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The Use of Betacyanin Compound Profile as the Basis for Classification (Family Relationships) of Six Genera in Family Amaranthaceaea

NanikLutfiyah
Universitas Darul Ulum Jombang, Jl.Gus Dur (ex Merdeka No 29 A 61413 (032-853436)
Jombang - East Java – Indonesia

Abstract: *This study aims to determine the content of the betacyanin in six genera of Amaranthaceae (Achyranthes, Aerva, Althernantera, Amaranthus, Celosia, Gomphrena), and their classification based on betacyanin compound. The analysis of betacyanin compound applies spectrometry and LCMS. Cluster analysis is performed using NTSYSpc. The findings indicate that all taxa studied contains betacyanin compound. The highest content is found in genus Alternantera up to 447.72 µgr/g in dry weight. For classification of family Amaranthaceae, betacyanin compound marker is divided into two clade where similarity values reaches 44.00%. Clade one is composed of two genera, Celosia and Alternanthera with similarity values up to 60.00%. Clade two consists of four genera Amaranthus, Alternanthera, Acyranthes, Gomphrena and Beta with a similarity value up to 60.00%.*

Keywords: *Amaranthaceae, Betacyanin, Classification, Morphology*

I. Introduction

Family Amaranthaceae plays an important role in human life. Some species have been used for years as horticulture, such as Amaranthuscaudatus(amarant or spinach). Some other species have also been used as ornamental plants, like Celosia (plumed cockscomb), Gomphrena (globe amaranth) and Alternantera (alligator weed). Some other species are used as herb garden, for example Aervalanata which is used as balm for feeling faint and swelling, and Achyranthesasperawhich is usable to wash eyes when they are lack of sight [1].

Most of species in Amaranthaceae have red fruit, red flower, or red vegetative parts. Red color is made by betalain pigments. Betalain is water-soluble compound. It is a kind of pigment that contains nitrogen and is detectable in 13 plant families in order Caryophyllales[2].

Betalain is specific compound in order Caryophyllales[3]. It is also usable as taxonomy indicator in family Amaranthaceae[4]. The analysis on family relationship, morphologically, is highly affected by environment [5]. Betalain has two categories: betacyanin (reddish to violet betalain pigments) and betaxanthin which appear yellow to orange. Betacyanin is betalain compound commonly found in family Amaranthaceae.

Information on family relationship is so required in plant breeding. In plant breeding, superior taxon is often generated by crossing of closely related taxa to produce desirable characteristics. The study aims to determine classification of six genera in family Amaranthaceae according to its relationship, by using profile indicator of betacyanin compound.

II. Materials And Methods

SelectionandSampling

Observed genera are Amaranthus, Celosia, Aerva, Alternanthera, Achyranthes and Gomphrena. Each genus is represented by one species. Sample from the plant is identified based on determination keys by [6] and [7]. Out group is selected from family Chenopodiaceae, i.e. Beta, because this family is the closest family from Amaranthaceae and more primitive than family Amaranthaceae.

Hatchery and Planting

Seed is scattered evenly on seed bed and buried by thin layer of soil. Each seed of plant species is sowed on the same container and labeled. When primary leaf grows or 2-3 weeks old, the plant will be relocated to polybag with a mixture of soil and compost inside of it. Ratio for the mixture is 3:1. After flowering stage of each plant occurs, three individual of each genus is selected as sample.

Extraction of Betacyanin Compounds

Around 4 gram old leaf sample is frozen in freezer for 24 hours, and dried up using freeze dryer for 24 hours. Dried leaf is pounded using pestle and mortal, and extracted by methanol 80% by 50 mm ascorbic acid up

to 5 ml. Homogenat, afterwards, is centrifuged at 2500 rpm speed for 5 minutes to separate the densities. Generated supernatant is then diluted by methanol 80% ascorbic acid up to 5 ml [8].

The Analysis of Betacyanin Content

The existence of total betacyanin generated by extraction process is measured spectrophotometer. Betacyanin existence is indicated by maximum absorption at 530 nm – 550 nm. Absorption is measured at maximum. Total betacyanin content is measured based on amaranthine molecule weight [9] , and made in equation as below as amaranthine is dominant betacyanin in family Amaranthaceaea.

$$AC_a = \frac{A.\ MW.\ Va.\ DF}{\varepsilon.\ Wa} \quad \text{……..……..…(Equation 1)}$$

AC_a = betacyanin compound in raw liquid extract (mg/ 100 g dry matter)
A = absorbency at 536 (λ max)
MW = 726.6 g/mol (amaranthine)
Va = total extract volume (ml)
DF = dilution factor
ε = molar extinction coefficient = 5.66×10^4 cm^{-1} mol^{-1} L
Wa= dry weight of extracted matter

The Analysis of Betacyanin Profile

To find out betacyanin compounds profile, this study applies LCMS. Through the analysis process, molecule weight of extracted compounds is detectable. LCMS autosampler uses Thermo Scientific brand Accela type at 10^o C and 10 µl in volume. UHPLC uses Thermo Scientific brand Accela type, isocratic takes 4 minutes in 100% solvent with 0.1 % formic acid in acetonitril at 250 µl per minutes speed in Hypersil Gold (50 x 2.1) column. MSMS uses TSQ Quantum Access Max (Triple Quadrupole) brand, scan event =1, MS acquire =1, scan type SRM (Selected reaction Monitoring), scan width (M/Z) 0.010, scan time 0.01, parent mass 178 with product mas 114 collision 20, parent mass 178 with product mas 72 collision 20.

Data Analysis

The data on morphological characteristics consisting of habitus, stem, leaf, flower, and seed is described and scored. Dendogram construction uses Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm by using NTSYSpc software version 2.02i. UPGMA algorithm uses similarity coefficient introduced by Sokal and Sneath (1973) in Radford (1981). Algorithm equation complies with Equation 2.

$$S_{jk} = 1 - \frac{\sum \frac{Xij - Xik}{Ri}}{n} \quad \text{.....Equation (2)}$$

S_{ij} = similarity value between taxa j and k
Xij = the ith characteristics of taxon j
Xjk = the ith characteristics of taxon k
Ri = the ith characteristics range
n = characteristics count

The data generated by LCMS is used as parameter where the clustering will be analyzed based on compound presence or absence. Compound is interpreted in binary data where the value is described 0 for an absent characteristics and 1 for present characteristics from the compared genera. Dendogram construction uses Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm in NTSYSpc software version 2.02i.

III. Research Findings And Discussion

Extraction of Betacyanin Compounds

Based on the observation using UV-VIS spectrophotometer, all leaf sample reach maximum wavelength at 536 nm. Max wavelength at 536 indicates the presence of betacyanin compound.

The highest betacyanin content is found in Alternantera leaf of 447.725 µgr/dry weight, and the second highest is Celosia, followed by Aerva, Gomphrena, Amaranthus and Achyranthes which has the lowest

betacyanin content. The highest betacyanin is supported by morphological characteristics, where leaves color of Alternantera and Celosia are red.

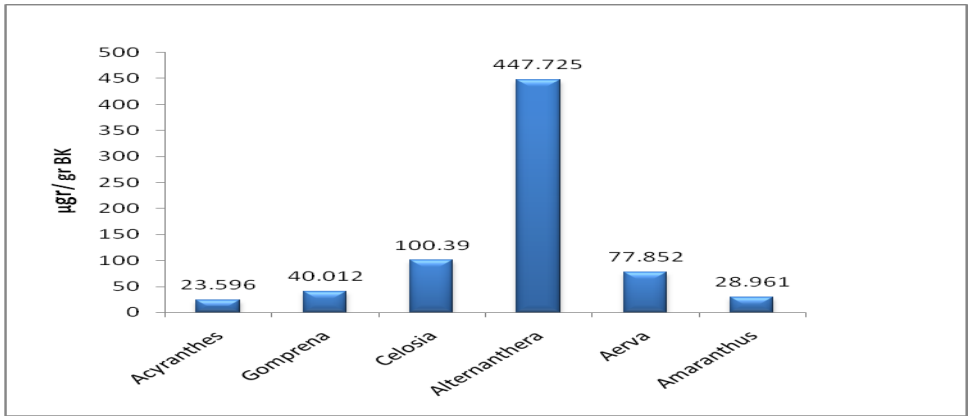

Figure 1. Betacyanin Content of the Six Genera in Family Amaranthaceae

Classification of Genus Amaranthus Based on Betacyanin Compound Profile

Analysis using LCMS is able to predict compound based on the molecule weight and fragmentation pattern. The analysis on genus Achyranthes found that it contains (M + H) 813 compound where the compound has no common name, and (M + H) 389 or so called betanidin compound. Genus Gomphrena has (M + H) 551 or called gomphrenin I compound (Strack D & Schliemann, 2003, and Kugler, et al., 2007) and (M + H) 398 or betanidin compound. Genus Celosia contains (M + H) 903 or celosianin II, 871 (iresin I), 727. According to Schliemann, et al. (2001), these compounds are amaranthine, 637 (phylocactin) and 389 (betanidin). Genus Alternanthera contains (M + H) 903 (celosianin II), 871 (iresin I), 727 (amaranthine), 695 (hylocerenin) and 551 (betanin). Genus Amaranthus contains betacyanin (M + H) 871 (iresin I), 727 (amaranthine), 551 (betanin), and 389 (betanidin). Genus Aerva contains betacyanin (M + H) 871 (iresin I), 743 (has no common name) and 389 (betanidin). Genus Beta contains (M + H) 871 (iresin I) compound, 743 (has no common name), 551 or betanin compound.

Table 1. Betacyanin Compound in FamiliaAmaranthaceae

Genus	(M+H) Compound	Fragment
Achyranthes	813 (-)	389
	389 (betanidin)	212
Gomphrena	551 (gomphrenin)	389
	389 (betanidin)	212
Celosia	903 (celosianin II)	859, 727, 389
	871 (iresin I)	727, 389
	727 (amaranthine)	389
	637 (phylocactin)	389
	389 (betanidin)	212
Alternanthera	903 (celosianin II)	859, 727, 551, 389
	871 (iresin I)	727, 551, 389
	727 (amaranthine)	683, 551, 389
	695 (hylocerenin)	551, 389
	551 (betanin)	389
Amaranthus	871 (iresin I)	727, 551, 389
	727 (amaranthine)	551, 389
	551 (betanin)	389
	389 (betanidin)	212
Aerva	871 (iresin I)	389
	743 (-)	699, 389
	389 (betanidin)	212
Beta	871 (iresin I)	551, 389
	743 (-)	551, 389
	551 (betanin)	389

Classification which is built on chemical compound for family Amaranthaceae has two clade: clade one has three genera, and clade two has four genera where the similarity value reaches 51.67%. The first clade has three genera, Celosia, Amaranthus and Alternanthera, where the similarity value reaches 65.00%. Special characteristics of this clade are the presence of (M + H) 727, or so called amaranthine. Among these three

genera, Alternantera is split up from two other genera, while Celosia are making group with Amaranthus with similarity index between both genera reaches 70.00%. The second clade has four genera: Aerva, Beta, Achyranthes and Gomphrena where the similarity value reaches 60.00%. All members of this clade have (M + H) 389 compound, or called betanidin, except Beta. This clade makes two ranks; the first rank consists of two genera, genus Aerva and Beta, where similarity index reaches 80%. The next rank consists of two genera, genus Gomphrena and Achyranthes, where the similarity index is also 80.00% (Figure 3).

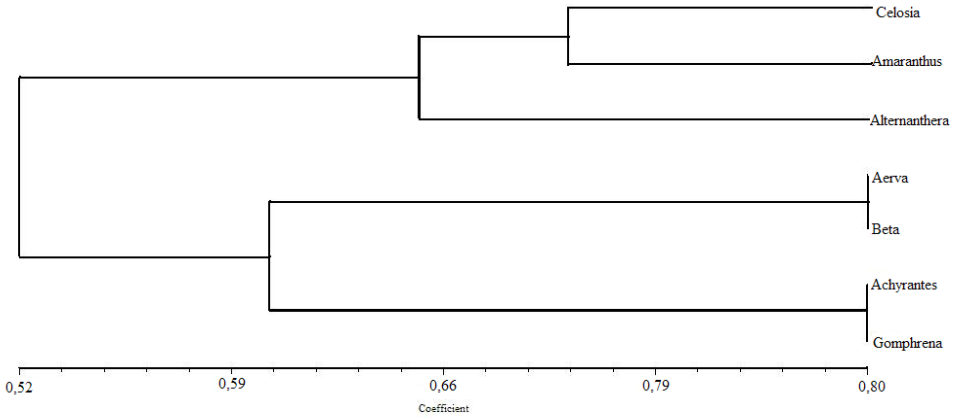

Figure 2. Classification for FamiliaAmaranthaceae Based on Betacyanin Compound Presence and Beta the out group

IV. Discussion

Similarity value constitutes family relationship closeness, the higher similarities, the closer family relationship will be. Based on betacyanin compound profile, the closest family relationship is between genus Achyrantes and genus Gomphrena with similarity value 80.00%.

Celosia and Althernanthera are in the same clade, supported by same morphological characteristics, i.e. red leaf color they have. The findings indicate that betacyanin compound is the special characteristics found in genus Gomphrena[10]; [11]. Phylocactin is betacyanin compound that becomes characteristics found in genus Celosia, hylocerenin, on the other hand, is betacyanin compound that becomes characteristics found in genus Alternanthera. (M+H) 813 is characteristics found in genus Achyranthes.

Based on the compound found in dendogram of family Amaranthaceae, it does not indicate that Beta is the out group, in fact, data found in this study affirms previous study that Beta, one of genera in family Chenopodiaceae, is close family from family Amaranthaceae[12] because characteristics of this genus are look alike in phytochemistry ways. FamiliaChenopodiacea and family Amaranthceae are both from the same ordo, Caryophyllales. Moreover,[13] writes about phylogenetic analysis result based on glucosyltransferaseenzym to produce betalain. He explains about a fact that Beta is in one group with Gomphrena and not the out-group of Gomphrena (Figure 3).

Figure 3. Phylogenetic Scheme Based on GlucosylTransferase in Plant's Betalain Production by Amino Acid Sequence

V. Conclusion

The highest betacyanin compound is found in genus Alternantera (447,72µgr/gr). Classification of family Amaranthaceae according to betacyanin compound marker is divided into two clade; clade one consists of three genera, Celosia, Amaranthus and Alternanthera. Clade two consists of four genera, Alternanthera, Achyranthes, Gomphrena and Beta. Classification for family Amaranthaceae based on morphological characteristics and betacyanin compound has two clade. Clade one has two genera, Celosia and Amaranthus. Clade two has four genera, Aerva, Achyranthes, Gomphrena and Alternanthera. Genus Achyrantes and genus Gomphrena has close relationship by 80.00% similarity value.

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