NUSANTARA BIOSCIENCE
Vol. 4, No. 1, pp. 16-21
March 2012

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n040104

Characterization of *Carica pubescens* in Dieng Plateau, Central Java based on morphological characters, antioxidant capacity, and protein banding pattern

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Manuscript received: 2 February 2012. Revision accepted: 20 March 2012.

Abstract. *Laily AN, Suranto, Sugiyarto. 2012. Characterization of* Carica pubescens *in Dieng Plateau, Central Java based on morphological characters, antioxidant capacity, and protein banding pattern. Nusantara Bioscience 4: 16-21. Carica pubescens* Lenne & K. Koch is a species of fruit plant firstly cultivated in South America and has adapted to the highland environment, such as Dieng Plateau, Central Java (±2000 m asl). It has a narrow habitat range and limited or unknown intraspecies variation. Therefore, important information about the characters of the plants at various altitudes is needed, so that it is possible to extend its distribution through transplantation to other areas. Characterization can be performed based on morphological characters, chemical content, and protein banding patterns. This study aimed to describe the morphological characters, the chemical content (antioxidant capacity), and the pattern of protein bands by staining, using coomassie brilliant blue on *C. pubescens* in the Dieng Plateau. The research was conducted in the villages of Kejajar (1400 m asl), Patak Banteng (1900 m asl.), and Sembungan (2400 m asl.). The observations of morphological characters were conducted in the field and continued in the laboratory. Morphological characters, the chemical content (antioxidant capacity), and the banding pattern of protein of *C. pubescens* were analyzed descriptively. The results showed that the morphological characters of *C. pubescens* in Dieng Plateau varied in stems, leaves and fruits. The antioxidant capacity decreased with decreasing habitat altitude, 2400 m asl. altitude> 1900 m altitude> 1400 m asl.. The Protein banding patterns did not vary, but the pattern in C. Papaya was different. The uniformity of the pattern of protein bands showed that genetic stability in *C. pubescens* was not affected by environmental factors.

Key words: *Carica pubescens*, morphological characters, antioxidant capacity, protein banding pattern

Abstrak. *Laily AN, Suranto, Sugiyarto. 2012. Karakterisasi* Carica pubescens *di Dataran Tinggi Dieng, Jawa Tengah berdasarkan sifat morfologi, kapasitas antioksidan, dan pola pita protein. Nusantara Bioscience 4: 16-21. Carica pubescens* Lenne & K. Koch merupakan jenis tanaman buah yang pertamakali dibudidayakan di Amerika Selatan dan beradaptasi pada lingkungan dataran tinggi, misalnya Dataran Tinggi Dieng, Jawa Tengah (±2000 m dpl.). *C. pubescens* memiliki daerah persebaran sempit dan variasi intraspesies terbatas atau belum diketahui. Oleh karenanya, diperlukan informasi mengenai karakter tanaman pada berbagai ketinggian sehingga dimungkinkan untuk memperluas daerah penyebaran melalui transplantasi di daerah lain. Karakterisasi dapat dilakukan berdasarkan karakter morfologi, kandungan kimia, dan pola pita protein. Penelitian ini bertujuan untuk mendeskripsikan karakter morfologi, kandungan kimia (kapasitas antioksidan), dan pola pita protein dengan pewarnaan coomassie brilliant blue pada *C. pubescens* di Dataran Tinggi Dieng. Penelitian lapangan dilakukan di Desa Kejajar (1400 m dpl.), Patak Banteng (1900 m dpl.), dan Sembungan (2400 m dpl.). Pengamatan karakter morfologi dilakukan di lapangan dan dilanjutkan di laboratorium. Karakter morfologi, kandungan kimia (kapasitas antioksidan), dan pola pita protein *C. pubescens* dianalisis secara deskriptif. Hasil penelitian menunjukkan bahwa karakter morfologi *C. pubescens* di Dataran Tinggi Dieng bervariasi pada batang, daun, dan buah. Kapasitas antioksidannya bervariasi dengan urutan dari ketinggian 2400 m dpl. > 1900 m dpl. > 1400 m dpl.. Pola pita proteinnya tidak bervariasi antar ketinggian, namun berbeda dengan C. papaya. Keseragaman pola pita protein menunjukkan kestabilan genetik *C. pubescens* tidak dipengaruhi oleh perubahan lingkungan.

Kata kunci: *Carica pubescens*, karakter morfologi, kapasitas antioksidan, pola pita protein

INTRODUCTION

The genus *Carica* of the family of Caricaceae has about 40 species, but only seven species are edible (Budiyanti et al. 2005). In Indonesia, one of the edible species is *Carica pubescens* Lenne & K. Koch which is cultivated only in the highland of Dieng, Central Java and locally known as *karika* or mountain papaya. *C. pubescens* is a species introduced from the Andes, South America which grows at the altitude of 2000 meters above sea level (m asl.), at low temperature and high rainfall.

Not all places in the Dieng Plateau are suitable for *C. pubescens*. *C. pubescens* does not grow well at the valley of Dieng at the altitude of ± 1400 m asl. as in Kejajar village, but it grows very well at the top of the Dieng at the altitude of ± 2400 m asl., like in the village of Sembungan.

Thus, the higher the place in the Dieng Plateau the more *C. pubescens* will be found; hence it has a narrow distribution range.

Variations in *karika* are believed to be influenced by environmental and genetic factors. Sitompul and Guritno (1995) say that the appearance of plant forms is controlled by the genetic properties of plants under the influence of environmental factors. Environmental factors believed to influence the occurrence of morphological changes in plants are temperature, soil type, soil conditions, altitude, and humidity. If the environment factors are more powerful than the genetic factors, then the plants in different places with different environmental conditions will have different morphologies (Suranto 2001). But if the influence of environmental factors is weaker than that of the genetic factors then there will not be any morphological difference despite being planted in different places.

The problem faced today is the lack of information regarding the characterization of *C. pubescens* in terms of morphological features, chemical content, and protein banding pattern. Morphological features can be used to characterize patterns of genetic diversity, but the nature which can be described is limited and likely to be influenced by environmental factors, so that molecular genetic identification is required to overcome these limitations (Rahayu et al. 2006). Information about the molecular characters can be gathered by knowing the protein banding pattern of *C. pubescens* while the chemical character can be determined by measuring the antioxidant capacity of these plants. This study aimed to describe the morphological characters, the chemical content (antioxidant capacity), and the pattern of protein bands by the staining of *C. pubescens* using coomassie brilliant blue in the Dieng Plateau, Central Java.

MATERIALS AND METHODS

Time and places

This study was conducted from July 2010 to February 2011. The field research on morphological characters of *C. pubescens* Lenne & K. Koch was conducted in the village of Kejajar (1400 ± 50 m asl.), Patak Banteng (1900 ± 50 m asl.), and Sembungan (2400 ± 50 m asl.) in the Dieng Plateau, Wonosobo district, Central Java. The field research on morphological characters of superior *C. papaya* was conducted in Boyolali, Central Java (1500 ± 50 m asl.). Antioxidant capacity and protein banding pattern were analyzed at the Sebelas Maret University, Surakarta, Central Java, Indonesia.

Procedures

Sampling

Samples of *C. pubescens* in three different heights and those of *C. papaya* were taken for morphological observations in the laboratory, the analysis of antioxidant capacity, and the pattern of protein bands. Samples were required for the laboratory observations on the morphology of leaves, flowers, and fruits.

The observation on morphological characters

Observations were conducted on 10 *C. pubescens* plants for the three different altitudes at the Dieng Plateau, with a comparison with the superior plant of *C. papaya* of Boyolali. Observation of morphological characters in the field was followed by observations in the laboratory. Parts of stems, leaves, flowers, fruits, and seeds of *C. pubescens* were observed and documented. The morphological characters of stems observed included the height, the diameter, the cross-sectional shape, the outer surface, the color, the branch, the trunk's appearance. The morphological characters of the leaves included color, the bone, the stalk's length, the leaf's diameter, and the leaf's blade. The morphological characters of the flowers were types of flowers, the basic form of flowers, the shape of the curve, the edge of the petals, the number of crowns, the number of stamens, the number of the ovule, the position of the stamens, the postion of the fruit in relation to the position of the base of the flower, and the shape of the flower. The morphological characters of the fruits observed were the color, the dominant of central cavity, the diameter, the length, and the length of stem, the shape of fruit, the lengthwise slice, and the crosswise slice. The characters of the morphology of seeds observed were the common form of the outside part of the seed, the engraving of the grain leather, and the color of the endosperm. The guideline for the observation of these morphological characters was taken from Tjitrosoepomo (1990), Muzayyinah (2008), and the Center for Plant Variety Protection of the Ministry of Agriculture of the Republic of Indonesia (2006).

Test of antioxidant capacity

A total of 100 g of fruit extracts of *C. pubescens* and *C. papaya* were weighed and then dissolved in 1 mL methanol. The main liquor was taken using a micro pipette with multilevel dilution to obtain the test solution concentration of 10 ug/mL, 5 mg/mL, 2.5 u/mL, and 1.25 ug/mL. One mL of each test solution was put into glass bottles and then added with 2 mL of DPPH (diphenyl picril hydrazil hydrate), then was left for 30 minutes. Methanol was used as a blank solution. DPPH absorbance was analyzed with a spectrophotometer of visible light at a wavelength of 517 nm.

The making of protein banding pattern

The analysis of the protein band profile was carried out according to the methods of Coats et al. (1990), using SDS-PAGE electrophoresis technique. The concentration of acrylamide for *stacking gel* was 3%, while for the *gradient gel* was 10%. Electrophoresis was run at a constant voltage of 110 VA, until the loading dye was near the bottom of the gel. The painting was done overnight using the solution of coomassie brilliant blue, followed by the laxative solution until the protein banding pattern emerged. Electrophoresis results were documented in a digital camera.

Data analyses

Morphological character data, the chemical content (antioxidant capacity), and the banding pattern of proteins in *C. pubescens* and *C. papaya* were analyzed

descriptively. The antioxidant capacities of the fruits of *C. pubescens* and *C. papaya* were analyzed based on the absorption percentage of DPPH. Antioxidant capability was measured as a decrease in DPPH solution absorbance due to the addition of the sample for 30 minutes. DPPH solution absorbance values before and after the addition of the extract were calculated as percent inhibition (% inhibition). Then the calculations was included in the regression equation with the extract concentration (mg/100 mL) as the abscissa (X axis) and the percentage of inhibition as the ordinate (Y axis). The value of IC50 derived from the calculation at the time of the percentage of inhibition was 50%. Y = ax + b (Cahyana 2002).

The banding patterns formed on the leaf organ samples of *C. pubescens* and *C. papaya* were analyzed based on whether or not the band appeared on the gel and also the thickness of the band which was formed, as has been done by Suranto (1991, 2001, 2002) and Triawati (2005). Banding patterns which were formed were drawn as zimograms. The diversity of banding pattern was determined by the value of Rf, which is the relative mobility values obtained from the comparison between the migration distance towards the migration of *loading dye*. The data were obtained in the form of qualitative data, so the data analysis were done descriptively based on the results of electrophoresis of the leaf's organ of *C. pubescens* and *C. papaya*.

RESULTS AND DISCUSSION

Morphological characters

C. pubescens can be found in the Dieng Plateau, central Java at an altitude of 1400 m asl. up to 2,400 m asl.. The word "*pubescens*" means hair (Center for Plant Variety protection of the Ministry of Agriculture of the Republic of Indonesia 2006). Morphological observations of *C. pubescens* found the presence of feathers in several organs of plants, among which was evident on the outer surface of the lower leaves (abaxial), leaf stalk, the outer surface of the flowers, both male flowers and female flowers. *C. pubescens* has more hair than another member of the genus *Carica*, namely *C. papaya* (Table 1).

Antioxidant capacity

The leaf's morphology of *C. pubescens* at different altitudes showed the presence of variations. The colors appeared thickner in plants that grew on the higher land. At the higher places, the green and the large size of the leaves increase the amount of chlorophyll and the area of cross-sectional sliced leaf's surface, so that the tree is able to harness the sun's rays that are not too high in terms of intensity for optimal photosynthetic activity. Morphological features of flowers as the proliferation of generative organs of plants did not show any variation. Plants at every altitude consistently showed all types of flowers, namely male, female, and hermaphrodite. Morphological characters suggest that environmental factors, namely the air pressure and extreme temperatures on the higher altitude of the Dieng Plateau support the growth and development of *C. pubescens.*

Antioxidant capacity was measured by counting the amount of reduced DPPH purple color intensity that is proportional to the reduction of DPPH solution concentration (Figure 1).

The amount of the inhibition's percentage of various concentrations of the extract that gave rise to IC 50 values indicated that the fruit of *C. pubescens* grown at different altitudes had different antioxidant activities. Of the three sample extracts derived from the three different altitudes, the extracts of *C. pubescens* at an altitude of 2400 m asl. had the highest antioxidant capacity with IC 50 of 0.983 mg/100 mL, followed by *C. pubescens* grown at an altitude of 1900 m asl. with IC50 of 1.2945 mg/100 mL, and *C. pubescens* grown at an altitude of 1400 m asl. with IC50 of 8.843 mg/100 mL, while in *C. papaya* was 5.326 mg/100 ml. The order of antioxidant capacity from the largest to the smallest was as follows: *C. pubescens* at an altitude of 2400 m asl.> *C. pubescens* at an altitude of 1900 m asl.> *C. papaya*> *C. pubescens* at an altitude of 1400 m asl..

All three kinds of fruit extracts of *C. pubescens* from the three different altitudes had antioxidant capacity. The response of plants as a result of environmental factors can be seen in the morphology and physiology. Plants that normally live in areas of high elevation are the type that can adapt to the climatic conditions of low temperature, high humidity and low sun light intensity.

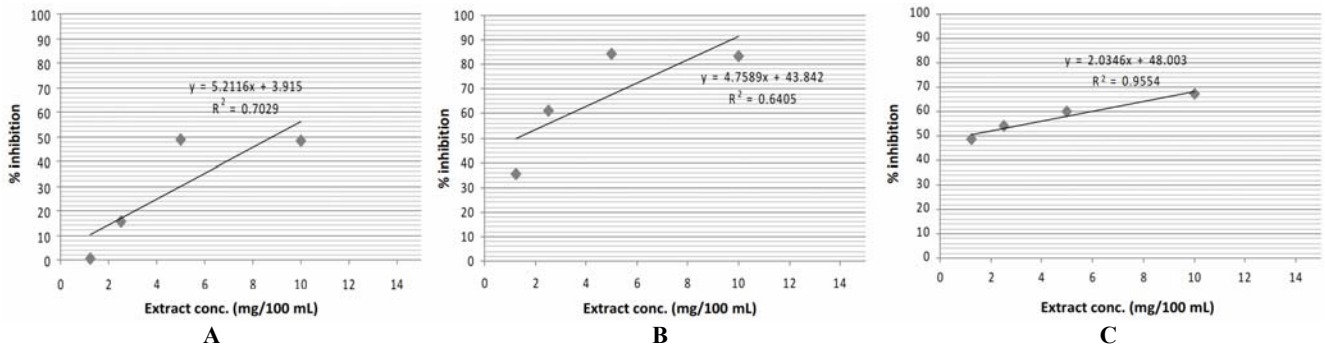

Figure 1. Linear regression curves for the determination of IC50 fruit extracts of *C. pubescens* growing at the altitude of: (a) 1400 m asl., (b) 1900 m asl., and (c) 2400 m asl.

Table 1. Morphological characteristics of *C. pubescens* in Dieng Plateau, Central Java

Plant organs	Morphological characters 1400 m asl	1900 m asl	2400 m asl
Stem			
Height (cm)	193,8	174,7	153,3
Diameter (cm)	11,2	11,6	10,8
Cross-sectional shape	round	round	Round
The outer surface	silken to rough and has pustules	rough, has pustules	silken to rough
Color	brown to dark brown, green, greenish brown and white glossy	greenish brown	dark brown, greenish brown
Branch	no branches, 2-4	4	6-8
How the branches look			
Leaves			
Color	dark green, yellowish green,	dark green, dark green	dark green
Leaf vein	finger-like, reddish or yellowish	finger-like, yelowish	finger-like, reddish
Length of stalk (cm)	33,65	44,45	44,54
Diameter (cm)	45,67	47,8	54,2
Leaf blade			
Flowers			
Flowers type	male, female, hermaphrodite	male, female, hermaphrodite	male, female, hermaphrodite
Shape of flowers receptacle	round	round	round
Shape of curve edge of sepals	spirostichous	spirostichous	Spirostichous
Number of petals	5	5	5
Number of stamens	5	5	5
Number of ovule	5	5	5
Position of stamens	above the ovary	above the ovary	above the ovary
Position of ovary towards receptacle of flowers	on the receptacle of the flower	on the receptacle of the flower	on the receptacle of the flower
Shape of flowers			
Fruits			
Color	bright-dark green on young fruits, and yellowish on ripe fruit	young-old green on young fruit, and yellow on ripe fruit	young-old green on young fruit, and yellow on ripe fruit
Dominant shape of central space	pentagon	pentagon	pentagon
Diameter (cm)	5,4	7,3	7,2
Length (cm)	8,1	8,8	8,6
Length of stalk (cm)	2,95	1,8	1,8
Shape of fruits			
Cross-sectional shape			
Longitudinal-sectional shape			

The production of flavonoids needs sugar as erythrose phosphoenolpyruvate and erythrose 4-phosphate that provide some carbon atoms required for the B- flavonoid ring as well as an acetate unit for the A flavanoid ring. Sugars, especially sucrose, can be obtained from the decomposition of starch or fat in storage organs during development of the sprouts or photosynthesis in cells that contain chlorophyll. Light also affects the composition of the chloroplast.

The antioxidant capacity test shows that the higher concentration of the standard vitamin C means more antioxidant activities. Using the regression equation, the obtained IC50 vitamin standard was -84.7875 C mg/100 mL. This value is lower than that in the IC50 fruit extract of *C. pubescens* and *C. papaya*. Fruit extracts of *C. pubescens* and *C. papaya* have a capacity of antioxidant because it contains flavonoids. Antioxidant capacity of Flavonoid is associated with the presence of phenolic hydroxyl group attached to the frame structure. Flavonoid compounds have been proven to be able to reduce free radical of DPPH. The activities are different, possibly because each extract that is believed to be flavonoid has a

hydroxyl group with different number and location of flavonoid skeleton. Flavonoids with free hydroxyl group have a radical capturing activity and the presence of more than one hydroxy group on ring B in particular will increase the antioxidant activity.

Protein banding patterns

The protein banding patterns were analyzed in the form of zimogram that was the typical electrophoresis results, so it can be used as a characteristic feature of the leaf's organ of *C. pubescens*. Data were analyzed qualitatively, based on whether or not the band appeared and whether thin and thick bands were found in the gel electrophoresis results. The diversity of banding pattern was seen from the Rf values that were formed. Rf value is the value of relative mobility explained by Ferguson, obtained from the comparison of the migration distance of protein towards the migration distance of *loading dye*.

The zymogram gained from *running* along the *karika* leaves of *C. pubescens* at an altitude of 1400 m asl. and the leaves of *C. papaya* is shown in Figure 3. These data show the similarity of protein banding pattern on the samples of *C. pubescens* from different altitudes. This suggests that the molecular basis of this plant is stable in response to various environmental factors. Environmental factors influence the morphology and physiology of the plant. The morphology of the plant is adapted to environmental conditions so that physiological processes can run optimally. Genetic variation is a key to optimal treatment towards the genetic resources. Morphological features can be used to characterize a species or an individual, but the nature described is only a small proportion of the genetic code. Therefore, the molecular characterization of genetic variation should be made from the protein banding pattern because it produces more accurate data since the protein is a late gene expression, relatively simple, and not easily changed.

Genetic differences and the environmental factors give the optimal growth of *C. pubescens* and *C. papaya*. The apparent variations in pattern of protein bands between *C. pubescens* and *C. papaya* showed the diversity of the synthesized protein, and it can be assumed there are differences in genetic makeup that encodes these proteins. The diversity of banding pattern of each species showed an encoding genetic diversity, since protein is a direct product of the gene in the form of amino acids. Amino acids are encoded by the DNA specifically for each type of protein. Resistance to damage may be caused by the pressure resistance of the protein molecule, being protected from damage by other molecules, a special structure, or a certain behavior patterns. Judged from the data pattern of protein bands, it appears that there is a striking difference between *C. pubescens* and *C. papaya*, on the level of molecular characteristics.

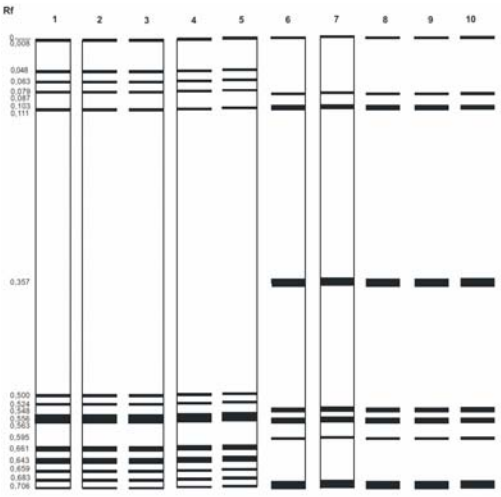

Figure 3. Zymogram protein banding pattern of the leaves of *C. pubescens* at 1400 m asl. and *C. papaya*

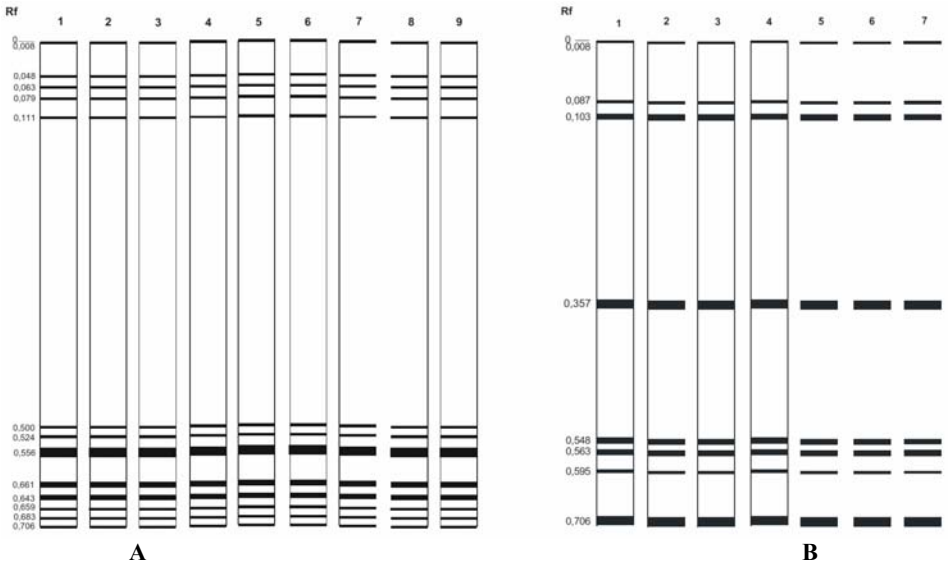

Figure 2. Zimogram protein banding pattern on the same scale: (a) *karika* leaves of *C. pubescens* at an altitude of 1400 m asl., 1900 m asl. and 2400 m asl., and (b) leaves of *C. papaya*. Note: 1: 2: 3: plants at an altitude of 1500 m asl., 4, 5, 6 plants at an altitude of 1900 m asl., and 7; 8; 9 plants at an altitude of 2400 m asl..

CONCLUSION

Morphological characters of *C. pubescens* in Dieng Plateau showed a variation in the stems, leaves and fruits. Antioxidant capacity of *C. pubescens* showed variations. The antioxidant capacity increased with increasing altitude. The banding patterns of protein of *C. pubescens* in Dieng Plateau did not show any variation. This suggests that the genetic stability is not affected by the environmental factors.

REFERENCES

Budiyanti T, Purnomo S, Karsinah, Wahyudi A. 2005. Characterization of 88 papaya accession collected by Fruit-Crops Research Institute. Buletin Plasma Nutfah. 11 (1): 21-27. [Indonesian]

Coats SA, Wicker L. 1990. Protein variation among Fuller Rose case population (Coleoptra: Curculionidae). Ann Entomol 83 (6): 1054-1062.

Muzayyinah. 2008. Plants terminology. Sebelas Maret University Press, Surakarta.

Center for Plant Variety Protection of the Ministry of Agriculture of the Republic of Indonesia. 2006. Guidance for testing the individual novelty, uniqueness, uniformity and stability. Center for Plant Variety Protection of the Ministry of Agriculture of the Republic of Indonesia, Jakarta.

Rahayu S, Sumitro SB, Susilawati T, Soemarno. 2006. Isoenzymic analysis to study genetic variation of Bali cattle in Province of Bali. Hayati 12: 1-5.

Sitompul SM, Guritno B. 1995. Analysis of plant growth. Gadjah Mada University Press, Yogyakarta.

Suranto. 1991. Studies of population variation in species of *Ranunculus*. [Thesis]. Departement of Plant Science, University of Tasmania, Hobart.

Suranto. 2001. Isozyme studies on the morphological variation of Ranunculus nanus populations. Agrivita 23 (2): 139-146.

Tjitrosoepomo G. 1990. Plants morphology. Gadjah Mada University Press, Yogyakarta.

Triawati RM. 2005. Study on the diversity of total protein banding pattern of leafhopper (*Nephotettix virescens*) of the endemic and non-endemic populations on rice tungro virus. [Hon. Thesis]. Faculty of Agriculture, Sebelas Maret University, Surakarta.