NUSANTARA BIOSCIENCE
Vol. 1, No. 2, pp. 78-83
July 2009

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n010205

Variation of morphology, karyotype and protein band pattern of adenium (*Adenium obesum*) varieties

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Manuscript received: 22 January 2010. Revision accepted: 19 March 2010.

Abstract. *Hastuti D, Suranto, Setyono P. 2009. Variation of morphology, karyotype and protein band pattern of adenium (*Adenium obesum*) varieties. Nusantara Bioscience 1: 78-83.* The aim of this research to find out the *Adenium obesum* variation from six varieties, namely: *obesum*, cery, *red lucas*, *red fanta* , white bigben and *harry potter* based on morphology, karyotype, as well as protein banding pattern. The chromosome preparation was made using semi-permanent squash method from the tip of root plant; while protein banding pattern was made using SDS-PAGE method. Qualitative data included shape and color of the leave and flower described from each variety. Data were presented in morphometry and analyzed using ANOVA and then followed by DMRT with 5% of confidence levels, indicated significance difference. Protein banding pattern, the root, stem, leave and all organs were analyzed using Hierarchical Cluster Analysis method with Average Linkage (between Groups) using SPSS 10.0. The result of the research shows that the six *A. obesum* varieties have morphological character with no variation of light green to dark green leave, not hairy, smooth leave bone, meanwhile for light red to dark red flower crown color although some of them are white and the same funnel color, yellow. All varieties of *A. obesum* have same number of chromosome, 2n = 22 and shows the difference ranging from 2.56 to 5.13 μm. In the banding pattern formed qualitatively, there is variation among the six varieties.

Keywords: *Adenium*, morphology, karyotype, electrophoresis.

Abstrak. *Hastuti D, Suranto, Setyono P. 2009. Variasi morfologi, karyotipe dan pola pita protein pada berbagai varietas kamboja jepang (Adenium obesum). Nusantara Bioscience 1: 78-83.* Penelitian ini bertujuan untuk mengetahui variasi *Adenium obesum* dari enam varietas yaitu *obesum*, cery, *red lucas*, *red fanta* , white bigben, dan *harry potter* berdasarkan sifat morfologi, karyotipe, serta pola pita protein. Preparat kromosom dibuat dengan metode squash semi permanen dengan bahan ujung akar tanaman dan pola pita protein dilakukan dengan metode SDS-PAGE. Data kualitatif meliputi bentuk dan warna daun dan bunga dari masing-masing varietas. Data morfometri antara varietas dianalisis dengan analisis sidaik ragam (ANAVA), dilanjutkan dengan uji jarak berganda Duncan (DMRT), pada taraf 5% terbukti terdapat beda nyata antar varietas. Pola pita protein akar, batang, daun serta semua organ dianalisis secara kualitatif menggunakan analisis kelompok hierarkhis *Average Linkage* (*between Groups*) dalam program SPSS 10.0. Hasilnya menunjukkan bahwa keenam varietas memiliki karakter morfologi yang bervariasi, yaitu warna daun hijau muda sampai hijau tua, tidak berbulu, tulang daun polos, sedangkan warna mahkota merah muda sampai merah tua, walaupun ada yang putih dan kuning. Jumlah kromosom semua sama yaitu n =22, dimana panjang absolut kromosom berkisar antara 2,56-5,13 μm. Pola pita protein yang terbentuk secara kualitatif terdapat variasi ketebalan di antara keenam varietas, yang menunjukkan adanya perbedaan kandungan proteinnya.

Kata kunci: *Adenium*, morfologi, karyotipe, elektroforesis.

INTRODUCTION

Dessert rose or Adenium (*Adenium obesum* (Forsk) Roem et Schult) is a tropical plant that can grow and thrive in a barren desert, earning the nickname of desert rose. These plants were originated from the deserts of Africa, scattered from Senegal to Ethiopia and from Somalia to Tanzania. It also grows wild in Saudi Arabia, Oman, and Yemen (Oyen, 2008). This plant is widely used as an ornamental plant, because besides it is easy to maintain, resistant to drought, this kind of flowers vary both in shape and color. This plant can be used as a drug because it contains toxins and plant crystalline glycoside that is useful as a means of relaxation (Chuhairy and Sitanggang 2004). These plants can be propagated vegetatively using stem and the consumer preferences of relatively rapid change are important in narrowing the genetic diversity and high impact to the decline of the economic value of plants, so that the necessary efforts to develop new varieties of *A. obesum* is being done. New varieties can be obtained by exploration of new varieties, crosses, or in a more modern, such as protoplast fusion and mutation (Soetarso et al. 1985).

High enough variation in shape, size, and color of flowers of *A. obesum*, showed a wide genetic diversity. This is an important factor in plant breeding programs, both for material and enlargement of the cross germplasm genetic diversity itself (Soetarso et al. 1985). Cross-crosses between species (intraspecific) or between the new properties (infraspecific) and uniquely different from both parent will further enrich the genetic diversity of these plants.

Morphological variation occurs due to genetic influences. Genome as a collection of biological information that regulates a variety of characters in an individual is stored in one set of chromosomes (Menadue and Crowden 1990; Wulandari et al. 2006). The number and shape of chromosomes in every cell of plant species are fixed. Each species has a characteristic number of chromosomes and each chromosome in one species also have a typical structure (Snustad 1997; Wulandari et al. 2006). Consistent chromosomes are widely used for the purpose of taxonomy, the diversity of kinship relationships and evolution, although in certain circumstances may occur variations. The differences in the chromosome are used to describe the diversity of genetic variation, whereas differences in plant morphological, physiological processes describe the biochemical diversity of gene products that are influenced by the environment (Stebbins 1951; Yuliastri et al. 2005). Molecular analysis is the exposure of genetic material using electrophoresis. The basic principle of electrophoresis is that every plant genome (enzyme/protein and DNA) has a different weight so that the speed of movement of the gel media were also different and this can be seen through the coloring (Sudarmono 2006). This study is aimed to determine variations in leaf and flower morphology, karyotype, and variations in protein band patterns of six varieties of *A. obesum*.

MATERIALS AND METHODS

Plant material

Frangipani plant japan (*A. obesum*) of six varieties of *obesum* (Var 1), *cery* (Var 2), *red lucas* (Var 3), *red fanta* (Var 4), white bigben (Var 5), and *harry potter* (Var 6). Electrophoresis using SDS-PAGE with protein Coomassie blue dye, marker proteins with M 4038 (Sigma, Germany).

Procedures

Observation of morphology of the leaves of *A. obesum* done by measuring the length, width, shape, hair condition, and leaves' bones. Sampling began with the fifth leaf from the tip-growing plant with optimum appearance, age and relative size of the uniform and never flowered. While the observations of flower morphology include color, diameter, and edge rates, as well as taking photos of the leaves and flowers (the Setyawan et al. 2002).

Observations of karyotype were carried out by planting the seeds *A. obesum* in previous week before the lab and seeds soaked in growth hormone (vitamin B1). A Preliminary Study of *A. obesum* was performed every 30 minutes and made prepared for semi-permanent. Retrieved optimum cleavage time hours 8:30 to 09:00 pm. Preparations made with squash semi-permanent method (Okada 1981; Darnaedi 1991 with the modification of Setyawan and Sutikno 2000). Root tip is cut along the 2-3 mm from the tip and then inserted into the bottle containing distilled water and stored flakon bottles in 5oC for 24 hours. After soaking distilled water, samples were stored in 2-3 mL Colchicine 0.2% and stored at 5oC for 2-4 hours. Colchicine 0.2% was discarded and washed with distilled water 3 times or until it is clean so it does not cover the chromosomes. After Colchicine is cleaned, it is replaced with 45% acetic acid and stored in 5^{o}C for 15 minutes. The 45% acetic acid is then washed with distilled water and removed 3 times. The distilled water is replaced by 1N HCl and included in the oven at 60^{o}C for +2 minutes. Discarded 1N HCl then washed with distilled water 3 times. After the distilled water removed is replaced with acetoorcein 2% for 48 hours at room temperature. Roots were taken with a brush/toothpick, deposited on glass objects, and then drops of glycerin and covered with a glass lid and tap on-tap with the tip of a pencil eraser rubber band until evenly crushed. Excess glycerin is removed with tissue paper and a glass lid sealed with clear nail polish. The observations, made with a light microscope to improve resolution of used oils and preparations are well emersion were photographed, enlarged captured so easily observed.

Protein banding pattern analysis was conducted using SDS-PAGE (Laemmli 1970). Preparation of extract buffer: 100 mM Tris HCl pH 8.5, 4% Mercaptoethanol, 20% glycerol. 30% polyacrylamide stock: 29 g acrylamide, 1 g bisacrylamide; plus aquabidest until volume of 100 mL. SDS PAGE 12%: 4.8 mL stock polyacrylamide; 3 mL of 1.5 M Tris pH 8.8; 0.12 10% SDS; plus aquabidest up to volume 12 mL. Stacking gel 3%: 2 mL stock polyacrylamide; 2.52 mL of 1.5 M Tris pH 6.8, 0.3 mL SDS 10%; plus aquabidest up to volume 20 mL. Electrode buffer: 3 g Tris, 14.4 g glycine, 10 mL 10% SDS. SDS sample buffer: 2.5 mL of 1.5 M Tris pH 6.8, 2 g SDS, 0.5 g DTT, 10 mg Bromphenol blue, 10 mL glycerol; plus aquabidest up to volume 20 mL. Coomassie blue dye: 0.1% Coomassie blue in 100 mL destaining. Destaining: 50% methanol, 10% glacial acetic acid, 40% aquabidest.

After all the solution is made, then the roots, stems, leaves, and all organs of each variety aquabidest are washed until they are clean and then cut into small pieces, then weighed with the weight of each 0.5 g, crushed with a mortar and pestle is mixed with the extract 1000 mL buffer. Once crushed and homogenized, include it in Eppendorf tubes. Centrifuge is prepared and when it is cold (temperature +0°C), then add it to Eppendorf tubes that are centrifuged at 12 000 rpm for 5 minutes. Supernatant was boiled for two minutes, for the protein to open.

Polyacrylamide gel consisting of 2 parts, the 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 was made by mixing 10 mL stock 12% SDS PAGE, plus 7 mL Temed, and 80 mL 10% APS. While the 3% stacking gel was prepared by mixing 5 mL of 3% stacking gel stock, plus 3.5 mL Temed, and 50 mL 10% APS. Polyacrylamide gel solution was mixed, after homogeneous separating gel electrophoresis included in the glass, after somewhat thickened, add saturated isobutanol. After that isobutanol discarded the stacking gel electrophoresis, insert it into the vessel just above the running gel. Sample comb mounted on the stacking gel and released after the compact. Supernatant was loaded into the hole as much as 10 mL samples using injection equipment (stepper), also loaded ladder (M 4038, Sigma, Germany). Before the installation of plate glass on the vessel

electrophoresis confirmed that circulator shows the temperature not exceeding 4°C. Next clip clamp and then the shield of plate glass tube was removed and the glass plate was mounted on the electrophoresis bath, face to face, with a notched glass plate located on the inside. Furthermore, the electrode buffer is filled again until the full and bathtub mounted back cover. Electrophoresis performed at 125 volts for 90 minutes or until the lower limit supernatant.

After electrophoresis was complete, the gel was taken and stained. Staining is done by putting gel electrophoresis that has been removed from the glass into the plastic tray, then pour a solution of blue and Coomassie have shaken overnight. After that, the gel was rinsed with destaining until clear. When it was clear, then the washing was stopped by replacing destaining with a solution of 10% glacial acetic acid.

Data analysis

Observations of morphology include the length and width of the leaf 's and flower's diameter. Colors, shapes, leaves, the condition of the bones, and the presence or absence of fur (hairiness) on the leaf's surfaces are described by descriptive method. Morphological observations are presented in the form of morphometry. To determine whether there is any difference in the length and width of the leaf's morphology and flower's diameter, ANOVA test is performed. The observation of karyotype microscopically with a magnification of 1000x, squash method was used for semi-permanent which includes the absolute number and length of chromosomes from *A. obesum* var. *obesum*. Observations are documented in the form of photographs. Data analysis was performed using the protein banding pattern of quantitative analysis that is based on whether or not the pattern of protein bands appear to calculate molecular weight and Rf based marker, and qualitative methods based on thin thick ribbon is formed. Estimated ribbon was formed into a table and made into a zymogram. To find out the close relationship between the varieties which appear, ribbons are interpreted by a dendrogram analysis.

RESULTS AND DISCUSSION

Morphology

Results of the research on the morphology of leaves and flowers of six varieties of *A. obesum* indicate a diversity of morphological traits. The diversity of these varieties includes the length and width of leaves, diameter and flower color of the six varieties of *A. obesum* as in Table 1 and Figure 1.

Table 1. The average results of morphological measurements of leaves and flowers of six varieties of *A. obesum*.

Variety	Length of leaves (cm)	Width of leaves (cm)	Diameter of flowers (cm)
1	8.15 ^{b}	2.91 ^{bc}	7.51 ^{e}
2	6.96 ^{a}	1.63 ^{a}	7.25 ^{de}
3	6.61 ^{a}	1.87 ^{a}	5.02 ^{a}
4	8.75 ^{b}	2.80 ^{bc}	6.02 ^{b}
5	6.44 ^{a}	2.61 ^{b}	7.02 ^{cd}
6	8.41 ^{b}	2.99 ^{c}	6.81 ^{c}

Note: 1. var. *obesum* 2. var. *cery*, 3. var. *red lucas*, 4. var. *red fanta* , 5. var. *white bigben*, 6. var. *harry potter*. The numbers figures followed by the same letters in columns and rows indicate no significant difference based on level 5% DMRT test.

Figure 1. Morphology of plants from six varieties of *A. obesum*. Note: 1. *obesum*, 2. cery, 3. *red lucas*, 4. *red fanta* , 5. white bigben, 6. *harry potter*.

Morphological observations of the six varieties of *A. obesum* about the length and width of leaf and flower diameter indicate significant differences. From the ANOVA test results, it shows that significant levels of leaf length are 0000 at level of 95%. This means that the length of the leaves looked a real difference. ANOVA Test towards the leaf's width also showed significant differences, namely a significant level of 0.000 on the level of 95%. Flower diameter showed a significant level of 0.000 at the level of 95%, this means flower diameter also showed significant differences. Through the morphology, it can be concluded that between the length and width of the leaf's and flower's diameter, there are significant differences.

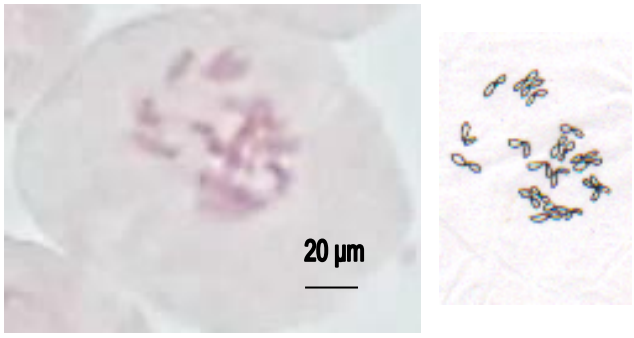
Having held further test of the length and width of the leaf's and flower's diameter by DMRT 5% level there are two variations for the length of the leaf, and there are three variations for the width of the leaf, and there are five variations for the flower's diameter. Thus, the diameter of the flower shown is more varied than the leaves.

Karyotype

Mitotic division consists of prophase, metaphase, anaphase, and telophase. These stages in natural conditions only last a few minutes. The experts give the term prometaphase to stage between prophase and metaphase. This stage is the most important condition for the study of cytology, because when prometaphase shape, number and size of the chromosome it is possible to study (Sabelli and Larkins, 2007; Sadava et al. 2009).

The observation of chromosomes among the six varieties, namely *A. obesum* var. *obesum* shown in Figure 2. In this study, the number of chromosome *A. obesum* var. *obesum* namely 2n = 22, and the absolute length of chromosomes ranged from 2.56 to 5.13 µm. These results are in line with other researchers who generally said that the number of chromosome *A. obesum* is 2n = 22 (Van der Laan and Arends 1985; Oyen, 2008). The position of the observed chromosomes is not so evenly spread, because there is overlap. Such a position is still happening even though at the time of mitotic division of the core wall is lost and the spread chromosomes in the cell space are expanding. This buildup determines the observation form, size, and number of chromosomes. In outline form, the size and number of chromosomes are calculated based on the chromosome with the highest frequency, while the number of chromosomes is counted as an approach to the basic number (x).

Chromosome's size can be described with an absolute length of chromosomes. This is consistent with the statement Buitendijk et al. (1997) who conducted the study on 12 species of Alstroemeria, and concluded that DNA content was correlated positively with the total length of the chromosomes. Along with Bennett (1987) that the amount of DNA contained in the cell nucleus is positively correlated with several parameters such as the total length of cells, and/or the volume of metaphase chromosomes during mitosis or meiosis.



A B

Figure 2. Chromosomes *A. obesum* var. *obesum.* Note: A. The original photo, B. Redrawn.

Banding pattern of protein

Root

Electrophoresis of protein band patterns of the roots of the six varieties of *A. obesum* is shown in Figure 3. In general, the pattern of protein bands of the six varieties of *A. obesum* is visibly different, which means there is no difference in protein content. The pattern of protein bands var. *obesum* (1) and var. *cery* (2) in general seems much thicker than that of other varieties. It shows a higher protein content than other varieties. While var. *white bigben* (5) the banding pattern formed is the thinnest and the least and this means that the protein content of these varieties are lower.

Protein band with molecular weight 6 kDa protein with the type of myosin (M 4038, Sigma) from two varieties of *red lucas* (3) and *red fanta* (4) shows the thinnest band compared with the four other varieties. Similarly, protein bands with molecular weight of 69.96, and 145 kDa on *white bigben* varieties (5) look the thinnest of all. The expression pattern of a thin ribbon does not mean that these varieties do not have certain types of proteins, but it may only have one protein alone.

The above results are made into a dendrogram as in Figure 4. The results show that the protein profiles in dendrogram of *A. obesum* are classified into 4 groups. Lucas red varieties (3) and *red fanta* (4) merge into one group, as well *obesum* (1) and *cery* (2) also are joined into one group, while the *harry potter* (6) and white bigben (5) respectively form a separate group.

Stem

The results electrophoresis protein banding pattern stems from the six varieties of *A. obesum* are shown in Figure 3. In general, the pattern of protein bands sixth stem varieties *A. obesum* visible differences, which means that there are differences in protein content. The pattern of protein bands formed from the six shaft varieties also showed a difference, except in *red lucas* (3) seems the thinnest when compared with the other. When viewed from the protein content also showed no obvious band differences. Protein band with a size 92, 109 and 119 kDa was not apparent in *red lucas* (3) and *red fanta* (4), while others appear thicker. This indicates that the protein content in these varieties is higher than the other. Based on marker proteins with M 4038 (Sigma), protein bands with

molecular weight of 6 kDa (myosin), and 66 kDa (carbonic anhydrase) is owned by all the varieties, while the protein with a molecular weight of 20 kDa (phosphorylase b) only owned *cery* (2) and white bigben (5). Dendrogram above results contained in Figure 4, where the protein profiles in stem banding pattern dendrogram, group *A. obesum* into 3 groups. Variety *obesum* (1) and *harry potter* (6) merge into one group, *cery* (2) and white bigben (5) to join in a group, as well as *red lucas* (3) and *red fanta* (4). Varieties that fall into the same group means having a lot of similarities than differences. Meanwhile, in a further grouping (Euclidean distance) there is little in common.

Leaf

The pattern of protein bands sixth leaf varieties *A. obesum* formed on electrophoresis with marker M 4038 (Sigma) is shown in Figure 3. Leaf's protein banding pattern of the six varieties of *A. obesum* in general shows differences, this means that there are differences in protein content. This shows that between varieties of one another, there are also differences in protein content. Variety *obesum* (1) and *cery* (2) appear thicker than the other varieties, this means that these varieties are higher protein content. But *red lucas* (3) and white bigben (5) express banding pattern that is thinner than other varieties. Such varieties show lower protein content.

Protein band with a size of 155 kDa is owned by all varieties, except *cery* (3), whereas the protein band with a size of 122 kDa is only owned by white bigben (5). Based on 4038 M protein markers (Sigma), protein bands with molecular weight 116 kDa (α-lactalbumin) is owned by the six varieties of *A. obesum*. The above results are made into a dendrogram as shown in Figure 4, where the protein profiles on the dendrogram group *A. obesum* are classified into 5 groups. Variety *obesum* (1) and *cery* (2) joined in one group, while the other varieties of *red lucas* (3), *red fanta* (4), white bigben (5), and *harry potter* (6) form a separate group.

Protein banding pattern of all organs of A. *obesum*

Protein banding pattern of all organs with the results of electrophoresis markers M 4038 (Sigma) is shown in

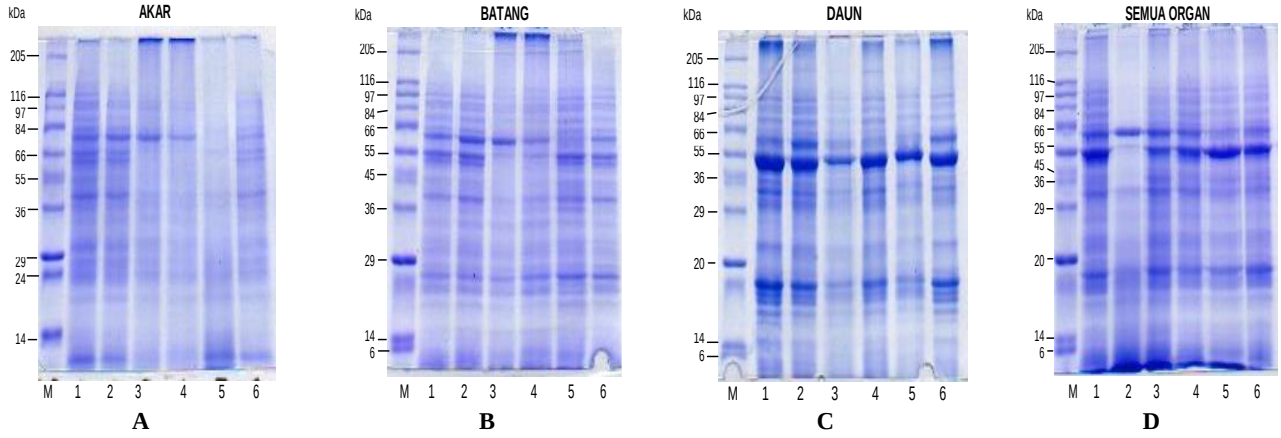


Figure 3. The pattern of protein bands roots *A. obesum.* A. Roots, B. Stem, C. Leaf, D. All organs. Note: M = Marker, 1. *obesum,* 2. *cery*, 3. *red lucas*, 4. *red fanta* , 5. *white bigben*, 6. *harry potter*

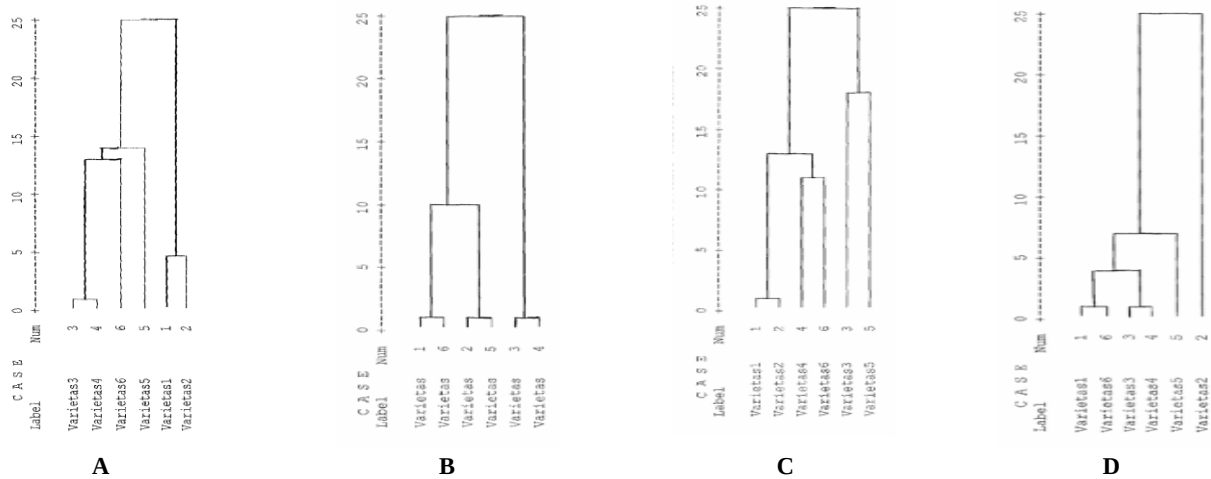


Figure 4. Protein banding pattern dendrogram root *A. obesum.* A. Roots, B. Stem, C. Leaf, D. All organs. Note: M = Marker, 1. *obesum*, 2. Cery, 3. *red lucas*, 4. *red fanta* , 5. *white bigben*, 6. *harry potter*

Figure 3. Protein banding pattern of all organs of the six varieties of *A. obesum* generally indicates differences. This means that there are differences in protein content. Variety *obesum* (1) and *red lucas* (3) show the banding pattern that is thicker than the others. Variety *cery* (2) expresses a relatively thin banding pattern compared with the others. The banding pattern formed on *cery* (2) shows at least, that is not owned seven banding pattern *cery* (2) while others have it all. Expression pattern of bands that are not clearly visible does not mean not having banding pattern of proteins with specific molecular weight. This possibly can happen in visual observation.

Based on 4038 M protein markers (Sigma), the protein band with a molecular weight of 6kDa (myosin), 20 kDa (phosphorylase b), and 66 kDa (carbonic anhydrase) is owned by all varieties, except *cery* (2). It does not have this last type of protein (66 kDa). The above results made dendrogram as Figure 4, where the protein profiles on the dendrogram group *A. obesum* into 4 groups. Variety *obesum* (1) and *harry potter* (6) joined in a group, *red lucas* (3) and *red fanta* (4) also joined into one group. While white bigben (5) and *cery* (2) respectively to form his own group. When compared with the pattern of protein bands on the roots, stems, leaves and especially, the varieties of *cery* (2) in all organs are the thinnest. This can occur because of sampling performed on all organs during the two-week-old plants, while sampling for the banding pattern of roots, stems, and leaves done in one-year-old plants. At a young age, plant's proteins do not form a complete or perfect form yet, so that the banding pattern of protein expression has not been optimum.

Diversity patterns of protein bands between varieties within a species are generally low, as well as a single protein band patterns of diversity within a species population. (Comas et al. 1979; Sammour and Sharaf el-din-1989; Vas et al. 2004). Variations generally only appear as a thick thin ribbon that shows the differences will need certain proteins in each individual. While inter-species are always more diverse (Sathaiah and Reddy 1985; González-Aguilera et al. 1986). This is due to the high level of exchange of genetic material between the individuals in it. Even if these plants are clonal and can be propagated vegetatively in which exchange of genetic material almost does not exist, the diversity of protein in it remains low, given the similarity of environmental causes a similar tendency toward mutation.

CONCLUSION

The longest is red leaf Fanta (8.75) cm and the shortest white bigben (6.44) cm, the widest leaves are *harry potter* (2.99) and the narrowest *cery* (1.63) cm. The diameter of the largest flower is *obesum* (7.51) cm and the smallest *red lucas* (5.02) cm. Light green leaf color to old, not hairy, bone plain leaf. While the flowers have the same basic color that is pink to dark red, although there are some that are white. Funnel on the flower's color, has the equation that is yellow. Inside the fruit are seeds of a stick with a length of about 1 cm of 60 to 80 pieces, at both ends there are hairs that serve as a tool for reproduction (dispersal) with the wind. Based karyotype chromosome, chromosome number Adenium *obesum* var. *obesum* is 22 pieces, the absolute length of chromosome that is between (2.56 m) to (5.13 m). Based on the analysis of protein band patterns in the roots, stems, leaves and all the organs it is shown that all had different protein content as indicated by the differences in expression patterns of protein bands thin thickness.

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