

## Variation of morphology, isozymic and vitamin C content of dragon fruit varieties

BANATI RAHMAWATI<sup>1,\*</sup>, EDWI MAHAJOENO<sup>2</sup>

<sup>1</sup> SMP Negeri 5 Surakarta, Jl. Diponegoro No. 45, Surakarta 57131, Jawa Tengah, Indonesia; Tel./Fax.: +92-271-634930, email: smpn5solo@smpn5solo.sch.id  
<sup>2</sup> Bioscience Program, School of Graduates, Sebelas Maret University, Surakarta 57126, Central Java, Indonesia

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**Abstract.** *Rahmawati B, Mahajoeno E. 2009. Variation of morphology, isozymic and vitamin C content of dragon fruit varieties. Nusanantara Bioscience 1: 131-137.* The aims of the research was to study the variation of morphology, the band pattern of isozyme, and vitamin C content of dragon fruit (*Hylocereus* spp.) varieties such as super red, red and white from Pasuruan (East Java), Sukoharjo and Klaten (Central Java), and Bantul districts (Yogyakarta). Morphological character were carried include fruit, stem, and flowers of each variety of dragon fruit. The isozymic pattern was analyzed using NTSYS 2.02i. The data matrix was counted based on the DICE coefficient. The clustering was done by applying UPGMA which counted through SHAN. Vitamin C content measured by titration method then analyzed descriptively. The results showed that the higher vitamin C content was found from super red of Pasuruan (6.00) and then followed by red color (5.376) and super red (5.113) both from Bantul. The morphological variation on the stem and petal colors, and fruits were also shown by the isozymic data of three varieties of dragon fruits collected from four separated locations. Esterase (EST) showed 18 bands and forming four (4) groups based on 75% genetic similarity index. The specific band occurred on *Rf* 0.633 of red varieties of dragon fruit from Bantul and on *Rf* 0.755 from Pasuruan. The specific band also occurs on *Rf* 0.347 of white variety from Bantul and on *Rf* 0.510 and on *Rf* 0.633 from Klaten. Glutamic oxaloacetic transaminase (GOT) enzyme shows 12 bands and also forming four groups with a little difference for member in the fourth group. The specific band occurs on *Rf* 0.321 of red color fruit from Pasuruan. The specific band also occurs on the white from Pasuruan on *Rf* 0.446 and on *Rf* 0.482. The variation of dragon fruits were also supported by isozymic data indicated that the morphological character were in accordance with the genetics data.

**Key words:** dragon fruit, *Hylocereus*, morphology, isozyme, vitamin C.

**Abstrak.** *Rahmawati B, Mahajoeno E. 2009. Variasi morfologi, isozim dan kandungan vitamin C pada varietas buah naga. Nusanantara Bioscience 1: 131-137.* Penelitian ini bertujuan untuk menguji keragaman variasi morfologi, pola pita isozim dan kandungan vitamin C pada buah naga (*Hylocereus* spp.) berdaging merah super, merah, dan putih dari Kabupaten Pasuruan (Jawa Timur), Sukoharjo dan Klaten (Jawa Tengah), serta Bantul (Yogyakarta). Data morfologi diuraikan secara deskriptif meliputi buah, batang, dan bunga dari setiap varietas buah naga. Data pola pita isozim dianalisis menggunakan program NTSYS 2.02i. Data matrik dihitung berdasarkan koefisien DICE. Pengelompokan dilakukan dengan UPGMA yang dihitung melalui SHAN. Kandungan vitamin C diketahui dengan metode titrasi dan dianalisis secara deskriptif. Hasil penelitian menunjukkan bahwa kandungan vitamin C tertinggi terdapat pada merah super Pasuruan (6,00), diikuti merah Bantul (5,376) dan merah super Bantul (5,113). Variasi morfologi terjadi pada warna batang, kelopak bunga dan rasa daging buah yang ditunjukkan juga pada pola pita isozim ketiga varietas dari empat lokasi pengamatan. Enzim esterase (EST) mengekspresikan 18 pita yang membentuk empat kelompok berdasarkan jarak kemiripan 75%. Pita spesifik muncul pada buah naga berdaging merah pada *Rf* 0,633 dari Bantul dan pada *Rf* 0,755 dari Pasuruan. Pita spesifik juga dimiliki untuk buah naga putih pada *Rf* 0,347 dari Bantul serta pada *Rf* 0,510 dan *Rf* 0,633 dari Klaten. Enzim glutamat oksaloasetat transaminase (GOT) mengekspresikan 12 pita dan memperlihatkan empat kelompok dengan keanggotaan sedikit berbeda di kelompok keempat. Pita spesifik muncul pada varietas buah naga berdaging merah pada *Rf* 0,321 dari Pasuruan. Pita spesifik juga muncul pada buah naga berdaging putih dari Pasuruan pada *Rf* 0,446 dan *Rf* 0,482. Terjadinya variasi pada buah naga yang di uji dan didukung oleh data isozim menunjukkan bahwa data genetik mendukung karakter morfologi.

**Kata kunci:** buah naga, *Hylocereus*, morfologi, isozim, vitamin C.

### INTRODUCTION

Dragon fruit has high economic value and is useful for treating various types of diseases (Suryono 2006). Dragon fruit is believed to able to lower cholesterol concentration, to balance blood sugar concentration, to prevent colon cancer, to strengthen kidney function and bone, to strengthen the brain workings, increasing the sharpness of the eyes as well as cosmetic ingredients (Suryono 2006).

Dragon fruit is rich in potassium (K), ferum (Fe), sodium (Na), calcium (Ca), and fiber which are good for health compared to other fruits (Sari 2009).

Recent studies of biological diversity get much attention both nationally and internationally. The assessment of biological diversity includes diversity between or within species or populations (Murphy et al. 1993; Karcicio et al. 2003; Yunus 2007a, b; Julisaniah et al. 2008). The studies of relationship of species have been

conducted up to the organizational structure and evolution of a genome (Purwanto et al. 2002). In order to obtain results that can strengthen taxon boundaries, examine relationship relationships, define and classify taxon particularly categories of species and taxon level below the species, it requires an accurate marker. The commonly used markers are morphological characteristics of plants. The weakness of morphological marker is that it is based on the nature of phenotypes, while the obtained genetic diversity is still a conjecture and is still influenced by environmental factors (Cahyari et al. 2004). A more accurate marker is molecular marker such as isozyme and DNA analysis.

The use of isozyme markers have many advantages because the isozymes are regulated by a single gene and have codominant character and inheritance, normally segregated according to Mendel ratio collinear with gene and is a direct product of genes. This markers are stable because they are not influenced by environmental factors, more quickly and more accurate because they do not need to wait for the plants to reproduce (Cahyarini et al. 2004). According Rahayu et al. (2006) the advantage of isozyme are, among others, producing more accurate data because the isozyme is the last gene expression, relatively simple, requiring relatively low cost compared to other molecular markers. Isozyme has several characteristics and advantages (Hadiati et al. 2002), among others: (i) the product of different alleles move at different positions in the gel, (ii) different alleles are usually inherited in an codominant way, free of epistasis, so that homozygous individuals can be distinguished from heterozygous ones, (iii) the position of the tape is often the product of a locus, making it possible to detect the number of genes encoding an enzyme by analyzing the banding pattern of the enzyme, (iv) the necessary equipment and materials are relatively inexpensive and the experiments can be done easily in the laboratory, (v) the number of samples can be analyzed in a short time, and (vi) it can be done on seed phase, so it saves time, place and money.

Vitamin C has a very important role in strengthening the immune system to fight infection. Animals and humans cannot synthesize Vitamin C, allegedly due to lack an enzyme needed to convert L-gulonic acid into ascorbic acid in foods, so that the intake of Vitamin C should be included in the diet (Airey 2005).

This study aims to determine variations in morphology, banding pattern isozyme and the content of Vitamin C in dragon fruit plant varieties with super red pulp, red pulp and white pulp taken from Pasuruan, Sukoharjo, Klaten and Bantul

## MATERIALS AND METHODS

### Materials

A morphology test is carried out on stems, fruit, and flower of dragon fruit plants with super red pulp (*Hylocereus costaricensis*), red pulp (*Hylocereus polyrhizus*), and white pulp (*Hylocereus undatus*). Isozyme banding pattern test uses shoots of the dragon fruit plant stems taken from Pasuruan (East Java), Sukoharjo and

Klaten (Central Java), and Bantul districts (Yogyakarta). The material test of vitamin C is a ripe dragon fruit.

### Morphological observation of dragon fruit plant

The observed variables of dragon fruit plants morphology are stems, flowers, and fruits. Observation of morphology refers to Kristanto (2008).

### Diversity of isozyme markers

**Sample subtraction.** Young stems of each sample plant are weighed with an analytical balance until it reaches the weight of 100 mg and are placed in the mortar to be extracted.

**Extraction of samples.** Young stems are pulverized by a mortar, and then are given with a solution of 1 mL of buffer extract and are pulverized again until smooth and then are put into the eppendoRf tube. The examiner then prepares the centrifuge to cool condition (temperature is  $\pm 0^{\circ}\text{C}$ ), and it is played with the speed of 700-1500 rpm for  $\pm 20$  minutes. Clear supernatant can be immediately used for electrophoresis or cooled at a temperature of  $20^{\circ}\text{C}$  for later use. The use of fresh ingredients give the best results (Arulsekhar and Parfit 1986).

**Preparation of polyacrylamide gel.** Polyacrylamide gel consists of 2 parts, namely 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%. Polyacrylamide materials is more profitable than the core gel due to their transparent characters so it can be scanned on visible light or on ultraviolet region. In general, acrylamide gel has no charge while the starch gel contains carboxyl in small proportion which at neutral pH will be negatively charged (Nur and Adijuwana 1989).

**Preparation of running gel.** All the ingredients are mixed, after a homogeneous mixture is inserted into the electrophoresis glass. On the edge of the left, right and bottom are mounted with bulkhead (shield tube). Furthermore, to create a flat surface of the gel, alcohol and water are added, then alcohol and the water sucked up by aspirator, so that the top of the running gel can be poured with a spacer gel.

**Preparation of spacer gel.** After the solution is mixed and homogeneous, this mixture is inserted in the glass just above the running electrophoresis gels, and then a comb is mounted on the spacer gel and electrophoresis glass is heated with neon lights for  $\pm 0.5$  to 1 hour until it is condensed. Once solidified, the comb is removed so that there are holes that will be filled with supernatant.

**Electrophoresis.** The process of electrophoresis uses a vertical electrophoresis type, completed with its power supply. The first step, the cover is opened and the electrophoresis bath tub is filled with a solution of buffer tanks electrode for as high as  $\pm 2$  cm. This solution serves as conductor of electric current during electrophoresis in a face to face condition, then is added with solution of running buffer tank to the inside of the plate that has been mounted against it, then the supernatant solution is filled into the sample hole for as much as  $5\ \mu\text{l}$  with a stepper. The remaining buffer tank is loaded up again till it reaches the electrophoresis bath tub and the cover is replaced. The

process of electrophoresis is performed with electric currents of ± 100 mA for 180-200 minutes.

**Coloring process.** Staining process is performed after the process of electrophoresis, the esterase enzyme dye (EST), and glutamic oxaloacetic transaminase (GOT).

**Observations gel.** After the coloring process and the image banding pattern on the gel is visible, then the fixation process is performed (gel is placed in a solution of ethanol 60% + distilled water and covered with the glass, then put into a refrigerator). The goal is to preserve the gel by stopping the chemical reactions that occur in the gel.

**Making dendrogram.** Banding pattern isozyme results are interpreted in zimogram electrophoresis, and then are converted into binary data, and are drawn their dendrogram. The measurement of migration distances (RF) is measured by measuring the visible distance of ribbon and then is divided by the longest migration distance.

**Vitamin C of dragon fruit**

For the analysis of Vitamin C, the iodine titration method referring to the Sudarmadji et al. (1984) is used. A total of 14.88 g of ripe dragon fruit is crushed till it becomes mush. It is added with 100 mL of aquadest. Then, the mush is refined to obtain filtrate. A total of 10 mL of filtrate is titrated with 0.01 of N standard iodine until the blue color is emerged within 15 seconds. Iodine titration volume is converted to ascorbic acid, where 1 mL of 0.01 standard iodine is equivalent with 0.88 mg of vitamin C.

**Data analysis**

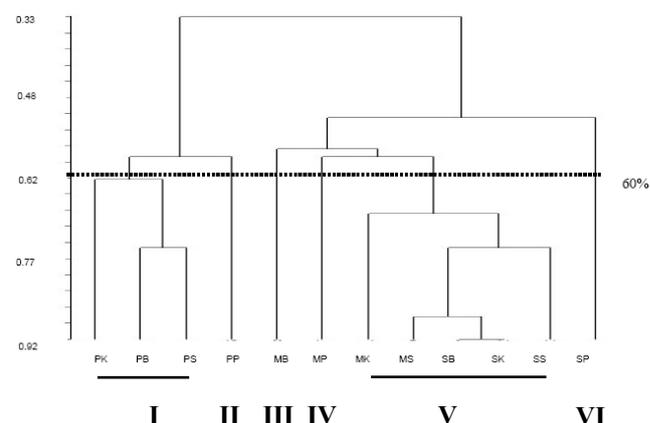
The morphological observation of dragon fruit is described descriptively. Banding pattern isozyme is analyzed qualitatively according to whether or not the banding appears on the gel and quantitatively according to the thickness of the ribbon formed. The diversity of banding pattern is determined based on the value of RF. The resulting binary data is created in the matrix equation. The data matrix is calculated based on the DICE coefficient. The grouping is done by UPGMA (unweighted pair group with arithmetic mean) which is calculated by SHAN on NTSYS program (Rolf 1993). The results of quantitative levels of Vitamin C are analyzed descriptively.

**RESULTS AND DISCUSSION**

**Morphology**

Morphological data as shown in Table 1 is then converted into binary data. The grouping is done by UPGMA is calculated by SHAN on NTSYS program (Rolf 1993).

Morphological dendrogram is shown in Figure 1.



**Figure 1.** Dragon fruit plants dendrogram based on morphological characteristics. Description: PK: white Klaten, PB: white Bantul, PS: white Sukoharjo, PP: white Pasuruan, MB: red Bantul, MP: red Pasuruan, MK: red Klaten, MS: red Sukoharjo, SB: red super Bantul, SK: red super Klaten, SS: super red Sukoharjo, SP: Super red Pasuruan.

The Similarity distance in morphology at 60% of Pasuruan super red varieties and Bantul red varieties always forms their own group. This is because the location of plantations is located near the beach with a sandy soil texture. Location of beach makes it capable of providing a more powerful influence than genetic influences. Coastal

**Table 1.** Morphology test results fleshy white dragon fruit plants, Red and Super red on four observation sites.

Fruit origin		Super red				Red				White			
		P	S	K	B	P	S	K	B	P	S	K	B
Stem shape	Triangle	√	√	√	√	√	√	√	√	√	√	√	√
	Quadrangle								√				
Fruit shape	Round	√	√	√	√	√	√	√			√		√
	Oval								√	√		√	
Hollow shaft	Shallow (5-15 mm)		√	√	√		√	√	√				
	Medium (16-21 mm)	√											
	Deep (22-28 mm)					√				√	√	√	√
Stem color	Whitish green	√	√						√				√
	Light green										√	√	
	Dark green			√	√	√	√	√		√			
Flower color	Pure white		√	√	√	√	√	√	√	√		√	√
	White	√									√		
Calyx color	Light green		√		√		√				√		
	Reddish green	√		√		√	√		√	√		√	√
Fruit color	Vermilion										√	√	√
	Red					√			√				
	Dark red	√	√	√	√		√	√		√			
Color of fruit flesh	White									√	√	√	√
	Red					√	√	√					
	Dark red		√	√	√		√						
	Blackish red	√											
Taste of fruit flesh	Sweet	√	√			√						√	
	Sweet sour			√	√	√	√	√	√	√	√		√
Color tassels	Red					√	√	√					
	Red with green tip	√	√	√	√	√						√	
	Red with yellow tip									√	√		√
Thorn	Dense	√	√	√	√	√	√	√	√				
	Sparse									√	√	√	√
Tassels	Dense	√	√	√	√	√	√	√	√				
	Sparse									√	√	√	√

Note: P: Pasuruan, S: Sukoharjo, K: Klaten, B: Bantul.

regions have a different climate, temperature, soil conditions, altitude and soil moisture. According to Suranto (2001), the emergence of variation can be caused by two factors: environmental and genetic factors. If genetic factors have a stronger influence than environmental factors, then if the plants live in an environment that is different with their native, it will show no morphological variation similar to their original place and if environmental factors are stronger than the influence of genetic factors, then the plant grew in different place will have a morphological variety.

Plant genetic traits are influenced by environmental factors. Phenotype in an individual is an interaction between genotype and its environment (Sitompul and Guritno 1995).

The gene properties are able to interact with their environment. In this case, environmental factors can influence the emergence of traits or characteristics of an individual. For example, two individuals have the same genes, but living a different environment then the second individual can only bring different characteristics and traits.

#### Isozymic banding pattern

Electrophoresis results show that the tested esterase isozyme can be visualized well, makes it possible to do genetic interpretation. Zimogram of esterase banding pattern isozyme of dragon fruit plants is shown in Figure 2. This data is made to dendrogram as in Figure 3.

The style of zimogram isoenzymes electrophoresis results can be considered as phenotypic traits, the genetic testing determines the pattern of its zimogram which is encoded by genes on the same loci and genes at different loci (Sudaryono 1989). In the gel, isoenzymes can be separated by using electrophoresis and the result is zimogram banding pattern. Zimogram patterned electrophoresis typical results that can be used as distinguishing features to reflect the genetic phenotype (Sriyono 2005).

Isozymes migration on the electrophoretic process moves from the negative pole to the positive pole. Esterase enzymes express 18 bandings. Bandings at  $R_f$  of 0.591 and 0.816 are owned by all the varieties of the four sampling sites. Red dragon fruit varieties of Bantul, express the specific band at  $R_f$  0.633 and at  $R_f$  0.755 for Red dragon fruit varieties of Pasuruan which is not owned by the red dragon fruit plants from 3 other locations. Specific band is expressed in the phenotype morphology of the oval shape fruit whereas fruit from other regions is round, while stem color is green while other plants are dark green on their stems.

Bantul white dragon fruit express specific band of ribbon at  $R_f$  0.347 which does not appear in other locations. Specific band is expressed at the phenotypic morphology, namely the color green of its stem, while the other is light green and round shape of fruit. Klaten also express a unique band at  $R_f$  0.510 and 0.633 that is not owned by any other location. Specific band is expressed at the morphological phenotype, namely the fruit flavor is sweeter than fruit from other locations and the tassels color is red with green tip while the other is red with yellow tip. Nandariyah et al. (2004) says that the cultivars that have

specific properties have a difference in the taste of fruit pulp, fruit pulp texture and leaf stalks that are not owned by any other cultivar.

Based on the research conducted by Nandariyah (2007) concerning the identification of diversity in cultivars of salak manggala, it is concluded that this cultivar has a specific band that is not owned by the other cultivars and it can be connected with a prominent characteristic of this cultivar, namely the nature of the leaf with curved tip and striated skin of fruit that is not found on other cultivars.

Quantitative traits are usually controlled by many genes and strongly influenced by the environment, on the other hand, the qualitative traits has a relation with the presence or absence of bands on certain migration distance that reflects the presence or absence of amino acids forming enzyme which is the product of the gene itself (Bailey, 1983 in Setianto 2001). The difference of ribbon thickness is because of the difference of migrated molecular weight, the greater the molecular weight, the harder it is separated properly, and thus it forms a thicker ribbon. Molecules that have a large ionic strength will migrate further than the one with lower ionic strength (Cahyarini 2004).

The groups which are separated at a similarity distance above 0.75 or 75% actually still have a close resemblance. Since the similarity distance is said to be far if the distance is less than 0.60 or 60% (Cahyarini 2004). The analysis shows that at the similarity distance which is less than 75% of the 12 groups of dragon fruit being studied, there are 12 divisions of groups (Figure 3).

The results show that the grouping varieties of Sukoharjo super red is separated from the other super red. This indicates that there is genetic variation in a dragon fruit with super red pulp from four locations. Banding pattern isozymes of Sukoharjo super red dragon fruit is slightly difference with Pasuruan dragon fruit which is the first local place for planting dragon fruit. It is possible that the varieties of dragon fruit from Sukoharjo have plasticity in response to the environment. According to Fitter (1998), the different responses to environment (nutrient) is related to heredity, so that plant breeders can create the response of crop fertilization. Claudia et al. (2002) explains that the environment which is too dominant affects the enzyme activity, such as heat, temperature, and pH. This is caused by damage to the function of enzymes due to environmental conditions. A research on the insect has shown that esterase is influenced by a particular dominant environment (Hadiati 2002).

The lower level of crop genetic similarity between accessions, the higher genetic diversity among accessions is. On the contrary, the higher the genetic similarity between accessions, the lower level of genetic diversity generated (Sulistiyowati 2008). The occurrence of genetic variability in populations of living species in nature can be caused by hybridization (sexual and somatic), natural mutations, and the transfer of genes from living species of the same or of different (transgenic). The Opportunities of the three factors are very much different (Baihaki 2002; Mansyah 2003).

According to the research of Pasquet et al. (1999) on wild Bambara peanut species and its cultivation, the low

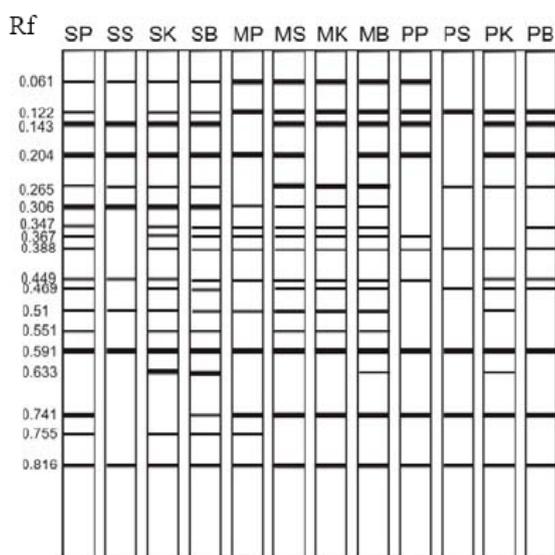
diversity of genes may be due to a strong autogamy pollination system in these species. Differences of environmental factors may emerge the isozyme expression (Supriyadi 2006). Sitompul and Guritno (1995) say that the appearance of crop plant genetic is controlled by nature under the influence of environmental factors. Dragon fruit breeding is mostly done by vegetative propagation rather than generative multiplication (Kris 2008).

Vegetative propagation produces the less varied offspring, so it is said that the genetic similarity of dragon fruit varieties is high. The high level of genetic similarity shows low levels of genetic diversity in dragon fruit. Maideliza and Masyurdin (2007) say that high gene flow is

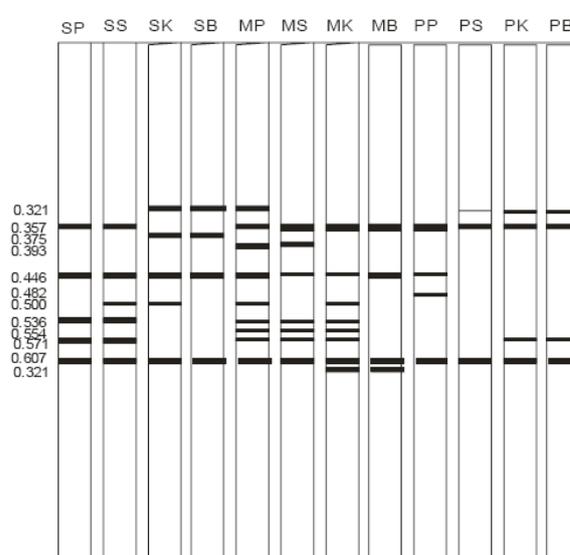
usually found on plants that are interbreed or reproduced by seeds. Dragon fruit plants are reproduced by stem cuttings and thus have a high level of genetic similarity. Dragon fruit plants are found scattered in some areas, there is possibility that they come from a single genetic source with different genetic types and then dispersed to various places with the help of humans.

**Enzyme Glutamate Oxaloacetic Transaminase (GOT)**

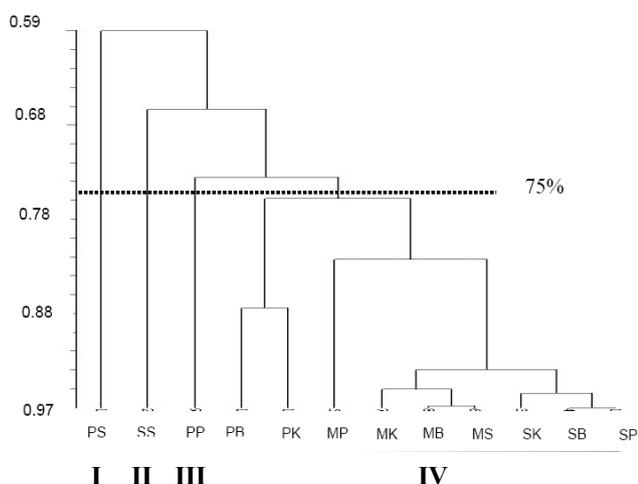
Banding pattern isozyme of Glutamate oxaloacetic transaminase (GOT) in the super red dragon fruit can be shown in zimogram in Figure 4, and is subsequently made to dendrogram as in Figure 5



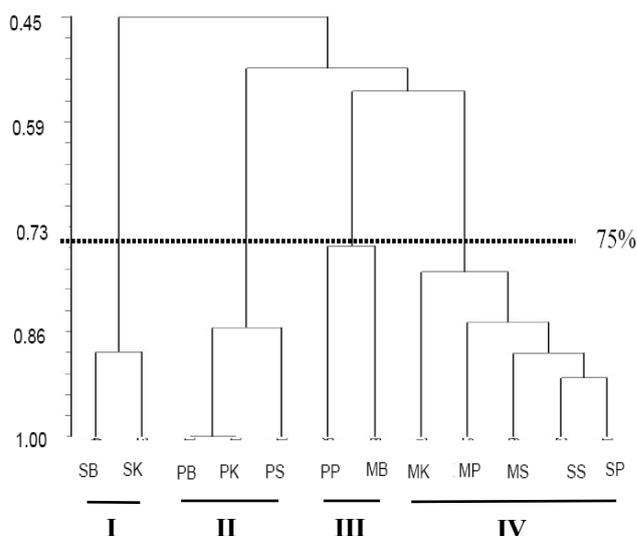
**Figure 2.** Esterase isozyme zimogram dragon fruit taken from four sampling sites. Note: Rf: The distance migration. Acronym stands for the same case with Figure 1.



**Figure 4.** GOT isozyme zimogram of dragon fruit from four sampling sites. Note: Rf: The distance migration. Acronym stands for the same case with Figure 1.



**Figure 3.** Dendrogram of dragon fruit plants esterase from four sampling sites. Note: Acronym stands for the same case with Figure 1.



**Figure 5.** GOT enzyme dendrogram dragon fruit plants from four sampling sites. Note: Acronym stands for the same case with Figure 1.

GOT isozyme in dragon fruit plants expresses 12 ribbons.  $R_f$  0.607 is at all dragon fruit plants from the 4 locations. Red dragon fruit varieties from Pasuruan express specific band that is at  $R_f$  0.321 which is not owned by the plants from 3 other locations. This specific band is expressed in the morphologic phenotype, namely the taste is sweeter and the stem has a deep groove, whereas on other varieties the stem groove is shallow. White dragon fruit varieties from Pasuruan also express specific band namely at  $R_f$  0.446 and at 0.48. This specific band is expressed in the morphologic phenotype i.e. the fruit pulp is dark red. At a similarity distance of 0.75 or 75% of similarity, the studied dragon fruit is divided into four groups (Figure 5).

This relationship analysis shows the existence of genetic variation that is high enough. Varieties that have genetic proximity, are probably derived from the elders who are closely related, on the contrary varieties which have relatively high genetic distances, are probably derived from an elder which has a distant relationships with elders of other varieties. The above results can be used as a reference in determining the parent for seed production. The more distant relationship between samples, the less successful the crossing is, but the possibility to obtain superior genotypes is greater if the crossing goes successfully. The more diverse the genetic is the higher the possibility to obtain superior genotypes. Marriage between individuals with close genetic similarity or equal relationship relationship has the effect of increased homozygosis, whereas marriage between individuals having large similarity distance or having far relationship relationship has the effect of increased heterozygosis.

Isozyme is a variation found in the same enzymes that have similar functions and are in the same individual. The enzyme is an amino acid chain in which the genetic information in it is the translation of RNA, whereas RNA is a direct transcription of DNA. Therefore, the variation in enzyme levels shows the variation in the level of DNA (genes) (Na'im 2000). Variations on banding pattern formed by GOT enzyme are less than those by esterase. Sriyono (2006) says that the difference in isoenzymes will produce different velocity when the condition of the electric field and the medium gel is semiporous, so every different enzymes will cause different banding pattern.

The classification of GOT enzyme shows that Pasuruan white varieties belong to the same group with the Bantul red. This is an interesting phenomenon. This seems to be possible because the pioneers of dragon fruit from Pasuruan, East Java, Sapta Surya also develops dragon fruit plantation in Kulonprogo and other Yogyakarta regions (Wijaya 2005). Dragon fruit seedlings introduced to Yogyakarta (Bantul) from Pasuruan is the result of the crossing between varieties of dragon fruit. To prove this phenomenon needs to be done further research using other enzymes or other more modern methods such as using DNA data, whether this occurs due to mutation or due to other factors. This phenomenon also occurs in red pineapple and green pineapple that have a different pigment but form similar banding pattern (Hadiati 2002), it also occurs in dark marie cactus and marie cactus which

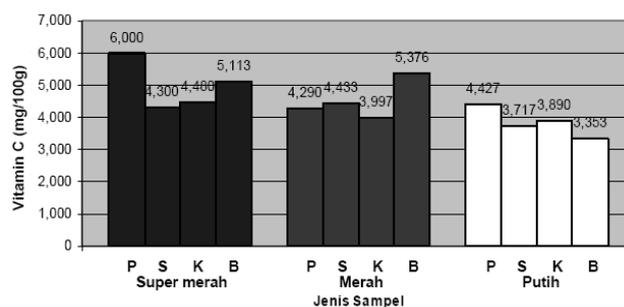
have different pigment but form the same banding pattern (O'Leary and Boyle 2000). In the science of plant breeding, plant introductions have an important role to increase genetic diversity in a region. There is a need for high yielding varieties by bringing in varieties from other areas to assist in the provision of high yielding varieties for farmers and as a germplasm collection (Allard 1998).

Novariant (2008) explains that the breeding is very dependent on the source of genetic diversity. Genetic diversity is not just about physical germplasm collection, but also assessment of the extent of genetic diversity is necessary in genetic manipulation activities towards assembling the desired varieties. Germplasm should be evaluated its genetic diversity as the basis for selection in the crossing process or the assembly of the desired variety of consumers.

Carvalho et al. (2004) explains that the polymorphism produced can be used as a basis for selecting the parent elders that can be used for plant breeding programs. To assemble the improved varieties, the thing need to be considered is the determination of crossing elders, so there is a requirement for information on the genetic distance and relationship relation. According to Hadiati (2002), in hybridization process, the farther the genetic distance between the elders is, the greater opportunity for new cultivars to be produced. In contrast, crossing process between closely related elders resulted in a narrow genetic variability.

#### Vitamin C contents of dragon fruit

The test results of Vitamin C content of dragon fruit from the 4 locations show differences in Vitamin C as shown in Figure 6. The highest level of Vitamin C content is Pasuruan super red dragon fruit (6:00 mg/100g), followed by Bantul red super (5113 mg/100g) and then Bantul red (5376 mg/100g). While the white pulpy dragon fruit contains Vitamin C which at least compared with other varieties.



**Figure 6.** Dragon fruit Vitamin C content of the super red, red and white of the four observation sites. Description: P: Pasuruan, S: Sukoharjo, K: Klaten, B: Bantul.

Super red dragon fruit from Pasuruan district contains the highest Vitamin C compared to the three other locations. This means that the dragon fruit grown in the district of Sukoharjo, Klaten, Bantul undergo a decrease of

its Vitamin C content. The cause is probably that a super red dragon fruit is more suitable to be planted in the area where the plant is first grown in Indonesia. Pasuruan is low-lying areas which are ideal for growing super red dragon fruit. Dragon fruit Vitamin C content of Pasuruan is not much different from one of Bantul because Bantul has similar rainfall with Pasuruan and it is also low-lying areas.

White pulp dragon fruit is Pasuruan also has the highest Vitamin C content when compared with the three other regions. This means that white pulp dragon fruit is also most suitable to be planted in the area of Pasuruan which has high light intensity due to its coastal region. and its low-lying area. According to Fitter (1998), the productivity of a community is a reflection of net photosynthesis of its component species, and strongly influenced by many factors other than light intensity. Nevertheless its total irradiation during one growing season at times when physiologically important is the important determinants for the production of maximum photosynthesis.

Kristanto (2008) suggests that the content of Vitamin C in dragon fruit ranging from 8-9 mg/100 g. The result of this study shows that Vitamin C content is lower. This is possible because the fruit is too ripe, resulting in lower number of Vitamin C. According to Winarno (1995) and de Man (1999), the content of Vitamin C in raw fruit is higher than in a mature one, and the more mature the fruit is the lesser the Vitamin C content.

## CONCLUSION

There is a morphological diversity of stem color, flower petal color, fruit pulp color, and taste of fruit on dragon fruit plants of super red, red, and white from the four sampling sites, Pasuruan, Sukoharjo, Klaten and Bantul. There is a variation of banding pattern isozyme on the varieties of dragon fruit. Based on the isozyme esterase, 18 bands are appeared and are classified into four groups. Based on GOT isozyme, 12 bands appear and are classified into four groups. There are differences in Vitamin C content of dragon fruit from the four sampling sites. Dragon fruit which has the highest content of Vitamin C are Pasuruan super red, Bantul super red, and Bantul red.

## REFERENCES

- Airey R. 2005. Natural Secrets relieve the symptoms of cough and cold. Erlangga. Jakarta. [Indonesia]
- Allard RW. 1998. Principle of plant breeding. John Wiley and Sons. New York.
- Arulsekar S, Parfit DE. 1986. Isozyme analisis prosedures for stones fruit, almond, grape, walnut, pistachio and fig. J Hort Sci 21 (4): 928-933.
- Baihaki A. 2002. Genetic engineering: the challenge and hope in genetic engineering. Unpad Press. Bandung. [Indonesia]
- Balen B, Marijana, Zadro I. 2004. Esterase activity and isoenzymes in relation to morphogenesis in *Mammillaria gracilllis* Pfeiff. tissue culture. Acta Bot Croat 63 (2): 83-91.
- Cahyarini RD, Yunus A, Purwanto E. 2004. Identification of the genetic diversity of some local varieties of soybean in Java based on isozyme analysis. Agrosains 6 (2): 79-83. [Indonesia]
- Claudia M, Takasusuki R, de Fatima PSMM, Conte H. 2002. Esterase-3 polymorphism in the sugarcane borer *Diatraea saccharalis* (Lepidoptera, Pyralidae). Genet Mol Biol 25 (1): 61-64.
- de Carvalho VM, de Fatima PSMM. 2004. Esterase polymorphism in remanant populations of *Aspidosperma polyneuron* Mull. Arg (Apocynaceae). Rev Arvore 28 (5): 625-631.
- de Man JM. 1999. Principles of food chemistry. 3rd ed. Aspen Publishers. Maryland.
- Fitter AH, Hay RKM. 1998. Environmental physiology of plants. Gajah Mada Universitas Press. Yogyakarta. [Indonesia]
- Gyorgy D, Pearson WN. 1964. The vitamin, chemistry, psychology methods. 2nd ed. Academic Press. New York.
- Hadiati S, Murdaningsih HK, Baihaki A, Rostini N. 2002. Variations of banding pattern and relationship of pineapple based on isozyme analysis. J Zuriat 13 (2): 65-72. [Indonesia]
- Hartati, Usdiyanti P. 1997. Study of genetic diversity of forest trees through isozyme analysis. Warta Biotek 11 (1-2): 1-3. [Indonesia]
- Julisaniah NI, Sulistyowati, Sugiharto. 2008. Cucumber (*Cucumis sativus* L.) relationship analysis using RAPD-PCR and isozyme methods. Biodiversitas 9 (2): 99-102. [Indonesia]
- Karcicio M, Izbirak A. 2003. Isozyme variations in some *Aegilops* L. and *Triticum* L. species collected from Central Anatolia. Turk J Bot 27: 433-440.
- Kristanto D. 2003. Dragon fruit cultivation in pots and in the garden. Penebar Swadaya. Jakarta. [Indonesia]
- Maideliza T, Mansyurdin. 2007. Allele diversity of wild yam (*Dioscorea bulbifera* L.) in West Sumatra. Makara Sains 11 (1): 23-27. [Indonesia]
- Murphy J, Philips TD. 1993. Isozyme variation in cultivated oats and its progenitor species *Avena sterilis*. Crop Sci 33: 1366-1372.
- Na'iem M. 2000. Applications isozymes as molecular markers for conservation and breeding program tree. ITTO Workshop. Yogyakarta. [Indonesia]
- Nandariyah, Soemartono, Artama WT, Taryono. 2004. The diversity of salak cultivars (*Salacca zalacca* (Gaertner) Voss) in Java based on RAPD. Agrosains 6 (2): 78-83. [Indonesia]
- Nandariyah. 2007. Identification of the genetic diversity of cultivars of salak Java based RAPD analysis. Agrosains 9 (2): 70-76. [Indonesia]
- Novariantio H. 2008. Superior coconut assembly through molecular techniques and their implications for rejuvenation of coconut in Indonesia. Pengemb Inov Pert 1 (4): 259-273. [Indonesia]
- Nur MA, Adijuwana H. 1989. Spectroscopic techniques in biological analysis. PAU Ilmu Hayat IPB. Bogor. [Indonesia]
- O'Leary MC, Boyle TH. 2000. Diversity and distribution of isozymes in a *Schlumbergera* (Cactaceae) clonal germplasm collection. J Amer Soc Hort Sci 125: 81-85.
- Pasquet RS, Schwedes S, Gepts P. 1999. Isozyme diversity in bambara groundnut. Crop Sci 39 (4): 1228-1236.
- Rahayu S, Sumitro SB, Susilawati T, Soemarno. 2006. Analysis of isoenzymes to study the genetic variation of Bali cattle in Bali Province. Berkala Penel Hayati 12: 1-5. [Indonesia]
- Rolf FJ. 1993. NTSYS-pc numerical taxonomic and multivariate analysis system. Exeter Software. New York.
- Sitompul SM, Guritno. 1995. Analysis of plant growth. Gajah Mada University Press. Yogyakarta. [Indonesia]
- Sudaryono T. 1989. Isozyme analysis in grapevines (*Vitis* sp.). [Thesis]. School of Graduates. Bogor Agricultural University. Bogor. [Indonesia]
- Suranto. 2001. Study on *Ranunculus* population: isozymic pattern. Biodiversitas 2 (1): 85-91. [Indonesia]
- Suryono J. 2006. Consuming dragon fruit to treat various diseases. Sinar Tani, 15-21 Februari 2006. [Indonesia]
- Wijayanti. 2005. Flap of a dragon in the land of Java. Trubus 36, Januari 2005. [Indonesia]
- Winarno FG. 1995. Food enzymes. Gramedia. Jakarta. [Indonesia]
- Yunus A. 2007a. Identification of genetic diversity jatropha (*Jatropha curcas* L.) in Central Java based markers isoenzymes. Biodiversitas 8 (3): 249-252. [Indonesia]
- Yunus A. 2007b. Studies of morphology and isoenzymes of physic nut (*Jatropha curcas* L.) as raw materials for renewable energy in Central Java. Enviro 9 (1): 73-82. [Indonesia]