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Morphological, anatomical and isozyme variation among giant taro (*Alocasia macrorrhizos*) accessions from Central Java, Indonesia

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Abstract. *Suratman, Pitoyo A, Kurniasari S, Suranto. 2016. Morphological, anatomical and isozyme variation among giant taro (*Alocasia macrorrhizos*) accessions from Central Java, Indonesia. Biodiversitas 17: 422-429.* The objective of this study was to evaluate morphological, anatomical and isozyme variation among giant taro (*Alocasia macrorrhizos* (L.) G.Don) accessions from Central Java (Indonesia). A total of 20 giant taro accessions were collected from different collection sites in Central Java. Identification of morphological characters was done by direct observation of roots, leaves, stems, and corms. Anatomical characters were observed from both paradermal and transverse sections of leaf. Identification of biochemical markers was done by using peroxidase and esterase isozyme system. The genetic similarity among giant taro accessions was measured by using Group Average Clustering. The results of the analysis of variance revealed highly significant differences for majority of the tested morphological and anatomical characters suggesting that there was a high degree of diversity among the giant taro accessions. Isozyme polymorphism was observed in giant taro accessions using peroxidase (two banding patterns) and esterase (four banding patterns). Based on the dendogram, giant taro accessions were segregated into two major clusters. In Cluster I, the closest relationship were showed in KTN 2 and WNG 1 accessions from Klaten and Wonogiri that had 80.95% of similarity coefficient. The five accessions (SKA, SKH, WNG 4, KRA 3, KRA 4) from Surakarta, Sukoharjo, Wonogiri and some parts of Karanganyar were clustered separately as Cluster II with similarity coefficient of 50%.

Keywords: *Alocasia macrorrhizos*, anatomy, Central Java, giant taro, isozyme, morphology

INTRODUCTION

Alocasia is recorded as the largest genus in the family Araceae which comprises more than 100 species of herbaceous, laticiferous, diminutive to gigantic, usually robust herbs (Boyce 2008). This genus inhabits wet disturbed sites, areas of regrowth, large canopy gaps, and roadside ditches, but there are also forest undergrowth species (Hay 1990; Ivancic et al. 2009). Giant taro (*Alocasia macrorrhizos* (L.) G.Don) is a species of the genus *Alocasia* and may have originated from Sri Lanka or India (Purseglove 1979; Plucknett 1984; Ivancic and Lebot 2000). From this area, it has spread to almost all tropical and subtropical regions (Groen et al. 1996; Lebot 1999; Matthews 2004; Nauheimer et al. 2012). The corm of giant taro is very rich in carbohydrates, which is mainly starch at 77.9% and 1.4% crude fiber, on Dry Matter (DM) basis. The corm is edible and also a good source of dietary protein, thiamin, riboflavin, sodium, iron, magnesium, kalium, phosphorus, zinc and a very good source of vitamin B6, vitamin B12, vitamin C, vitamin E, niacin, potassium, copper and manganese (Soudy et al. 2010; Manner 2011). This edible corm has been served either as staple food or mixed with other vegetables, usually after cooking (Kumoro et al. 2014). The utilization of the corms as a staple food in many parts of the tropics and sub-tropics providing about a third of the food intake of more than 400 million people (Soudy et al. 2010). The corms, cormels, stems and leaves also can be used as vegetable and animal fodder. For medical puposes, the chopped roots and leaves are used as a rubefacient and juice from the petiole is used againts coughs (Groen et al. 1996). Giant taro is also cultivated and introduced as a tropical ornamental plant, which a number of varieties have been recognised (Furtado 1941).

The genetic diversity of giant taro accessions from Central Java (Indonesia) is poorly documented. In order to ascertain the level of genetic variation among and within species, populations or accessions, a variety of morphological, anatomical, biochemical and molecular markers are used. Morphological markers are routinely used for estimating genetic diversity of plants since they are inexpensive, simple and fast (Jingura and Kamusoko 2015). Anatomical characters are also valuable in taxonomy and identification of groups of plant (Rahayu et al. 2012; Chikmawati 2013).

Isozyme as the classical biochemical marker can be used to determine genetic variation of cultivars, natural populations and accessions in germplasm collections, if the morphological characters appear to overlap due to strong influence of environment (Suranto 2001; Fernandez de Souza and Primo 2001; Padmanaban et al 2013). Isozymes have several advantages over traditional markers such as morphological or anatomical traits to study polymorphism because they are not influenced by environmental factors making identification possible in early stages of development (Torres 1990). A range of enzyme loci also can be studied easily using a small quantity of material with minimum preparation and cost (Johnson et al. 2010; Kovacevic et al 2010).

Information on genetic diversity and relationship among and between individuals, accessions, populations, varieties, and species of plant are also important for plant breeders in guiding the improvement of plants (Dharmar and De Britto 2011). This information can provide predictive estimation of genetic variation within species thus facilitating breeding material selection (Qi et al. 2008).

The objective of this study was to evaluate morphological, anatomical and isozyme variation among giant taro accessions from Central Java (Indonesia). This is the first study to combine morphological, anatomical and isozyme markers to evaluate genetic variation in giant taro accessions from Java, especially in Central Java, Indonesia.

MATERIALS AND METHODS

Plant materials

A total of 20 giant taro accessions were collected from different collection sites in Central Java (Table 1, Figure 1). Plants were then transplanted into polybags and kept in screen house in Department of Biology, Universitas Sebelas Maret for 8 weeks before young corms were collected. The plantation site is situated at 126 m asl altitude, 28 ºC of temperature, 8200 lux of ligh intensity, 85% of air humidity and 50 % of soil humidity.The young corms of each accession were then used for isozymes extraction.

Morphological analysis

Identification of morphological characters (both quantitative and qualitative) was done by direct observation of vegetative structures such as roots, corms, stems and leaves of the giant taro plant. The observed of morphological characters were plant height, leaf length, leaf width, petiole length, petiole width, sheath length, sheath width, corm length: width ratio, root length: width ratio, abaxial secondary veins

Table 1. The geographic variation of giant taro (*A. macrorrhizos*) accessions originated from Central Java, Indonesia with climatic data for each collection site

No.	Acces-sions	Collection site	Alt. (m. asl.)	Temp. (ºC)	Light int. (x 1000 lux)	Air humid. (%)	Soil humid. (%)
1	BYL 1	Boyolali	548	27	17.2	84	28
2	BYL 2	Boyolali	607	26	13.5	82	10
3	BYL 3	Boyolali	802	23	2.4	80	18
4	BYL 4	Boyolali	970	22	7.4	83	30
5	KTN 1	Klaten	475	26	8.3	91	10
6	KTN 2	Klaten	457	28	9.8	69	30
7	KTN 3	Klaten	726	25	1.7	80	10
8	KTN 4	Klaten	802	23	1.7	100	15
9	WNG 1	Wonogiri	225	30	53.8	65	50
10	WNG 2	Wonogiri	381	28	5.4	84	80
11	WNG 3	Wonogiri	600	25	7.4	81	75
12	WNG 4	Wonogiri	677	23	6.9	78	82
13	KRA 1	Karanganyar	396	29	7.6	64	55
14	KRA 2	Karanganyar	641	28	9.6	66	10
15	KRA 3	Karanganyar	740	26	5.2	69	76
16	KRA 4	Karanganyar	905	23	77.8	32	60
17	SRG 1	Sragen	197	23	2.4	91	80
18	SRG 2	Sragen	304	23.3	3.2	100	75
19	SKH	Sukoharjo	119	28	50.8	54	30
20	SKA	Surakarta	119	29	3.9	80	50

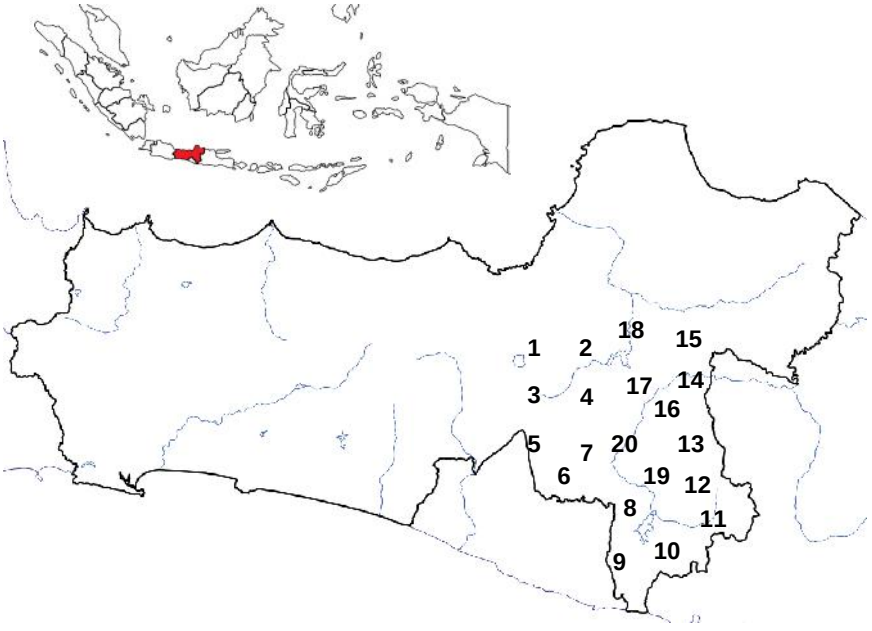

Figure 1. Map of the collection areas for giant taro (*A. macrorrhizos*) accessions studied in Central Java. The number (1 to 20) indicated location of each collected accession

Anatomical analysis

Leaf anatomy was observed from both paradermal and transverse sections. The leaf paradermal and transverse section were carried out as described by Chikmawati (2013). The section were observed under light microscope. The observed characters were stomatal density, stomatal index, stomatal length; stomatal width; abaxial epidermis thickness, adaxial epidermis thickness, mesophyll thickness, palisade thickness, palisade ratio, number of calcium oxalate crystal.

Isozyme analysis

Gel and buffer preparation

Acrylamide gel electrophoresis and buffer solutions (extraction buffer, tank buffer, running buffer) were prepared and carried out as described by Suranto (2001) and Setyawan et al. (2014).

Isozyme extraction

Young corms of giant taro were ground in mortar using 0.15-0.35 m l of extracting solution and then transferred to a 1.5 ml microtube. Samples were centrifuged at 3500 g for 15 minutes, and supernatant was transferred to new microtube. The supernatants were then applied into the well of acrylamide gel. The extracting solution consisted of 0.018 g of cysteine, 0.021 g of ascorbic acid, 5 g of sucrose, diluted in 20 ml of borax buffer pH 8.4 (tank buffer).

Electrophoresis

From the centrifuged samples about 200 µl of supernatant was taken and 5µl of bromophenol blue (tracking dye) was added to each sample. About 10-15 ul of prepared samples (for peroxidase) and 15-24 ul (for esterase) was taken and loaded into each well of the gel. Loaded samples were electrophoresed at a constant current of 5 mA for peroxidase and 7 mA for esterase at room temperature for about 60 minutes. Electrophoresis was stopped when the bromophenol blue marker dye had traveled about 56 mm from the well toward the anode (Suranto 2001; Padmanaban et al. 2013; Setyawan et al. 2014).

Staining procedures

After electrophoresis, the gels were stained for the appropriate enzyme as described in Suranto (2001) and Setyawan et al. (2014) with some modifications. Peroxidase staining was prepared by diluting 0.0125 g of O-dianisidine into 25 ml of acetone. Then 50 ml of 0.2 M acetate buffer pH 4.5 was added and 2 drops of H_2O_2 lastly given. Esterase staining was prepared by dissolving 0.0125 g of α-naphthyl acetate in 2.5 ml acetone.After that 50 ml of 0.2 M phosphate buffer pH 6.5 and 0.0125 g of Fast Blue BB Salt were added. Gels were immersed in these staining solutions until bands appeared.

Data analysis

Analysis of variance was performed for quantitative morphological and anatomical observation data in order to test the significance of variation among accessions. The data from zymograms were entered as a matrix of presence/absence of bands for each enzyme. The genetic similarity among giant taro accessions based on morphological, anatomical and isozyme markers was measured by using Group Average Clustering which were integrated in the program Numerical Taxonomy and Multivariate Analysis System (NTSYS) version 2.10. (Rohlf 1998).

RESULTS AND DISCUSSION

Morphological analysis

The analysis of variance revealed significant differences among accessions for all of the tested quantitative morphological traits suggesting that there was a high degree of phenotypic diversity among the accessions. Plant height, leaf length, leaf width, petiole length, sheath length, root length: width ratio showed wide variation while petiole width, sheath width, corm length: width ratio showed a narrower range of phenotypic variation (Table 2).

Plant height exhibited wide range of variation and ranged from 52.5 cm (KRA 2) to 130.5 cm (BYL 3) with an average 89.31 cm. The leaf length varied significantly among accessions and displayed a range from 14.5 cm (KRA 2) to 51.4 cm (BYL 3), with an average 31.26 cm. Leaf width differed significantly among tested accessions and was highest in the accession KRA 1 (46.8 cm) and lowest in the accession SKA (15.3 cm) with an average 29.02 cm. Petiole length also exhibited wide differences among accessions and ranged from 21 cm (KRA 2) to 83 cm (BYL 3) with an average 49.79 cm. Petiole width values showed narrower variation and ranged from 0.8 cm (KRA 2) to 2.87 cm (BYL 3) with an average 1.75 cm. Sheath length displayed wide range of variation and ranged from 10 cm (SKA) to 45.5 cm (BYL 3) with an average 27.22 cm. Sheath width values exhibited narrower differences among accessions and ranged from 1.18 cm (KRA 2) to 4.68 cm (BYL 3) with an average 2.76 cm. Corm length: width ratio displayed narrower differences among accessions and varied from 1 to 4. Root length: width ratio showed wide range of variation and ranged from 23 (WNG 4) to 152 (KTN 3) with an average 88.5. For qualitative morphological characters, most of examined accessions showed flattened abaxial secondary veins, except in WNG 3 accession which has prominet abaxial secondary veins.

Anatomical analysis

Analysis of variance for anatomical characters revealed that there was significant variation for all the tested characters among giant taro accessions, except in case of palisade ratio. However, accessions variation for palisade ratio was non-significant (Table 3).

Comparing the stomatal densities and stomatal index, there was significant variability among the tested accessions. SRG 1 accession displayed the highest value of stomatal density (24.89/mm^2) whereas the lowest one can be found in SKA accession (15.24/mm^2) with an average 20.84/mm^2. The highest stomatal index value was

distributed in WNG 3 accession (40) whereas the lowest one in the KRA 2 accession (15) with an average 24.05.

Leaves are transversally arranged into one layer of upper (adaxial) epidermis cells, mesophyll cells, and one layer of lower (abaxial) epidermis cells. The mesophyll consisted of spongy tissue. Of all examined giant taro accessions was remarkable in having significant variation in leaf tissue layer thickness such as abaxial epidermis thickness, adaxial epidermis thickness, mesophyll thickness and palisade thickness.

Table 2. Morphological character variation among giant taro accessions from Central Java, Indonesia

No.	Accessions	PlH	LfL	LfW	PtL	PtW	ShL	ShW	CoR	RoR	AbV
1	BYL 1	81.2a	31a	29.4a	48a	1.69a	25a	3.66b	1a	127bc	Flat
2	BYL 2	105b	32.8a	30.4a	43.3a	1.94b	23.2a	2.36a	1a	101b	Flat
3	BYL 3	130.5b	51.4b	46.1b	83b	2.87b	45.5b	4.68b	1a	112b	Flat
4	BYL 4	99.9b	36.9b	33.3b	63b	1.72a	34a	3.60b	1a	100b	Flat
5	KTN 1	80.6a	28.3a	27.2a	47a	1.75a	23a	2.55a	1a	46a	Flat
6	KTN 2	102b	37b	38b	61.2b	2.20b	32.5b	3.22b	1a	33a	Flat
7	KTN 3	107b	33.2a	28.2a	56b	1.72a	33b	2.42a	2a	152c	Flat
8	KTN 4	101b	40.5b	36.3b	59b	2.07b	31b	2.99a	1a	82a	Flat
9	WNG 1	102.8b	39.5b	30ab	64.4b	2.36b	39b	3.92b	1a	131c	Flat
10	WNG 2	104.3b	35.4ab	31.3a	47a	1.75a	29a	2.55a	1a	81a	Flat
11	WNG 3	89a	23.2a	22.7a	48.5a	1.62a	25a	2.07a	2a	91ab	Prominent
12	WNG 4	71.6a	29.6a	28a	44a	1.62a	26a	2.87a	2a	23a	Flat
13	KRA 1	105b	46b	46.8b	59b	2.45b	31b	2.87a	2a	52a	Flat
14	KRA 2	52.5a	14.5a	15.6a	21a	0.8a	10.2a	1.18a	1a	91ab	Flat
15	KRA 3	119.7b	40b	36b	73b	2.07b	45b	3.98b	4ab	75a	Flat
16	KRA 4	64a	25a	22a	37a	1.31a	20a	2.48a	4ab	83a	Flat
17	SRG 1	68.5a	23a	19a	46.5a	1.40a	24a	2.48a	1a	141c	Flat
18	SRG 2	71a	19.7a	20.2a	30.5a	1.31a	17a	1.91a	2a	66a	Flat
19	SKH	64a	21.6a	24.5a	40.5a	1.34a	21a	1.91a	1a	128bc	Flat
20	SKA	66.5a	16.5a	15.3a	24a	0.92a	10a	1.43a	1a	48a	Flat
	Average	89.31	31.26	29.02	49.79	1.75	27.22	2.76	1.55	88.15	

Note: * PlH = plant height (cm); LfL = leaf length (cm); LfW = leaf width (cm); PtL = petiole length (cm); PtW = petiole width (cm); ShL = sheath length (cm); ShW = sheath width (cm), CoR = corm length: width ratio; RoR = root length: width ratio; AbV = abaxial secondary veins. ** Values followed by the different lower-case letter in the same column are significantly different (Duncan multiple range test, $p < 0.05$)

Table 3. Anatomical character variation among giant taro accessions from Central Java, Indonesia

No.	Accessions	StD	StI	StL	StW	AbT	AdT	MeT	PaT	PaR	CaO
1	BYL 1	26.44b	32bc	45.08b	30.51b	44.75a	43.79ab	213.22b	61.72ab	0.44a	3a
2	BYL 2	20.14ab	19a	51.86b	32.54b	55.93b	37.41a	206.78ab	52.29a	0.40a	3a
3	BYL 3	16.89a	20a	49.49b	31.19b	49.83ab	40.78a	216.61b	65.00ab	0.44a	3a
4	BYL 4	19.14ab	22a	42.37a	27.46b	47.46a	39.83a	197.29a	52.50a	0.44a	3a
5	KTN 1	21.66ab	24ab	46.10ab	27.80b	52.54b	38.97a	223.05b	73.71b	0.40a	4a
6	KTN 2	19.63ab	22a	46.44ab	32.54b	52.54b	41.72ab	243.39b	75.34b	0.44a	5ab
7	KTN 3	28.28b	27ab	52.88b	30.85b	52.20b	42.59ab	217.63b	65.78ab	0.44a	4a
8	KTN 4	21.19ab	22a	51.53b	32.20b	55.25b	40.95a	220.68b	64.57ab	0.36a	4a
9	WNG 1	25.67b	36b	40.68a	30.85b	47.12a	44.74ab	213.90b	62.41ab	0.44a	4a
10	WNG 2	28.08b	21a	56.95b	32.88b	58.98b	42.33ab	221.02b	61.47ab	0.44a	4a
11	WNG 3	17.93a	40c	34.58a	17.63a	46.44a	37.84a	174.92a	45.00a	0.50a	8b
12	WNG 4	18.73ab	23a	50.51b	36.61b	47.80a	36.72a	199.32a	45.26a	0.44a	3a
13	KRA 1	21.28ab	24ab	44.07a	32.88b	55.93b	41.47ab	219.32	62.41ab	0.40a	2a
14	KRA 2	16.99a	15a	63.39c	27.12b	44.75a	41.38ab	203.73ab	56.64b	0.44a	3a
15	KRA 3	13.62a	23a	46.78ab	29.83b	41.02a	38.36a	212.20b	79.48b	0.40a	3a
16	KRA 4	17.39a	21a	50.51b	30.51b	57.97b	35.69a	164.75a	59.83a	0.44a	4a
17	SRG 1	24.89ab	21a	49.15b	30.85b	49.15ab	40.69a	198.64a	63.62ab	0.44a	2a
18	SRG 2	23.62ab	29b	44.07a	31.19b	45.42a	47.84ab	227.46b	74.48b	0.40a	3a
19	SKH	19.06ab	16a	49.49b	32.54b	51.19b	42.16ab	159.66a	54.48a	0.50a	3a
20	SKA	15.24a	22a	46.44ab	29.15b	44.41a	39.57a	174.24a	60.60ab	0.50a	3a
	Average	20.84	24.05	48.21	30.42	50.33	40.80	207.03	61.89	0.44	3.58

Note: * StD = stomatal density (pore/mm^2); StI = stomatal index; StL = stomatal length (µm); StW = stomatal width (µm); AbT = abaxial epidermis thickness (µm); AdT = adaxial epidermis thickness (µm): MeT = mesophyll thickness (µm); PaT = palisade thickness (µm); PaR = palisade ratio, CaO = number of calcium oxalate crystal (no/mm^2). ** Values followed by the different lower-case letter in the same column are significantly different (Duncan multiple range test, $p < 0.05$).

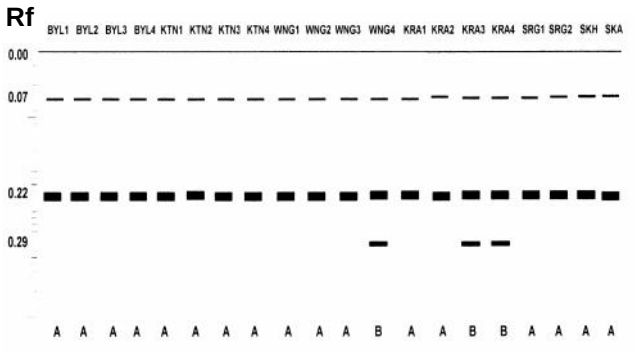

Figure 1. Peroxidase isozymic banding pattern of giant taro accessions from Central Java, Indonesia

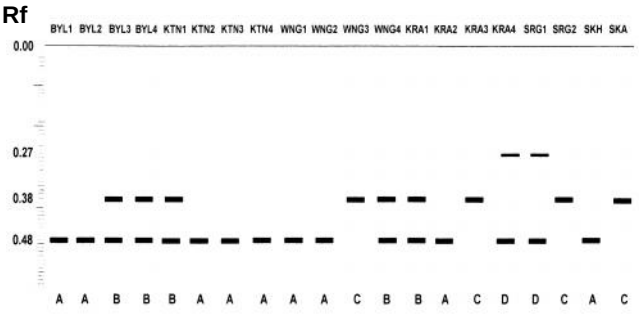

Figure 2. Esterase isozymic banding pattern of giant taro accessions from Central Java, Indonesia

The number of crystal of calcium oxalate per mm^2 also showed significant variation among accessions. The highest number of calcium oxalate was observed in WNG 3 accession ($8/mm^2$), whereas the lowest one was distributed in SRG 1 and KRA 1 accessions ($2/mm^2$).

Isozym analysis

The two enzymatic systems showed a total of six banding patterns, distributed in the whole set of samples as peroxidase with two banding patterns and esterase with four banding patterns. Peroxidase showed three anodic bands, resulting in two patterns zymogram (banding pattern A and B) which distributed in different Rf value varying from 0.07 to 0.29. Banding pattern A consisted of two bands which located at Rf 0.07 and Rf 0.22 whereas banding pattern B consisted of three bands which located at Rf 0.07, Rf 022 and Rf 0.29 from anodal zone (Figure 1).

Two isozymic banding patterns of peroxidase also distributed separately in giant taro accessions (Figure 2). Banding pattern A was seen in majority accessions and occured in 17 tested accessions (BYL 1, BYL 2, BYL 3, BYL 4, KTN 1, KTN 2, KTN 3, KTN 4, WNG 1, WNG 2, WNG 3, KRA 1, KRA 2, SRG 1, SRG 2, SKH, SKA) whereas banding pattern B only distributed in three accessions (WNG 4, KRA 3 and KRA 4). Therefore, peroxidase was considered as a suitable marker for these accessions.

Three bands of esterase at different Rf values varying from 0.27 to 0.48 were observed, which allowed to distinguish four pattern zymograms (banding pattern A, B, C and D) (Figure 2). Banding pattern A only consisted of one band which located at Rf 0.48. Banding pattern B consisted of two bands which located at Rf 0.38 and Rf 0.48. Banding pattern C consisted of one band which located at Rf 0.38. Banding pattern D consisted of two bands which located at Rf 0.27 and Rf 0.38 from anodal zone.

Banding pattern A occured in majority accessions and distributed in nine tested accessions (BYL 1, BYL 2, KTN 2, KTN 3, KTN 4, WNG 1, WNG 2, KRA 2, SKH) whereas banding pattern B in five accessions (BYL 3, BYL 4, KTN 1, WNG 4, KRA 1), banding pattern C in four accessions (WNG 3, KRA 3, SRG 2, SKA) and banding pattern D only lied in two accessions (KRA 4, SRG 1).The observed banding pattern D only distributed in two accessions, so esterase can be considered as a suitable marker for these accessions.

Relationships

In order to study the relationship among accessions, genetic similarity based on morphological, anatomical and isozyme markers was used to predicted a dendrogram for the giant taro accessions from Central Java using NTYSYS software. Based on the dendogram at a level of 50 % similarity, it showed distinct separation of twenty giant taro accessions from Central Java into two major clusters (Figure 3). Cluster I comprised most of tested accessions which originated from Boyolali, Klaten, Sragen, and some parts of Wonogiri and Karanganyar. The closest relationship were showed between KTN 2 and WNG 1 accessions from Klaten and Wonogiri that had 80.95% of similarity coefficient. The five accessions (SKA, SKH, WNG 4, KRA 3, KRA 4) from Surakarta, Sukoharjo, Wonogiri and some parts of Karanganyar were then clustered separately from the another as Cluster II with similarity coefficient of 50% and considered to be genetically unique.

Discussion

Analysis of quantitative morphological characters variation above provide an indication of genetic diversity present among accessions, and such methods have been successfully used to measure phenotypic diversity in germplasm collections. Phenotypic variations provided a good opportunity for genetic improvement (Sabaghnia et al. 2014). In this study, only one qualitative morphological characters was observed i.e. abaxial (lower) secondary veins. The observed abaxial secondary veins showed narrower variation among accessions. These results further indicated the existence of variability among accessions for this trait although their variation was considered low.

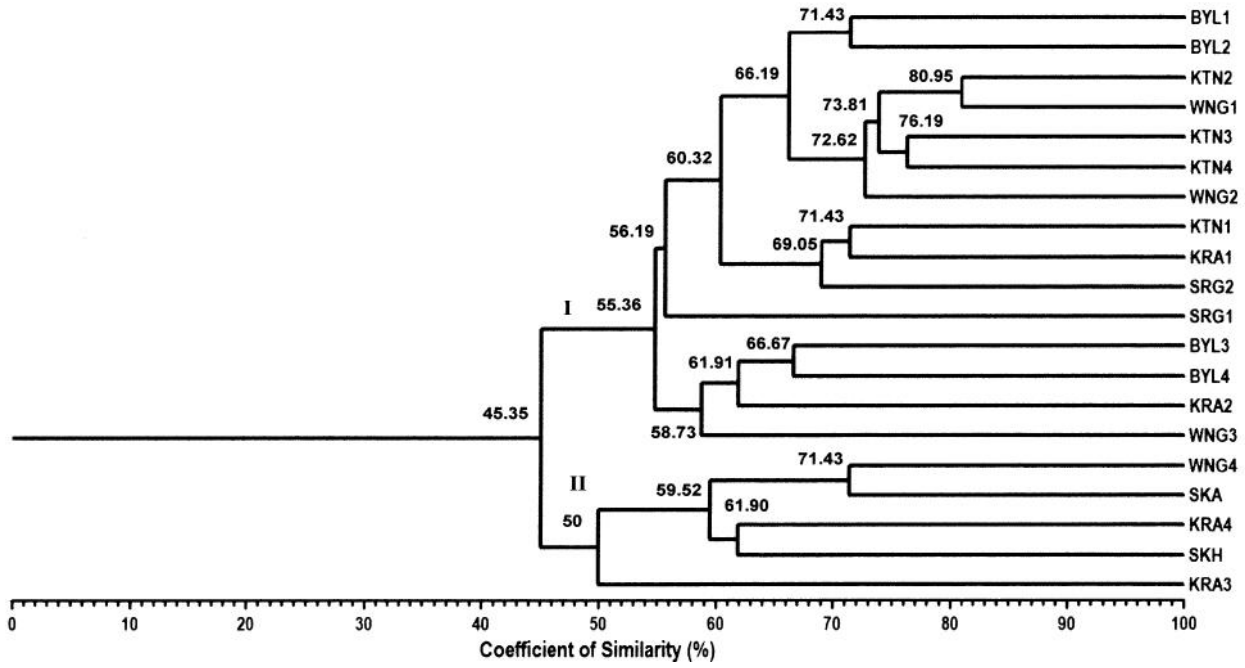

Figure 3. Relationship dendrogram among 20 giant taro accessions from Central Java, Indonesia using morphological, anatomical and isozyme markers

The pattern of variation exhibited for various characters were substantially different. The incidence of highly significant variation among the accessions for the majority of the studied morphological characters is a sign of the presence of high degree of genetic variation implying great potential of the accessions in future breeding programs through selection (Nkansah et al. 2013; Roy et al. 2013). Therefore, this indication showed that there is enough scope for selection of desirable genotypes, where variability exists.

Morphological markers have been commonly used as a first step in germplasm characterisation, but the time required for processing of candidate accessions is significant. Despite this limitation, morphological characters is still useful for preliminary evaluation because it is fast, simple, and can be used as a general approach for assessing genetic diversity among morphologically distinguishable accessions (Beyene et al. 2005).

Stomata of giant taro were found only in lower (abaxial) epidermis layer and distributed among kidney-shaped guard cells. Stomata shape was tetracyclic, with 4 subsidiary cells. In most species, frequency of stomata in the lower epidermis are more than the upper epidermis (Muradoglu and Gundogdu, 2011).

The higher stomatal density or stomatal index can be used as an indicator for higher transpiration rate, highest metabolism and absorption of mineral and water. Stomata characteristics such as frequency and dimensions can be affected by type of species and environmental factors (Munir et al. 2011). Although stomatal features can be affected by multiple ecological factors, as they are directly exposed to the environment, but stomatal differentiation and development are determined by genetic factors (Hetherington and Woodward 2003).

The leaf tissue layer thickness such as abaxial epidermis thickness, adaxial epidermis thickness, mesophyll thickness and palisade thickness exhibited siginificant variation among all examined accessions. The difference in layers thickness of leaves might be attributed to the responses toward environmental factors (Donovan et al. 2007). High levels of genetic variation stimulated the populations or accessions more flexible to fit a variable environment influence.

The number of crystal of calcium oxalat per mm^2 also showed significant variation among accessions. In this study, the observed calcium oxalate presented as fine needle-like crystals or raphides. Calcium oxalate content depends on the cultivars, fertilizers and environmental condition, especially during drought (Bradbury and Holloway, 1988).

Some anatomical characters might be influenced by environment factors but majority of the tested anatomical characters showed highly significant variation among all tested accessions.This information indicated that there is enough scope for selection of accessions on the basis of these characteristics for genetic improvement.

The morphological variation, as a product of genotype and the environment, is an important parameter, but much diversity, which remains unexpressed morphologically, can be revealed by biochemical methods. Study of isozymic variation is one such important and powerful procedure that has often been employed for this purpose (Smila et al. 2007; Johnson et al. 2012).

Isozyme polymorphism was observed in giant taro accessions from Central Java using peroxidase and esterase systems. Peroxidase and esterase have been widely utilized to assess the genetic similarity and to reveal the variation of organisms at the various taxonomic levels. Peroxidase is an easily detected enzyme because of extraordinary activity on plant tissue but in this study peroxidase showed lower variations of isozymic banding pattern among giant taro accessions. Collares et al. (2004) reported that the leaves showed more polymorphism in peroxidase zymograms than that observed in shoot and root samples. In our study, isozymes were extracted from young corms, therefore genetic variation derived from isozymic banding pattern of peroxidase was considered low, due to the small number of polymorphism. Isozyme extraction from leaf tissue of giant taro was difficult to be conducted because of its highly content of mucilage and fenol.

The esterases are a complex and heterogeneous group of enzymes, catalyzing the hydrolysis of the ester link (Smila et al. 2007). Esterase showed most isozymic banding pattern variations compared than peroxidase in this study. According to Desborough and Peloquin (1967), isozymes of esterase in tubers are reliable and valuable as a biochemical markers. Therefore, esterase is considered as a useful diagnostic tool in this study for identification or assesment of genetic variation in view of the extensive polymorphism for this enzyme.

There were some observed bands (both peroxidase and esterase) are fairly thick, but there are also thin bands overlooked in this study. The difference of isozymic banding thickness is probably due to the differences in the copy number of the gene. A thick band may also be caused by two bands coincide, which indicates heterozygote for two alleles of the monomer, and a thin band indicating homozygote (Setyawan et al. 2014).

The availability of isozyme banding pattern has substantially increased our knowledge of the genetics of plant accessions. Differences in isozyme profiles can be used to reveal genetic diversity among accessions. The observed polymorphic zones reflect the validity of the isozyme data to study the genetic diversity at intraspecific levels in giant taro accessions from Central Java. Sher et al. (2010) stated that isozymes are still useful markers for genetic polymorphism identification due to its simplicity and validity for describing genetic structure of groups of plants.

However, the relationship dendrogram showed that the grouping was inappropriate with geographical origins. It is explicit that there is no relationship between geographic distribution and genetic diversity in this study. Thus, the grouping did not always indicate the geographical origins similarity, but possibly showed the genetic similarity (Tikader and Kamble 2008).

One of the main applications of these clusters is the estimation of the genetic similarity among accessions and identification of parents for performing appropriate crosses, and reaching maximum heterosis in hybridization programs (Lombardi et al. 2014; Suratman et al. 2015). Selection of better accessions can be made for species improvement based on its genetic similarity percentage. Two similar genetically accessions or more but possessing distinct characters can be chosen for this purpose.

In this study, morphological, anatomical and isozyme markers showed that this method is informative and can be used to determine genetic variation and the relationships among accessions. The information about genetic similarity will be helpful to avoid any possibility of elite germplasm becoming genetically uniform (Fadoul et al. 2013). Thus, information about genetic diversity through morphological, anatomical and isozyme markers obtained in this study could be valuable for breeding strategies of giant taro in Java. From a conservation perspective, sampling many accessions from all possible agroecologies would be an effective strategy of capturing genetic variation for future collections (Beyene et al. 2005).

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