabak macao are shown in Figures 2 and 3 and Tables 1 and 2.

Adira-1

Adira-1 of northern Ngawi (50 m asl). The characteristics are: has roots, outer brown and yellow skin color, edible taste, stem segments with the length of 2-4 cm, an oval shape for the leaves, red color for the stalks and no flowers. Of the five samples can be gained the

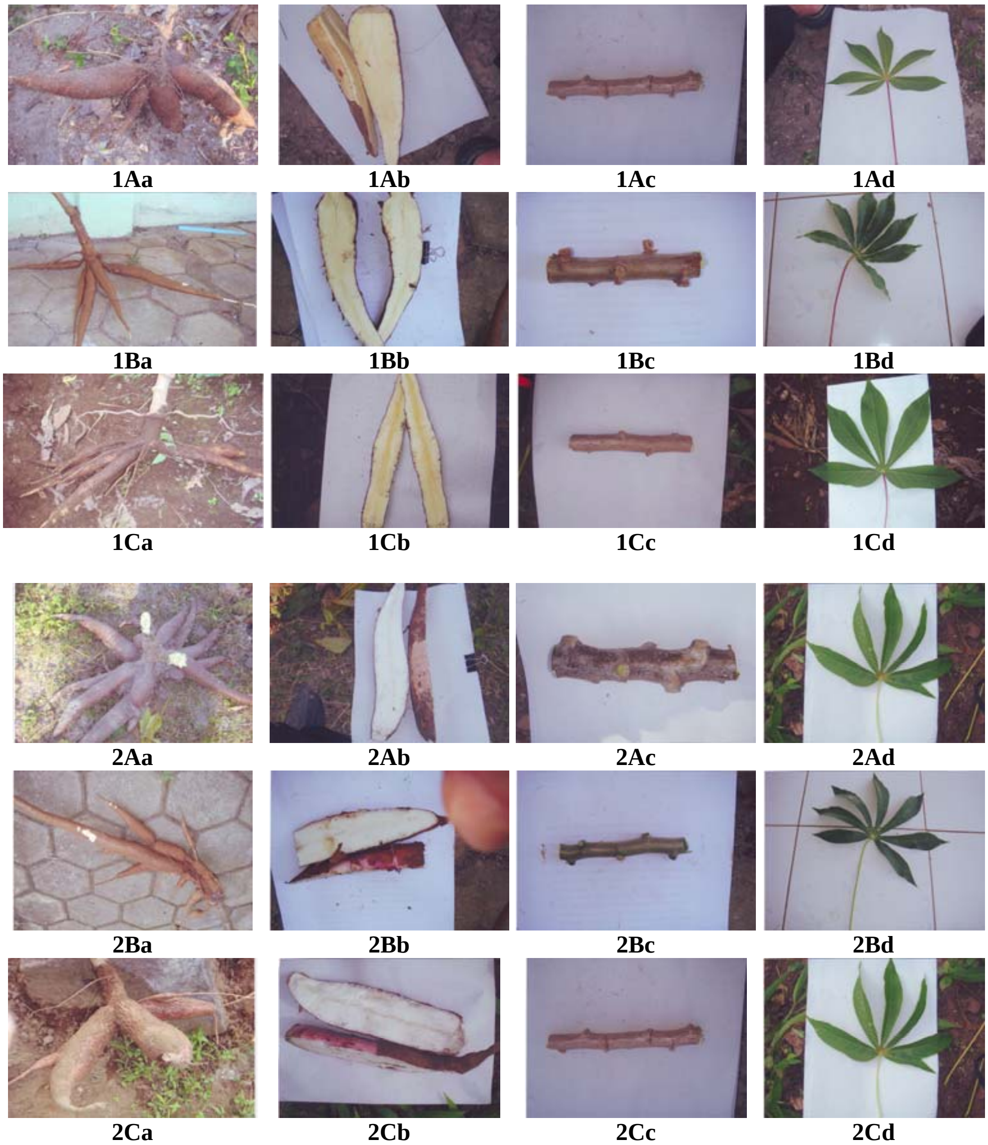

Figure 2. Morphology of cassava, the varieties of Adira-1 and Cabak Macao from various areas of the heights. Note: 1. Adira, 2. Cabak macao; A. 50 m asl, B. 300 m asl, C. 1000 m asl; a. tuber roots b. tuber color, c. stem, d. leaf.

following average: The length of the root is19.84 cm. The length of one segment to another is 2.32 cm. The diameter of the stem is 2.38cm. The length of the leaf is 9.72 cm. The length of the stem is 13.84 cm. The location of the study is Karangjati, Ngawi, with the average rainfall of 1800mm/year, the average temperature of 350C, 6 for the pH of the soil , with grumusol for the type of the soil..

Adira-1 of central Ngawi (300 m asl). The characteristics are: has roots, with brown skin color for the outside part and yellow for the inside, yellow for the tuber, and edible. 2-4 cm for the length of the stem, with yellow color and oval for its leaf's shape. The color of the stem is red and the type of the flower is kind of combination of many shades of brown color. Of the five samples can be gained the following average: The length of the root is 35.28 cm. The length of one segment to another is 3.18 cm. The diameter of the stem is 2.92 cm. The length of leaf is14.64 cm. The length of the stem is 21 , 48 cm. The research's location in Kendal, Ngawi with the rainfall of 1885 mm rain/year, the average temperature of 250C, 6 for the soil's PH, brown Mediterranean for the type of the soil.

Adira-1 of southern Ngawi (1000 m asl). The characteristics are: has roots, with the brown skin color for the outside part and yellow for the inside, yellow for the tuber and edible. 2-4 cm for the length of the stem with yellow color and oval for its leaf's shape. The color of the stalks is red and no flowers. Of the five samples can be gained the following average: The length of the root is 22.55 cm. The length of one segment to another is 3 cm. The diameter of the stem is 2.28 cm. The length of the leaf is 14.88 cm. The length of the stem is 23.04 cm. The research's location is in Jamus Ngawi, with the average rainfall of 4473 mm/year, the average temperature of 100ºC, 6 for the soil's pH, and the soil's type is brown lithosols.

Cabak macao

Cabak macao of northen Ngawi (50 m asl). The characteristics are: has roots, with the brown skin color for the outside part and red for the inside, white color for the tuber, and edible. 2-4 cm for the length of the stem, blackish green color, oval for the leaf's shape, light green for the stalk's color, and no flowers. Of the five samples can be gained the following average: The length of the root is 47.44 cm. The length of one segment to another is 2.96 cm. The diameter of the stem is 3.92 cm. The length of the leaf is 17.44 cm. The length of the stem is 26 , 6 cm. The research's location is in Karangjati, Ngawi, with the average rainfall of 1800 mm/year, the average temperature of 350C, 6 for the soil's pH, and grumusol taupe for the soil's type.

Cabak macao of Central Ngawi (300 m asl). The characteristics are: has roots, with the brown skin color for the outside part and red for the inside, white color for the

Table 1. The morphology observation of *M. esculenta*, varieties Adira-1 and Cabak macao (planted in June 2008 - August 2009).

Morphological characteristics		Adira 1			Cabak makao		
		Southern Ngawi	Central Ngawi	Northern Ngawi	Southern Ngawi	Central Ngawi	Northern Ngawi
Root	Outer skin (brown)	√	√	√	√	√	√
	Inner skin (red)	-	-	-	√	√	√
	Inner skin (yellow)	√	√	√	-	-	-
	Tuber (yellow)	√	√	√	-	-	-
	Tuber (white)	-	-	-	√	√	√
	Taste (good)	√	√	√	√	√	√
Stem	yellow	√	√	√	-	-	-
	Darken green	-	-	-	√	√	√
Leaves	Shape (palm)	√	√	√	√	√	√
	Petiole (red)	√	√	√	-	-	-
	Petiole (pale green)	-	-	-	√	√	√

Table 2. The average morphological characteristics measurement (cm) of *M. esculenta*, varieties Adira 1 and Cabak macao based on altitude.

Altitude	Length of tuber		Internode distant		Stem diameter		Length of leaves		Length of petiole	
	Ad	Cm	Ad	Cm	Ad	Cm	Ad	Cm	Ad	Cm
50 m dpl	19.84	47.44	2.32	2.96	2.38	3.92	9.72	17.44	13.84	22.36
300 m dpl	35.28	41.6	3.18	3.4	2.92	3.46	14.64	25.28	21.48	27.48
1000 m dpl	22.55	38.6	3	3.16	2.28	1.96	14.88	18.2	23.04	22.36

Note: ad: Adira, cm: Cabak macao.

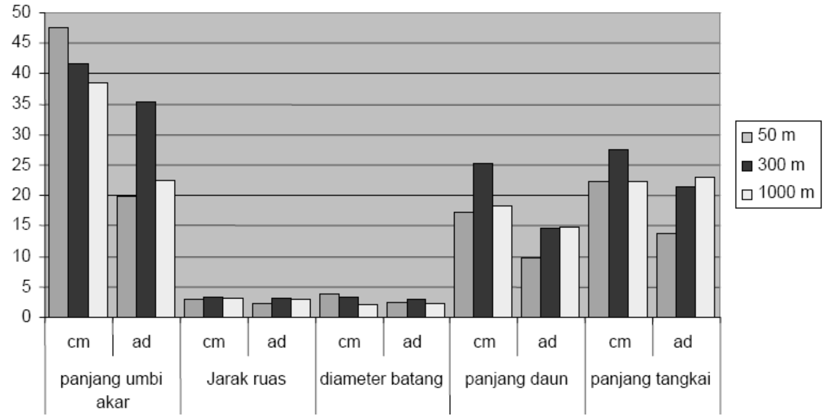

Figure 3. Comparative morphology of cassava varieties, Adira-1 and Cabak macao. Note: ad: Adira, cm: Cabak macao.

tuber, and edible. 2-4 cm for the length of the stem, blackish green color, oval for the shape of the leaf, light green for the color of the stalks, and green for its compound flowers. Of the five samples can be gained the following average: The length of the root is 41.60 cm. The length of one segment to another is 3.4 cm. The stem's diameter is3.46 cm. The length of the leaf is 25.28 cm. And the length of the stem is 27.48 cm. The research's location in Kendal, Ngawi with the rainfall of 1885 mm rain/year, the average temperature of 250ºC, 6 for the soil's pH, brown Mediterranean for the type of the soil.

Cabak macao of southern Ngawi (1000 m asl). The characteristics are: has roots white bulb with the brown skin color for the outside part and red for the inside, white color for the tuber, and edible. 2-4 cm for the length of the stem with blackish green as its color, light green its leaf's color and brown for its compound flowers. Of the five samples can be gained the following average: The length of the root is 38.6 cm. The length of one segment to another is 3.16 cm. The diameter of the stem is 1.96 cm. The length of the leaf is 18.2 cm. And the length of the stem is 22.36 cm. The research's location is in Jamus Ngawi with the average rainfall of 4473 mm/year, the average temperature of 10ºC, 6 for the soil's pH, and the soil's type is brown lithosols.

Morphological observations cassava, for the varieties of Adira-1 and Cabak macao from three areas of research with different altitudes of 50 m asl, 300 m above sea level, and 1000 m above sea level on the length of the root, the length of one segment to another , the diameter of the stem, the length of the leaf and the length of the stem, indicated variations in morphological levels. This is shown by data Table 2 and Figure 3. It means that the environmental factors in this case is the altitude of the area has effects on the morphological variations, especially for the varieties of Adira-1 and Cabak macao in Ngawi.

Based on data in Table 2 and Figure 3 for the varieties of Adira-1 can be gained some following data: for the measurement of the length of the root it can be concluded that there are significant differences that show that the altitude of where the cassava is planted determines the length of the roots. The longest root is found in the study sample planted in the area with the height of 300 m above sea level (35.28 cm). For the measurement of the length of one segment to another it is also found some differences but not as significant as the measurement foe the length of the roots. The longest is found in the study sample in the area with the height of 300 m above sea level (3.18 cm).

Altitude also affects the diameter of the trunk but not significant. For the length of the leaf it is also obtained data some differences in the results, but there are similar data in the study sample at the height of 300 m and 1000 m above sea level that means that altitude in anyway also affects the morphology, particularly the variations for the length of the leaves. Although not absolute, the altitude also affects the length of the stalk. The data of longest stalks is obtained in the study sample in the area with the height of 1000 m asl (27.48 cm).

Similarly, data from Tables 2 (Figure 3) for Cabak macao can be obtained the following data: The altitude also affects the morphological variations of the length of the root. The longest root is found in the sample at a height of 50 m above sea level (47.44 cm). The altitude also affects the length of one segment to another even though not really significant. The longest is found in the altitude of 300 m above sea level (3.4 cm). The diameter of the stem is also influenced by the altitude though not significant. The altitude shows significant influence on the morphological variations particularly on the longest leaf's length obtained in the study sample with a height of 300 m asl (25.28 cm) that is almost equal to the height of 50 m and 1000 m asl and the longest stem length data in the study sample in the area with the height of 300 m asl (27.48 cm) and almost the same with the study sample in the area with a height of 50 m and 1000 m asl.

At the organism level, phenotype is something that can be seen, observed and measured. It is a natural characteristic for individuals. Phenotype is determined by some genotypes of individuals, in some cases by the environment where these individuals live, the time and in some cases also by the interaction between the genotype itself and the environment. Time is usually classified as environmental aspects (of life) this can be written as follows: P = G + E, with P means phenotypes, and E means the environment. Observation of phenotypes can be simple for instance to observe the color of flowers or the stalks or can very complicated that requires special tools and methods (Cheverud 1982).

For the same type of cassava found in the three research's locations with the height of 50 m, 300 m, and 1000 m asl showed no significant morphological variations, except for length of the root, leaf and stalk. This variation is related to the growth of each plant. The cassavas found at the altitude of 300 m asl have bigger size then the ones at the other two places with the same age. The differences that emerged are related to the physical/environmental factors where the cassava is planted. the research location with the height of 300 m asl is a good and ideal place for the growth of ideal crops.

Temperatures that are too low or too high can affect the opening of stomata which in turn affects the photosynthesis process (Levitt 1980). Temperatures above 30° C tend to cause cassava stomata to open properly, so that the photosynthesis works effectively and the plants grow faster (Bueno 1986). While temperatures below 20°C tend to cause the stomata to close (Akparobi et al, 2002a, b). Low temperatures slow down the growth of cassava (El-Sharkawy 2004). In addition, the response of stomata to temperatures is also strongly influenced by water content and humidity in plants (Berry and Bjorkman 1980).

According to Park et al. (1997) and Sulistyono et al. (1999) anytime plants deal with environmental pressure, they always make an adaptation. They may make adjustments through changes in morphological and physiological characteristics. Suchs an adjustment is made by for instance making the leaves wider but at the same time thinner (Taiz and Zeiger 1991).

Phenotype/morphological aspects in living creatures is a combination of genotype and environmental factors (Prawoto et al. 1987). The physical environment of the northern of Ngawi is different from the one of the Central and South Ngawi (in terms of altitude, rainfall, temperature and soil type). Then the altitude for instance influences a lot towards the phenotypes that arise in the form of morphological characters in the study samples (cassava, varieties of Adira-1 and Cabak macao), except for certain characteristics such as the color of the outer and inner part of the roots, the stem's color and the taste. This can happen because phenotypes that appear are not necessarily morphological, they can be physiological. Changes in physiological characteristics only influence the system so that the cell performance can not be detected on morphological levels.

Another possibility that caused the characteristics of the study samples of the varieties of Adira-1 and Cabak macao in northern, central and the south part of Ngawi despite different environments is because genetic factors may have a stronger influence than that of the environmental factors. As stated by Suranto (2001) that the emergence of variations can be caused by two factors namely environmental factors and genetic factors. If genetic factors have a stronger influence than environmental factors, then individuals living in different environments may not show morphological variations.

Anatomy

Analysis is based on cross-sectional slice on the anatomical parts of cassava, for the varieties Adira-1 and Cabak macao, covers cross-sections of roots, stems, and leaves for the species planted in area with the different heights: 50 m asl, 300 m asl and 1000 m asl presented in Figure 4.

Adira-1

Roots. Based on the results of the cross sectional slice with an enlargement of 4x10 mm, at an altitude of 50 m asl, 300 m asl and 1000 m asl, it can be gained that there is no difference/almost the same as between the one in the areas with the altitude of 50 m asl, 300 m asl and 1000 m asl.

Stems. The result of cross-sectional slice with 2.4 magnification x10 mm. There is little difference for the density between cells shown from the plants in different altitude of 50 m asl, 300 m asl, and 1000 m asl. The altitude seems to influence the density between cells but not really significant.

Leaves. parenchyma cells of the leaves are found almost the same/no significant difference. The carrier tissues (phloem and xylem) show a state that is not much different either at the altitude of 50 m asl, 300 m asl, or 1000 m asl (Figure 4).

Cabak macao

Roots. Analysis based on the anatomy of roots, cassava, the varieties of Cabak macao, with enlargement of 4x10 mm2. It can be found that there is no difference in density.

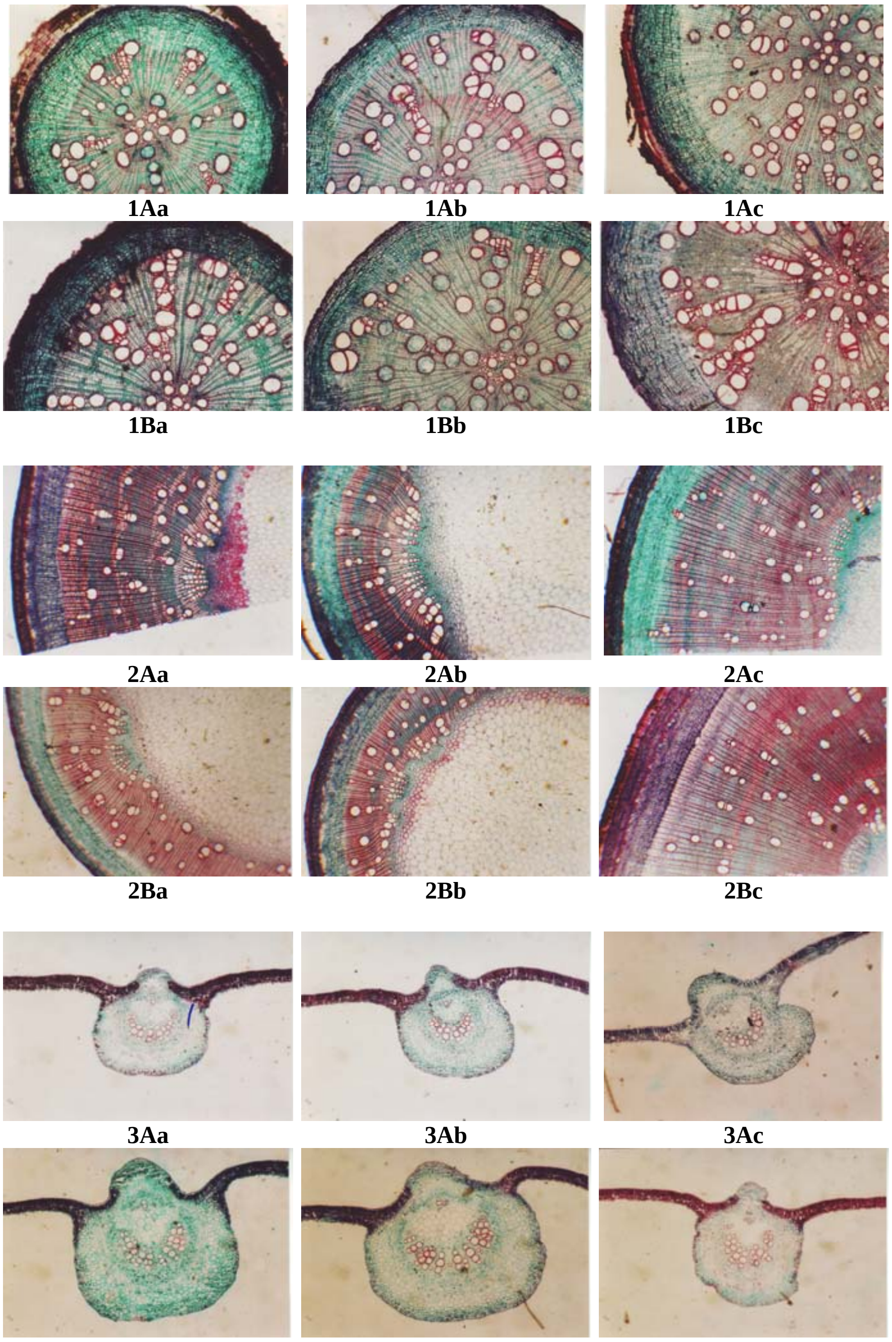

Figure 4. Cross sections of roots, stems, leaves of cassava varieties Adira-1 and local Cabak macao based on altitude. Note: 1. Root, 2. Stem, 3. Leaves. A: Adira-1, B: Cabak macao; a. 50 m asl, b. 300 m asl, c. 1000 m asl.

The structure of the root tissues shows the similar look of one from another.

Stems. Analysis on the stems to the variety of Cabak macao with 4x10 mm magnification. It shows that the distance or density between cells of the plants grown in the areas with the altitude of 50 m asl and 300 m asl appear smaller than at an altitude of 1000 m asl. It means that altitude only has insignificant effect on the anatomy of the stem. Altitude also has little effect on the distance or density between cells.

Leaves. Analysis of the cross sectional slice of the leaves, focusing on the bone, with 4x10 magnification, shows similar looks in terms of structures both at the altitude of 50 m, 300 m, and 1000 m asl. Cells around the carrier tissues around shows no significant difference either at the altitude of 50 m asl, 300 m asl or1000 m asl (Figure 4). Based on the above results all samples shows similar looks and characteristics, although they are planted in areas with different altitudes.

It can be understood that the three research's locations are still in one region that is in Ngawi, so it is possible that each sample of existing research in these three sites belongs to the same family that has no genetic difference whatsoever.

Genetic factors have stronger influence than that of the environmental ones, so that the plants belonging one and the same genetic characteristics show similar looks even when planted in different areas with different environmental factors. This is supported by results based on morphological variation indicating that cassava with the same variety found in different locations did not show variations in morphological levels.

Appearance of a phenotype depends on the nature of the relationship between genotype and environment. In fact, the development of an organism is influenced by the state of the environment and gene interactions. Living organisms are always responsive to the environment during its development. In a broad sense, environmental factors including both outside and inside factors, affect how a phenotype looks. Both of these factors can provide a major influence on the phenotype (Crowder 1997).

The result of cross-sectional analysis/anatomy of roots, stems, leaves for the varieties of Adira-1 and Cabak macao, in the areas with the altitudes of altitude of 50, 300, and 1000 m asl in the district Ngawi can be described as follows: the distance between cells of the roots cross did not show significant differences. There was no difference in the density between cells in the stems. There was no difference in that of the leaves, too. The final conclusion of this discussion is that the altitude where the cassavas are planted has no significant effect on the anatomy of the stems, roots and leaves.

The pattern of protein bands

According Suketi (1994) proteins or enzymes can be separated by using electrophoresis and the result is zimogram banding pattern. Zimogram electrophoresis results of a typical patterned so that it can be used as a characteristic phenotype to reflect genetic opener. In the electrophoresis process of gel used polyacrylamide gel was used. The percentage of polyacrylamide in the electrophoresis is 7%, usually done in a tris glycine buffer at the pH of 8.1. In certain cases the comparison between polyacrylamide and pH is various (Suranto 2001). Electrophoresis is processes in which molecules of enzymes/proteins that have electricity moves through the electric field. The speed of the molecule/protein of the enzymes depends on the amount of the electric current. The separation of one molecule/protein of enzymes from another by the electrophoresis process is influenced by two factors: the amount of the electric current and the size of the particles

The results of the electrophoresis process on the cassava's leaf for the varieties of Cabak Adira-1 and Macao with code S 8445, is shown in Figure 5.

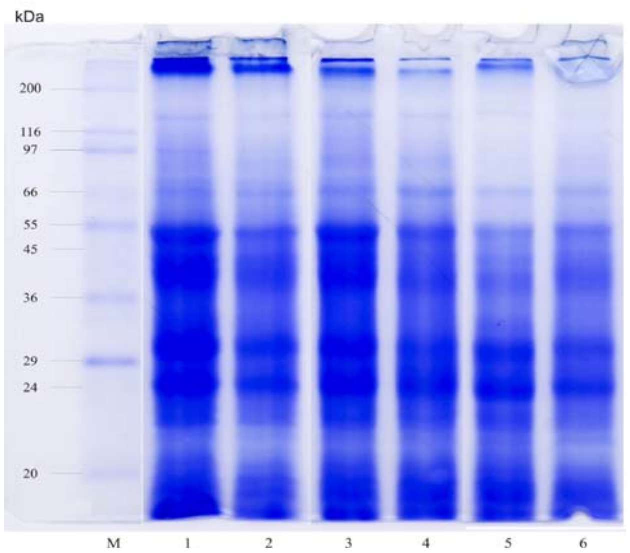

Figure 5. Protein banding pattern of cassava leaf varieties of Adira-1 (1, 3, 5) and Cabak macao (2, 4, 6). Note: 1: Adira-1 50 m asl, 2: Cabak macao 50 m asl, 3: Adira-1 300 m asl, 4: Cabak macao 300 m asl, 5: Adira-1 1000 m asl, 6: Cabak macao 1000 m asl., M = proteins marker (S 8445, Sigma).

Based on zimogram, the variety of Adira-1 (Figure 5, nos. 1, 3, 5) expresses 20 bands; nos. 1, 2 (thick) MW was not detected, no. 3 MW 158 kDa, no. 4 MW 92.6 kDa, no. 5 MW 88.2 kDa, no 6 MW 70.4 kDa, no 7 MW 66 kDa, no. 8 MW 63.8 kDa, no. 9 (thick) MW 55 kDa, no. 10 (thick) MW 45 kDa, no. 11 (thick) MW 44 kDa, no. 12 (thick) MW 42 kDa, No. 13 (thick) MW 38.3 kDa, no. 14 (thick) MW 30.4 kDa, no. 15 (thick) BM 25.8 kDa, no. MW 1623.7 kDa, no. 17 MW 20 kDa, nos. 18, 19, 20 MW was not detected. Same banding pattern expressed both in height (50 m, 300m 1000 m asl).

Cabak macao (Figure 5, nos. 2, 4, 6) expresses 20 bands. No.1, 2 (thick) was not detected, no 3 MW 158 kDa, no. 4MW 92.6 kDa, no. 5 MW 88.2 kDa, no 6 MW 70.4 kDa, no. 7 MW 66 kDa, No. 8 MW 63.8 kDa, no. 9 MW 55 kDa, no. 10 MW 45 kDa, no. 11 MW 44 kDa, no. 12 MW 42 kDa, no. 13 MW 38.3 kDa, no. 14 MW 30.4 kDa, no. 15 MW (thick) MW 25.8 kDa, no. 16 MW 23.7 kDa, no. 17 MW 20 kDa, nos. 18, 19, 20 MW was undetected. Band s were expressed equally well at a height of 50, 300, 1000 m asl.

Protein banding patterns for the varieties of Adira-1 and Cabak macao in the areas with the altitude of 50 m asl (no. 1, 2) and 300 m asl (no. 3, 4) in general seem much thicker than in those of the varieties of Adira-1 and Cabak macao at the altitude of 1000 m asl (no 5, 6). This shows a higher protein content that is possibly because at an altitude of 50 m and 300 m asl more sunlight is accessed that facilitates the better photosynthesis process, including the formation of proteins. The features of study samples of the protein bands (Adira-1 and Cabak macao) at an altitude of 50, 300, 1000 m asl did not show any difference/variation. The difference is only on the thickness bands due to the differences in the number of migrated protein molecules or the differences in the content/the quantity of protein. The thickness of the bands does not indicate the difference of the molecular weight, but only shows the differences in the content/the quantity of migrated proteins (Maryati 2008).

Apparently, the limited number of samples tested may cause the disappearance of protein polymorphism in cassava, since several other studies have shown the existence of polymorphism in cassava and its relatives with the marker of proteins such as in the researches conducted by Nassar (2003), De Souza (2002), and Nassar et al. (2010). On the other hand, studies using isozym, which is equivalent to the protein, to study the diversity of cassava also show the polymorphisms in the population. Sumarani et al. (2004) found that 37 polymorphic bands appear on the test of 218 accessions of wild cassava with esterase enzyme. Lefevre and Charrier (1993) found that from 365 cultivars and 109 accessions of wild cassava in Africa there are 17 bands of polymorphism generated by 10 enzymes dye. In Parana Brazil, Resende et al. (2000), found 28 loci polymorphisms of local cassava samples with four enzyme systems. Research by Montarroyos et al. (2005), on 28 accessions of cassava in Pernambuco, Brazil showed the existence of 6 and 8 isozyme banding patterns with GOT and peroxidase. Genetic diversity with isozyme in populations of cassava were also found by Hussain et al. (1987), Ramirez et al. (1987), and Sarria (1993).

CONCLUSION

The altitudes at which the plants are planted affect the variation of morphology, the length of the root, tuber and stalk. The longest samples are dominated by the ones from the height of 300 m asl because of the height is a good habitat and an ideal place for planting cassava. Anatomical observations indicate that the altitudes have no effect on the anatomy of the roots, stems and leaves of the plants. Analysis of protein band patterns showed that there were no differences in protein band profiles of cassava samples from different varieties (Adira-1 and Cabak macao) or different altitudes (50 m, 300 m and 1000 m asl). The difference is only the thickness of the bands due to the differences in the content/quantity of migrated protein molecules.

REFERENCES

Akparobi SO, Ekanayake IJ, Togun AO. 2002. Genotypic variability for cassava tuberous root development in two low altitude and mid altitude savanna sites of Nigeria. African J Root Tuber Crops 5 (1): 24-28.

Akparobi SO, Togun AO, Ekanayake IJ, Idris R. 2002. Effect of low temperatures on dry matter partitioning and yield of cassava clones. Trop Sci 42: 22-29.

Allem AC (2002) The origin and taxonomy of cassava. In: Hillocks RJ, Thresh JM, Bellotti AC (eds). Cassava: biology, production and utilization. CABI. New York.

Allem AC. 1994. The origin of *Manihot esculenta* Crantz (Euphorbiaceae). Genet Resour Crop Evol 41: 133-150.

Artama WT. 1991. Rekayasa genetika. PAU – Bioteknologi. Universitas Gajah Mada. Yogyakarta. [Indonesia]

Balitkabi. 2009. Cultivation technique of cassava. Crops Research Institute for Legumes and Tubers. Malang. [Indonesia]

Berry JA, Bjorkaman O. 1980. Photosynthetic response and adaptation to temperature in higher plants. Annu. Rev. Plant Physiol. 29: 345-378

Bueno A. 1986. Adequate number of environments to evaluate cassava cultivars. Rev. Bras. Mandioca 5: 83-93.

Cheverud JM. 1982. Phenotypic, genetic, and environmental morphological integration in the cranium. Evolution 36 (3): 499-516.

CIAT (Centro Internacional de Agricultura Tropical) (1993). Cassava: The latest facts about an ancient crop. CIAT. Cali, Colombia.

Cock JH. 1985. Cassava: New potential for a neglected crop. Westview Press. Boulder, Colorado.

Crowder. LV. 1997. Plant genetics. Gadjah Mada University Press. Yogyakarta. [Indonesia]

De Souza CRB, Carvalho LJCB, De Almeida ERP, Gander ES. 2002. Identification of cassava root protein genes. Plant Foods Human Nutr 57 (3-4): 353-363.

El-Sharkawy MA. 2004. Cassava biology and physiology. Plant Mol Biol 56:481-501.

Harnowo D, Subandi, Saleh N. 2006. The prospect of strategy and technology development of cassava. Agribusiness and food security. Food Crops Research and Development. Bogor. [Indonesia]

Heyne K. 1987. Tumbuhan Berguna Indonesia. Jilid I-IV. Koperasi Karyawan Departemen Kehutanan. Jakarta. [Indonesia]

Lefèvre E, Charrier A. 1993. Heredity of seventeen isozyme loci in cassava *(Manihot esculenta* Crantz). Euphytica 66: 171-178.

Levitt J. 1980. Responses of plants to environmental stresses. Academic Press. New York.

Maryati KT. 2008. Characterization of white grub (Melolonthidae: Coleoptera) on salak cultivation based on the characteristic morphology and protein banding patterns. [Thesis]. School of Geaduates, Sebelas Maret University. Surakarta. [Indonesia]

Montarroyos AVV, de Lima MAG, dos Santos EO, de Franca JGE. 2003 Isozyme analysis of an active cassava germplasm bank collection Euphytica 130 (1): 101-106.

Nassar NM, Hashimoto DY, Fernandes SD. 2008. Wild Manihot species: botanical aspects, geographic distribution and economic value. Genet Mol Res 7 (1): 16-28.

Nassar NMA, Bomfim N, Chaib A, Abreu LFA, Gomes PTC. 2010. Compatibility of interspecific *Manihot* crosses presaged by protein electrophoresis. Genet Mol Res 9 (1): 107-112.

Nassar NMA, Carvalho CG, Vieira C. 1996. Overoming crossing barrers between cassava, *Manihot esculenta* Crantz and a wild relative, *M. pohlii* Warma. Braz J Genet 19 (4): 617-620.

Nassar NMA. 1978. Wild *Manihot* species of Central Brazil for cassava breeding. Canadian J Plant Sci 58: 257-261.

Nassar NMA. 1992. Cassava, *Manihot esculenta* Crantz, genetic resources: origin of the crop, its evolution and relationships with wild relatives. Geneti Mol Res 1 (4): 298-305

Nassar NMA. 2003. Gene flow between cassava, Manihot esculenta Crantz, and wild relatives. Genet Mol Res 2 (4): 334-347.

Nweke FI. 1996. Cassava processing in sub- Saharan Africa: Implications for expanding cassava production. IITA Res 12: 7-14.

Office of Agriculture, Plantation and Horticulture, Ngawi District. 2009 Growing of cassava (*Manihot esculenta* Crantz) Office of Agricultural, Plantation and Horticulture, Ngawi District. Ngawi. [Indonesia]

Olsen KM, Schaal BA. 1999. Evidence on the origin of cassava: Phylogeographyof *Manihot esculenta*. Proc Natl Acad Sci USA 96: 10 5586-105591.

Park YL, Chow WS, Anderson JM. 1997. Antenna size dependency of photoinactivation of photosystem II in light-acclimated pea leaves. Plant Physiol 115: 151-157.

Prawoto, Sujoko, Maryam S. 1987. Evolution. Open University. Jakarta. [Indonesia]

Presidential Regulation (Perpres) 2006. Regulation of the President of the Republic of Indonesia No. 5 of 2006 about the National Energy Policy. [Indonesia]

Ramirez H, Hussain H, Roca W, Bushuk W. 1987. Isozyme electrophoregrams of sixteen enzymes in five tissues of cassava (*Manihot esculenta* Crantz) varieties. Euphytica 36: 39-48.

Resende AG, Filho PSV, Machado MDFPS. 2000. Isozyme Diversity in Cassava Cultivars (*Manihot esculenta* Crantz). Biochem Genet 38 (7-8): 203-216.

Sarria R, Ocampo C, Ramirez H, Roca WM. 1993. Genetic of esterase and glutamate oxaloacetatae transaminase isozymes in cassava (*Manihot esculenta* Crants). In: Roca WM, Thro AM (eds). Proc 1st Intl Sci Meeting of the Cassava Biotechnology Network. CIAT, Cartagena, Columbia, 25-28 August 1992.

Schägger H, Aquila H, Von Jagow G. 1988. Coomassie blue-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for direct visualization of polypeptides during electrophoresis. Analytic Biochem 173 (1): 201-205.

Subandi. 2007. Superior variety of nuts and tubers. Crops Research Institute for Legumes and Tubers. Malang. [Indonesia]

Suketi K. 1994. Characterization studies of durian clonal seedling based on leaf morphology and isozyme banding pattern. [Thesis]. School of Graduates, Bogor Agricultural University. [Indonesia]

Sulistyono E, Sopandie D, Chozin MA, Suwarno. 1999. Adaptation to shade of upland rice: morphological and physiological approach. Komunikasi Pertanian 4 (2): 62-68. [Indonesia]

Sumarani GO, Pillai SV, Harisankar P, Sundaresan S. 2004. Isozyme analysis of indigenous cassava germplasm for identification of duplicates. Genet Resour Crop Evol 5 (2): 205-209.

Suranto. 2001. Isozymes studies on the morphological variation of *Rannunculus nanus* population. Agrivita 3 (2): 139-146

Taiz L, Zeiger E. 1991. Plant physiology. Benyamin/Cumming. Tokyo

Tarkka, M. T., Vasara, R., Gorfer, M., and Raudaskoski, M. 2000. Molecular characterization of actin genes from homobasidiomycetes: two different actin genes from *Schizophyllum commune* and *Suillus bovinus.* Gene 251:27-35.

Veltkamp HJ, Bruijn GH. 1996. *Manihot esculenta* Crantz. In: Flach M, Rumawas F (eds). Plant Resources of South-East Asia 9: Plants yielding non-seed carbohydrates. Pudoc. Wageningen.

Wargiono J, Hasanudin A, Suyamto. 2006. Technology support for cassava bio-ethanol industry. Center for Food Crops Research and Development, Crops Research Institute for Legumes and Tubers. Malang. [Indonesia]