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Morphological, anatomical and isozyme variability among taro (*Colocasia esculenta*) accessions from southeastern part of Central Java, Indonesia

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Abstract. *Pitoyo A, Prameta AA, Marsusi, Suratman, Suranto. 2018. Morphological, anatomical and isozyme variability among taro (*Colocasia esculenta*) accessions from southeastern part of Central Java, Indonesia. Biodiversitas 19: 1811-1819.* The objective of this study was to evaluate morphological, anatomical and isozyme variability among taro accessions from southeastern part of Central Java (Indonesia). A total of 20 taro accessions were collected from a wide range of sites during field surveys. Morphological characters measurements were taken on vegetative structures such as roots, stems, leaves, 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. A UPGMA dendrogram among accessions was constructed based on the genetic similarity matrix by applying a cluster analysis using a computer programme, NTSYS Version 2.00. The analysis of variance for morphological and anatomical characters revealed that there was significant difference for majority of the tested traits indicating that there was a variability among the taro accessions. Polymorphism was observed using isozymes of esterase (12 banding pattern) and peroxidase (8 banding pattern). Based on the dendrogram at a level of 62 % similarity, taro accessions were segregated into two major clusters. In Cluster I, the closest relationship was shown between SKH and SKA accessions that had 96 % coefficient of similarity. The ten accessions from Klaten, Sragen, and Karanganyar were then clustered separately as Cluster II with coefficient of similarity 73.52 %.

Keywords: anatomy, southeastern part of Central Java, isozyme, morphology, taro, *Colocasia esculenta*

INTRODUCTION

Taro (*Colocasia esculenta* (L.) Schott), belonging to the family Araceae, is an important root crop especially in the humid tropics and sub-tropics. It is one of the few crops that can adapt well to different agro-climatic conditions (Kreike et al. 2004; Asha Devi, 2012). Taro is an ancient crop of uncertain geographical and genetic origins in Southeast Asia (Matthews 2014). Presently, taro is naturalized as a tuber crop and leafy vegetable. However, it attains the importance of staple food in many African, Oceanic and Asian cultures (Purseglove 1972; Safo Kantaka 2004).

The edible part of taro such as corms, leaves, and petioles provide a good source of carbohydrate, protein, dietary fibre, minerals (calcium, phosphor, magnesium and iron) and vitamins (thiamin, riboflavin, niacin, ascorbic acid) (Wilson and Siemonsma 1996; Safo Kantaka 2004; Huang et al. 2007; Rao et al. 2010; Amagloh and Nyarko, 2012; Darkwa and Darkwa, 2013). The corm is usually sliced and fried into taro chips and is used in the preparation of soups, beverages, and puddings. The starch is used in baby foods and as cereal substitute. Taro leaves and leaf stalks are used as a leafy vegetable and potherb for soups and sauces. Taro leaves and corms are also credited for having medicinal values. In Mauritius, the boiled young leaves are eaten to treat arterial hypertension and liver affections, whereas juice is applied externally to treat eczema. In India, China and New Guinea, the corms are used to treat stomach-ache, diarrhea, and as a poultice on sores and skin diseases (Wilson and Siemonsma 1996; Safo Kantaka 2004).

Taro is an excellent multipurpose food crop for subsistence agriculture and home gardens, giving food security. Genetic improvement of taro has the potential to overcome production constraints, particularly resistance to pests and diseases. The success of genetic improvement of a crop, however, depends on the availability of genetic resources and their diversity (Okpul et al. 2004).

The assessment of genetic diversity is required in the crop breeding program. In order to assess genetic variability of plants, a variety of morphological, anatomical, biochemical and molecular markers are used. The traditional technique used to assess genetic variation among and within species, populations or accessions is based on differences in morphological traits (Acquaah 2012). Morphological character is still routinely used for preliminary evaluation because it is fast, simple, inexpensive and can be used as a general approach for assessing genetic diversity of plants (Beyene et al. 2005; Jingura and Kamusoko 2015). Anatomical characters are also valuable in taxonomy and identification of groups of plant (Mavi et al. 2011; Faria et al. 2012; Rahayu et al. 2012; Kumar et al. 2014; Arshed and Agoo 2017).

Isozymes are known as the classical biochemical marker and can be used to determine genetic variation and

relationship among cultivars, varieties, natural populations and accessions in germplasm collections (Fernandez de Souza and Primo 2001; Padmanaban et al 2013; Rizk and Soliman 2014). They are not influenced by environmental factors, making identification possible in early stages of development. Isozymes also can be studied easily, without requiring prior knowledge of the genome, using a small quantity of material with an efficient and inexpensive technique (Torres 1990; Johnson et al. 2010; Kovacevic et al 2010; Kumar et al. 2013).

Information on genetic diversity and relationship among and between individuals, accessions, populations, varieties, and species of plant are then important in guiding the improvement of plants, thus facilitating breeding material selection (Qi et al. 2008; Dharmar and De Britto 2011; Tang et al. 2014).

The objective of this study was to evaluate morphological, anatomical and isozyme variability among taro accessions from the southeastern part of Central Java (Indonesia). The combination of morphological, anatomical and isozyme markers to evaluate genetic variation in taro accessions from Java is the first study that is reported from this region.

MATERIALS AND METHODS

Plant Materials

A total of 20 taro accessions in the form of living plant collection were collected from a wide range of sites during field surveys (Table 1, Figure 1). The collected plants were then transplanted to the polybag and kept in screen house in the Department of Biology, Universitas Sebelas Maret for 12 weeks. The plants were grown under controlled environmental conditions with temperature regime of 28°/20 °C day/night, a relative air humidity of 80%, at a photon flux density of about 8.200 lux and at 126 m asl altitude. The young leaves of each accession were then used for isozymes extraction.

Figure 1. Map of the collection areas for taro (C. *esculenta*) accessions studied in southeastern part of Central Java. The number (1 to 20) indicates location of each collected accession

Table 1. The geographic variation of taro (*C. esculenta*) accessions originated from southeastern part of Central Java with climatic data for each collection site

No. code	Accessions	Collection site	Altitude (m asl.)	Air temperature (°C)	Light intensity (lux)	Air humidity (%)	Soil humidity (%)
1.	BYL 1	Boyolali	548	27	17.200	84	20
2.	BYL 2	Boyolali	583	26	13.500	82	10
3.	BYL 3	Boyolali	664	33	33.500	65	20
4.	BYL 4	Boyolali	800	28	5.400	74	20
5.	KRY 1	Karanganyar	275	30	7.600	50	50
6.	KRY 2	Karanganyar	449	28	9.600	74	10
7.	KRY 3	Karanganyar	641	29	5.200	66	70
8.	KRY 4	Karanganyar	845	28	7.800	60	10
9.	KTN 1	Klaten	491	26	18.900	91	11
10.	KTN 2	Klaten	492	26	12.900	84	15
11.	KTN 3	Klaten	726	25	1.700	80	10
12.	KTN 4	Klaten	802	25	1.700	96	15
13.	SRG 1	Sragen	197	27	2.400	91	80
14.	SRG 2	Sragen	304	23	3.200	100	75
15.	SKH	Sukoharjo	126	33	24.200	49	80
16.	SKA	Surakarta	119	30	3.900	80	50
17.	WNG 1	Wonogiri	233	27	54.900	69	10
18.	WNG 2	Wonogiri	381	28	5.400	84	80
19.	WNG 3	Wonogiri	600	25	7.400	81	75
20.	WNG 4	Wonogiri	677	27	6.900	68	82

Morphological characters analysis

Morphological characters measurements were taken on vegetative structures such as roots, stems, leaves, and corms of the taro plant. Measurements included plant height, leaf length, leaf width, sheath length, sheath width, petiole length, petiole width, root length, root width, and corm length: width ratio.All measurements were averaged and averages for each plant were used in subsequent analyses.

Anatomical characters analysis

The anatomical characters were observed from both paradermal and transverse sections of the leaf. The preparation of microscope slide sections was carried out as described by Munir et al. (2011), Arzani et al. (2013) and Kumar et al. (2014). Light microscopic (Model: Olympus, magnification of 10 x for ocular and a 40 x for objective) observations were used to observe the specimens from both studies. The observed anatomical characters were stomatal index, stomatal density, stomatal length, stomatal width, abaxial epidermis thickness, adaxial epidermis thickness, mesophyll thickness, palisade thickness, and palisade ratio. All measurements were averaged and averages for each plant were used in analyses of variance.

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), Setyawan et al. (2014) and Suratman et al. (2016).

Isozyme Extraction

A total of 0.15 g young leaves of taro were ground in mortar using 600 µL of extracting solution and then transferred to a 1.5 mL microtube. Samples were centrifuged at 12000 rpm for 5 minutes, and supernatant was transferred to new microtube.

Electrophoresis

About 200 µL of supernatant was taken and 5µL of bromophenol blue (tracking dye) was added to each sample. About 10-24 µL of prepared samples (10-15 µL for peroxidase and 15-24 µL for esterase) was taken and loaded into each well of acrylamide 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 systems (esterase and peroxidase) as described by Suranto (2001), Setyawan et al. (2014) and Suratman et al. (2016) with some modifications. Gels were immersed in the staining solutions until bands appeared. After appearance of the bands, the gel was transferred to a fixative solution that contained 100 mL of 50% methanol, 20 mL of 10% acetic acid and 40 mL of distilled water. The gel was stored at 4^0C in refrigerator (Tiwari and Bakshi 2015).

Data analysis

Analysis of variance was performed for observed quantitative morphological and anatomical characters data in order to test the significance of variability among accessions. The data from zymograms were entered as a matrix of presence/absence of bands for each enzyme system. A similarity dendrogram among accessions was constructed on the basis of genetic similarity matrix based on morphological, anatomical and isozyme markers by applying an Unweighted Pair Group Method with Arithmetic Averages (UPGMA) cluster analysis using a computer programme, Numerical Taxonomy and Multivariate Analysis System (NTSYS) Version 2.00 (Rohlf 1998).

RESULTS AND DISCUSSION

Morphological Analysis

The analysis of variance for morphological characters revealed that there was significant difference ($p < 0.05$) among taro accessions for majority of the tested quantitative morphological traits except for variation of sheath width, petiole width, root width and corm length: width ratio, indicating the existence of substantial amount of variability for the characters among the accessions. Plant height, leaf length, leaf width, sheath length, petiole length and root length showed wide variation while sheath width, petiole width, root width and corm length: width ratio showed a narrower range of phenotypic variation (Table 2).

Plant height exhibited wide range of variation among accessions and ranged from 25 cm (WNG 1) to 79.5 cm (KTN 1), the average being 48.75 cm. The leaf length varied significantly among accessions and displayed a range from 9.8 cm (SKH) to 50 cm (BYL 1), the average being 24.62 cm. Leaf width differed significantly among tested accessions and ranged from 8 cm (SKA) to 40.3 cm (BYL 1), the average being 20.96 cm. Sheath length also exhibited wide differences among accessions and ranged from 10 cm (SKA) to 44 cm (KTN 2), the average being 28.02 cm. Petiole length showed wide differences among accessions and ranged from 30.2 cm (WNG 1) to 84 cm (KTN 2), the average being 57.99 cm. Root length displayed a range from 9.5 cm (SKA) to 46 cm (BYL 3), the average being 23.35 cm. Sheath width values exhibited narrow differences among accessions and ranged from 0.28 cm (SKA) to 2.48 cm (KTN 1), the average being 1.56 cm. Petiole width values showed narrow variation and ranged from 0.31 cm (SKA) to 2.58 cm (BYL 1), the average being 1.34 cm. Root width displayed narrow differences among accessions and varied from 0.06 cm (WNG 3, WNG 4) to 0.41 cm (BYL 2, BYL 3, SRG 1, SKH), the average being 0.22 cm. Corm length: width ratio also displayed narrow differences among accessions and varied from 1 to 4.

Table 2. Morphological character variation among taro accessions from southeastern part of Central Java (Indonesia)

Accessions	PlH	LfL	LfW	ShL	ShW	PtL	PtW	RtL	RtW	CoR
BYL 1	60.5b	50.0c	40.3c	30.8b	1.76b	63.4ab	2.58c	44.5b	0.35b	4ab
BYL 2	47.6ab	31.0b	30.0b	35.0b	1.31b	56.3ab	2.32c	41.4b	0.41b	2a
BYL 3	43.3a	12.0a	11.3a	18.9a	1.52b	45.3a	1.21ab	46.0b	0.41b	2a
BYL 4	43.3a	38.3b	28.4b	13.5a	2.08bc	59.5ab	1.24ab	28.9ab	0.29ab	1a
KRY 1	60.3b	19.5a	16.5a	31.0b	1.80b	69.5b	1.6b	20.0a	0.09a	1a
KRY 2	45.0ab	24.7ab	23.1ab	35.0b	1.80b	63.5ab	1.43b	26.5ab	0.09a	1a
KRY 3	59.0b	21.6a	21.0ab	21.9ab	1.90b	69.3b	1.43b	21.0a	0.15a	2a
KRY 4	52.0ab	32.5b	25.0ab	30.0b	1.60b	65.4b	1.75b	20.0a	0.10a	1a
KTN 1	79.5b	30.5b	24.5ab	32.0b	2.48c	82.3b	1.15ab	18.0a	0.16a	4ab
KTN 2	63.0b	32.9b	29.8b	44.0b	2.23c	84.0b	1.12ab	20.2a	0.16a	4ab
KTN 3	65.0b	36.0b	30.3b	38.6b	2.08bc	70.2b	1.17ab	18.0a	0.15a	2a
KTN 4	65.0b	29.5b	23.6ab	40.0b	1.44b	65.7b	1.24ab	15.0a	0.25ab	2a
SRG 1	62.0b	20.0a	15.6a	36.7b	1.60b	68.0b	1.27ab	27.0ab	0.41b	2a
SRG 2	70.0b	18.4a	15.0a	38.1b	2.17bc	73.6b	0.98a	28.9ab	0.29ab	1a
SKH	31.5a	9.8a	8.3a	23.8ab	0.44a	38.0a	1.14ab	18.9a	0.41b	2a
SKA	26.0a	10.0a	8.0a	10.0a	0.28a	30.5a	0.31a	9.5a	0.29ab	1a
WNG 1	25.0a	22.0ab	20.0ab	19.7a	0.74ab	30.2a	1.14ab	20.0a	0.10a	1a
WNG 2	37.5a	10.1a	9.3a	20.8a	1.60b	45.4a	1.36ab	15.2a	0.10a	2a
WNG 3	30.5a	31.0b	29.4b	19.7a	0.74ab	34.3a	1.33ab	18.0a	0.06a	1a
WNG 4	37.0a	12.5a	9.8a	20.8a	1.60b	45.3a	1.11ab	10.0a	0.06a	1a
Average	48.75	24.62	20.96	28.02	1.56	57.99	1.34	23.35	0.22	2.50

Note : * PlH = plant height (cm); LfL = leaf length (cm); LfW = leaf width (cm); ShL = sheath length (cm); ShW = sheath width (cm); PtL = petiole length (cm); PtW = petiole width (cm); RtL = root length (cm); RtW = root width (cm); CoR = corm length: width ratio.
** 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 taro accessions from southeastern part of Central Java (Indonesia)

Accessions	StI	StD	StL	StW	AbT	AdT	MeT	PaT	PaR
BYL 1	15.72ab	7.44a	32.48b	21.22ab	33.32b	36.38b	438.46c	115.46b	0.50a
BYL 2	14.93ab	11.00a	29.06ab	22.63ab	25.84a	24.40a	335.89b	79.07a	0.44a
BYL 3	16.28ab	11.67a	27.97a	20.91a	29.26ab	22.66a	323.09b	63.60a	0.57a
BYL 4	19.41b	12.44ab	25.21a	19.62a	24.77a	28.16ab	338.50b	96.72ab	0.50a
KRY 1	11.91a	8.67a	27.97a	19.41a	23.92a	30.76ab	335.86b	87.13ab	0.44a
KRY 2	14.23a	10.00a	25.63a	19.20a	25.20a	27.46a	397.43c	100.43b	0.50a
KRY 3	17.52ab	15.00ab	24.79a	19.83a	24.57a	28.12ab	412.83c	85.40ab	0.44a
KRY 4	14.26a	10.00a	25.21a	19.41a	26.91a	20.48a	189.76a	51.19a	0.50a
KTN 1	21.18b	18.44b	23.20a	18.99a	23.27a	24.40a	335.86b	92.90ab	0.44a
KTN 2	16.49ab	11.00a	28.40ab	20.90a	29.04ab	25.71a	284.60ab	82.37a	0.40a
KTN 3	15.76ab	8.67a	26.70a	26.28b	29.70ab	23.77a	233.36a	69.94a	0.50a
KTN 4	15.76ab	11.67a	27.76a	19.41a	25.82a	20.70a	269.20ab	82.13a	0.50a
SRG 1	17.11ab	8.89a	28.83ab	20.91a	27.74ab	25.27a	292.30ab	84.30ab	0.44a
SRG 2	9.54a	10.33a	25.00a	19.62a	29.90ab	31.59b	271.86ab	69.92a	0.44a
SKH	10.42a	19.11b	25.63a	19.62a	22.62a	32.91b	271.76ab	67.54a	0.40a
SKA	15.78ab	13.67ab	25.42a	18.36a	26.49a	32.70b	266.73ab	73.19a	0.44a
WNG 1	15.37ab	11.00a	27.96a	22.84ab	28.19ab	24.20a	289.73ab	99.32ab	0.50a
WNG 2	14.42ab	9.56a	27.32a	20.69a	25.61a	22.01a	172.70a	79.08a	0.57a
WNG 3	17.67ab	7.67a	33.54b	23.51ab	33.12b	20.24a	205.13a	61.63a	0.57a
WNG 4	20.45b	7.44a	25.00a	19.62a	27.32ab	21.58a	220.56a	74.93a	0.44a
Average	18.09	11.18	27.15	20.65	27.13	26.18	294.28	80.81	0.48

Note : * StI=stomatal index; StD = stomatal density (pore/mm^2); 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.
** Values followed by the different lower-case letter in the same column are significantly different (Duncan multiple range test, $p < 0.05$)

Anatomical analysis

The analysis of variance for anatomical characters revealed that there was significant variation ($p < 0.05$) for all the tested characters among taro accessions, except for the difference of palisade ratio. However, accessions variation for palisade ratio was non-significant (Table 3).

Comparing the stomatal index, stomatal densities, stomatal length and stomatal width, there was significant

variability among all tested accessions. The lowest stomatal index value was distributed in SRG 2 accession (9.54) whereas the highest one was in the KTN 1 accession (21.18), the average being 18.09. BYL 1 accession displayed the lowest value of stomatal density (7.44 pores/mm^2) whereas the highest one could be found in SKH accession (19.11 pores/mm^2), the average being 11.18 pores/mm^2. Stomatal length also exhibited differences among accessions and ranged from 23.20 µm (KTN 1) to 33.54 µm (WNG 3), the average being 27.15 µm. Stomatal width differed significantly among tested accessions and was highest in the accession SKA (18.36 µm) and lowest in the accession KTN 3 (26.28 µm), the average being 20.65 µm.

Abaxial epidermis thickness exhibited variation and ranged from 22.62 µm (SKH) to 33.32 µm (BYL 1), the average being 27.13 µm. The adaxial epidermis thickness varied significantly among accessions and displayed a range from 20.24 µm (WNG 3) to 36.38 µm (BYL 1), the average being 26.18 µm. Mesophyll thickness differed significantly among tested accessions and was highest in the accession BYL 1 (438.46 µm) and lowest in the accession WNG 2 (172.70 µm), with the average being 244.48 µm. Palisade thickness also exhibited wide differences among accessions and ranged from 51.19 µm (KRY 4) to 115.46 µm (BYL 1), the average being 80.81 µm. Palisade ratio displayed narrow differences among accessions and varied from 0.40 to 0.57, the average being 0.48.

Isozyme Analysis

Polymorphism was observed in taro accessions from Central Java using isozymes of esterase and peroxidase. Esterase and peroxidase have been widely utilized to assess the genetic similarity and to reveal the variation of organisms at the various taxonomic levels. The two enzymatic systems showed a total of 20 banding pattern, distributed in the whole set of samples as esterase with 12 banding pattern and peroxidase with 8 banding pattern.

A total of 12 pattern zymograms (banding pattern A-L) of esterase at different Rf values varying from 0.08 to 0.95 were observed (Figure 2). Banding pattern A was distributed in two accessions (KTN 1, KTN 2) and consisted of two bands which were located at Rf 0.1 and Rf 0.35. Banding pattern B was distributed in three accessions (KTN 3, KTN 4, KRY 1) and consisted of two bands which were located at Rf 0.1 and Rf 0.3. Banding pattern C was distributed in two accessions (SRG 1, SRG 2) and consisted of two bands which were located at Rf 0.1 and Rf 0.25. Banding pattern D was distributed in three accessions (KRY 2, KRY 3, KRY 4) and consisted of two bands which were located at Rf 0.1 and Rf 0.27 from anodal zone. Banding pattern E was only distributed in WNG 4 accession and consisted of three bands which were located at Rf 0.08; Rf 0.21 and Rf 0.95. Banding pattern F was distributed only in WNG 3 accession and consisted of five bands which were located at Rf 0.08; Rf 0.21; Rf 0.29; Rf 0.45 and Rf 0.95. Banding pattern G was distributed in two accessions (WNG 2, BYL 1) and consisted of five bands which were located at Rf 0.08; Rf 0.21; Rf 0.29; Rf 0.30 and Rf 0.95. Banding pattern H was distributed only in WNG 1 accession and consisted of four bands which were located at Rf 0.08; Rf 0.21; Rf 0.29 and Rf 0.30. Banding pattern I was distributed only in BYL 2 accession and consisted of four bands which were located at Rf 0.08; Rf 0.21; Rf 0.29 and Rf 0.95.Banding pattern J was only distributed in BYL 3 accession and consisted of four bands which were located at Rf 0.08; Rf 0.21; Rf 0.29; and Rf 0.45. Banding pattern K was distributed only in BYL 4 accession and consisted of six bands which were located at Rf 0.08; Rf 0.21; Rf 0.29; Rf 0.35; Rf 0.45 and Rf 0.95. Banding pattern L was distributed in two accessions (SKH, SKA) and consisted of three bands which were located at Rf 0.08; Rf 0.21; and Rf 0.25.

Peroxidase analysis showed eight patterns zymogram (banding pattern A-H) distributed in different Rf value varying from 0.08 to 0.73. Eight isozymic banding pattern of peroxidase also was distributed separately in tested taro accessions (Figure 3). Banding pattern A was distributed only in KTN 1 accession and consisted of two bands located at Rf 0.08 and Rf 0.3. Banding pattern B was distributed in two accessions (KTN 2, KTN 4) and consisted of two bands located at Rf 0.3 and Rf 0.4 from anodal zone. Banding pattern C was distributed in seven accessions (KTN 3, SRG 1, SRG 2, KRY 1, KRY 2, KRY 3, KRY 4) and only consisted of one band located in Rf 0.3. Banding pattern D was distributed in three accessions (WNG 2, WNG 3, WNG 4) and consisted of two bands located at Rf 0.18 and Rf 0.73. Banding pattern E was distributed only in WNG 1 accession and consisted of four bands located at Rf 0.18; Rf 0.4; Rf 0.6 and Rf 0.73. Banding pattern F was distributed in two accessions (BYL 1, BYL 2) and consisted of five bands located at Rf 0.18; Rf 0.4; Rf 0.56; Rf 0.6; Rf 0.73. Banding pattern G was distributed in two accessions (BYL 3, BYL 4) and consisted of three bands located at Rf 0.18; Rf 0.4 and Rf 0.73. Banding pattern H was distributed in two accessions (SKH, SKA) and consisted of two bands located at Rf 0.18 and Rf 0.4.

Similarity among taro accessions

Based on the dendrogram at a level of 62 % similarity, it showed distinct separation of 20 taro accessions from Central Java into two major clusters (Figure 4). Cluster I comprised most of tested accessions which originated from all Boyolali accessions (BYL 1, BYL 2, BYL 3 and BYL 4), all Wonogiri accessions (WNG 1, WNG 2, WNG 3 and WNG 4), Sukoharjo (SKH accession) and Surakarta (SKA accession). □

The closest relationship showed between SKH and SKA accessions that had 96 % similarity coefficient. The ten accessions that remained from all Klaten accessions (KTN 1, KTN 2, KTN 3, KTN 4), all Karanganyar accessions (KRY 1, KRY 2, KRY 3, KRY 4), and all Sragen accessions (SRG 1, SRG 2) were then clustered separately apart from the other as Cluster II with similarity coefficient of 73.52 % and considered to be genetically unique.

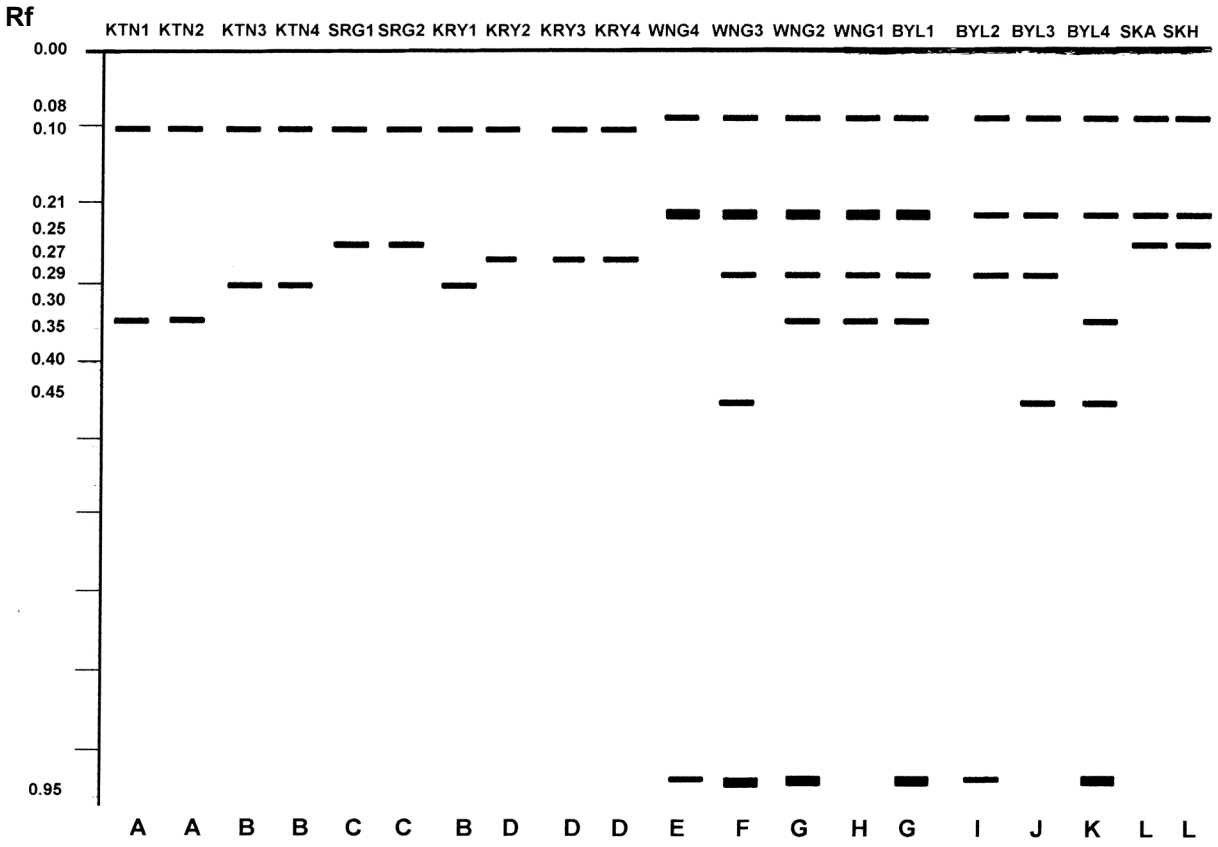

Figure 2. Esterase isozymic banding pattern of 20 taro accessions from southeastern part of Central Java (Indonesia)

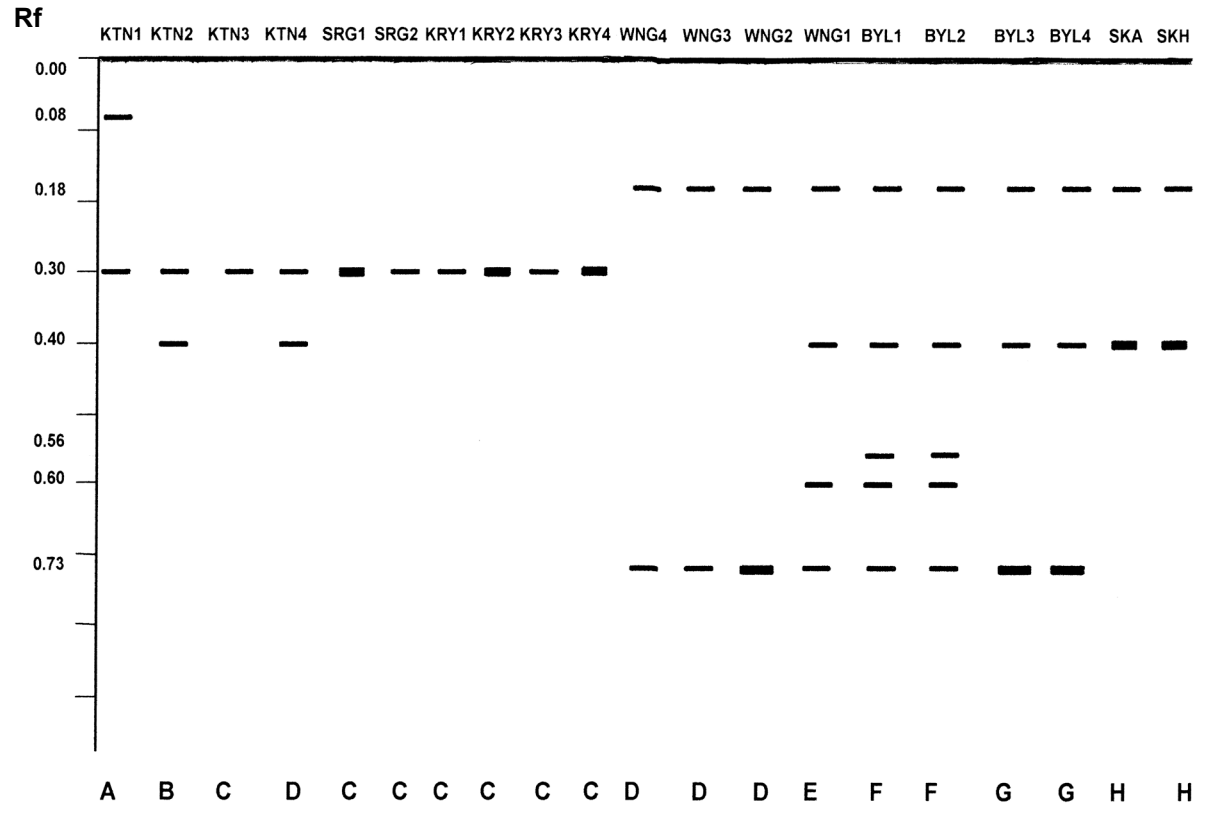

Figure 3. Peroxidase isozymic banding pattern of 20 taro accessions from southeastern part of Central Java (Indonesia)

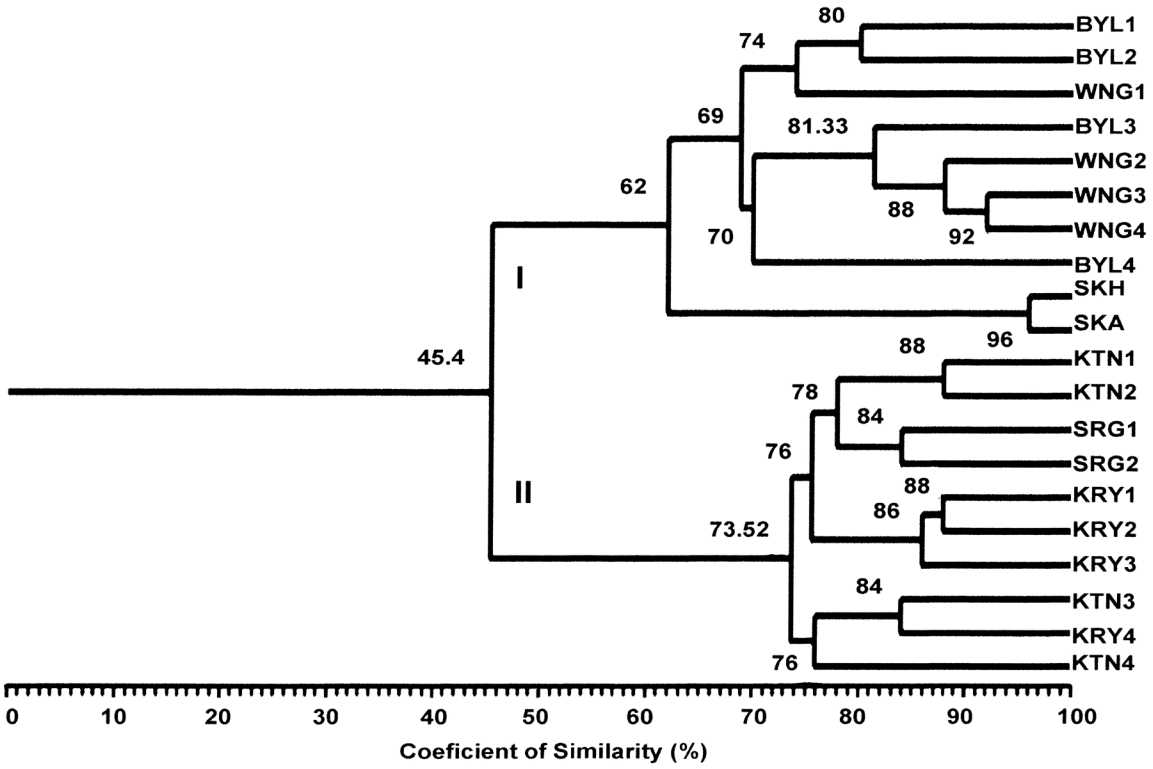

Figure 4. Relationship dendrogram among 20 taro accessions from southeastern part of Central Java (Indonesia) using morphological, anatomical and isozyme markers

Discussion

Analysis of variance for morphological characters used in the study indicates high genetic diversity among taro accessions. Thus, such methods have been successfully used to measure phenotypic diversity in germplasm collection. This indicates the existence of large diversity in taro, especially for quantitative characters. Indeed, these taro accessions collected from a wide range of sites during field surveys, when cultivated under the same microclimate, conserved the characteristics acquired previously. This indication then provided vital information on the morphological diversity (Mbouobda et al. 2007).

In general, this study revealed that the taro accessions showed variability in the majority of tested morphological characters. Morphological characters such as plant height, leaf length, leaf width, sheath length, petiole length, and root length also had high values. This implies that these characters could be employed for distinguishing variability in the accessions. Therefore, this indication showed that there is enough scope for selection of desirable characters, where variability exists (Beyene et al. 2013; Suratman et al. 2016).

The existence of 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; Sabaghnia et al. 2014).

Genetic variability as reflected from morphological characters is the raw material of crop breeding on which selection acts up to evolve into a superior genotype. Thus, the higher amount of variations expressed for a character in the breeding material, then the scope for its improvement through selection is greater (Osawaru et al. 2013).

Analysis of variance for anatomical characteristics revealed that there was significant variability for all the characters, except for variation of palisade ratio. The stomatal index, stomatal densities, stomatal length, and stomatal width varied significantly among all tested accessions. Stomata characteristics such as frequency and dimensions can be affected by type of species and environmental factors. Thus, the higher stomatal density or stomatal index can also be used as an indicator for higher transpiration rate, highest metabolism and absorption of mineral and water (Dong and Zhang 2000; Munir et al. 2011). Although stomatal features can be affected by complex environmental factors, stomatal differentiation and development are certainly determined by genetic factors (He et al. 1998; Hetherington and Woodward 2003). It has been reported that some stomatal features can be used as a selection marker for breeding program (Yang et al. 2004).

The leaf tissue layer thickness such as abaxial epidermis thickness, adaxial epidermis thickness, mesophyll thickness, and palisade thickness also showed variability among all examined accessions. The difference in leaf tissue layer thickness might be attributed to the

responses toward environmental factors (Brouillette et al. 2006; Donovan et al. 2007; Noman et al. 2014). Thus, these leaf anatomical characteristics can be used as a selection marker for genetic improvement of plants, especially to improve their adaptability to adopt varied environmental conditions.

The majority of the tested anatomical characters showed highly significant variation among all tested accessions although some anatomical characters might be influenced by environmental factors. This information indicated that there is enough scope for selection of accessions on the basis of these characteristics for genetic improvement (Kumar et al. 2014, Suratman et al. 2016).

Isozyme profiling of two enzyme systems viz. esterases and peroxidases enzyme were exploited to find out the variability among taro accessions. It was found that esterase and peroxidase isozymes are effective in differentiation among these accessions. Esterase showed most isozymic banding pattern variations (with 12 isozymic banding pattern) compared to peroxidase (with 8 isozymic banding pattern). Then, esterase exhibited significant variation among accessions in terms of number of bands and their thickness. Therefore, esterase is a useful diagnostic tool in this study for assessment of genetic variation in view of the extensive polymorphism for this enzyme (Tiwari and Bakshi 2015). Esterase is considered to be one of the most suitable enzyme systems for differentiation of group of plants (Rakshit et al. 2011; Sumathi and Balamurugan 2014).

Polymorphism is essential in use of isozymes as genetic marker. A large number of polymorphic zones reflect the validity of the isozyme data to study the genetic diversity (Kumar et al. 2013). 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.

The results from this present study concluded that the specific banding pattern observed in esterases and peroxidases can be used for accessions differentiation. Then, difference in the isozymes profile can reveal genetic diversity among accessions. However, an adequate level of genetic diversity is very essential for effective selection in a breeding programme.

The UPGMAdendrogram in this study showed that each group was comprised of geographically related accessions. However, the grouping did not always indicate the geographical origins similarity, but possibly showed the genetic similarity (Tikader and Kamble 2008). The genetic variability in taro accessions may be partly explained as a result of abiotic and biotic factors. Geographical, climatic or reproductive variables explain the partitioning of the diversity observed which may aid in improving the strategies for maximizing the efficiency of germplasm collection and preservation for breeding of taro accessions (Suratman et al. 2013).

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. Selection of better accessions can be made for species improvement based on its genetic similarity percentage. Two genetically similar accessions or more and possessing suitable characters for breeding activities can be chosen for this purpose (Prabha et al. 2010; Lombardi et al. 2014; Suratman et al. 2015).

Thus, genetic characterization based on morphological, anatomical and isozyme markers obtained in this study could be valuable for understanding of genetic variability among the examined taro accessions. It will also provide an important input into determining resourceful management strategies and help breeders in the taro improvement programme (Setyawan et al. 2014).

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