BIODIVERSITAS
Volume 22, Number 4, April 2021
Pages: 2104-2116

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d220459

Morphological and anatomical characters variation of *Indigofera* accessions from Java, Indonesia

MUZZAZINAH[1,♥], SURATMAN[2,♥♥], NURMIYATI[1], SRI RETNO DWI ARIANI[3]

[1]Department of Biology Education, Faculty of Teaching Training and Education, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia. Tel.: +62-271-669124, ♥email: yayin_pbio@fkip.uns.ac.id

[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia. Tel./fax.: +62-271-663375, ♥♥email: suratman@staff.uns.ac.id

[3]Department of Chemistry Education, Faculty of Teaching Training and Education, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia

Manuscript received: 4 August 2020. Revision accepted: 29 March 2021.

Abstract. *Muzzazinah, Suratman, Nurmiyati, Ariani SRD. 2021. Morphological and anatomical characters variation of* Indigofera *accessions from Java, Indonesia. Biodiversitas 22: 2104-2116.* The objective of this research was to evaluate morphological and anatomical character variation of *Indigofera* accessions from Java, Indonesia. A total of 15 accessions from three species of *Indigofera* were collected from centers of Batik manufacturing in Java located in the following locations: West Java (Pangandaran), Yogyakarta (Bantul, Kulonprogo), Central Java (Sragen, Sukoharjo, Karanganyar) and East Java (Tuban). There were 14 tested morphological characters based on measurement of vegetative and generative organs. Anatomical characters were observed from paradermal sections and there were 12 tested characters. Analysis of variance was performed for observed morphological and anatomical characters data in order to test the significance of variation among accessions using SPSS software. The characters' mean values then were used to perform principal component analysis and cluster analysis using PAST software. A cluster analysis was conducted by applying an Unweighted Pair Group Method with Arithmetic Averages (UPGMA) in order to group the accessions based on morphological and anatomical character similarity. The analysis of variance for the evaluated morphological and anatomical characters revealed highly significant differences among accessions for all of the tested characters suggesting that there was a high degree of phenotypic diversity among *Indigofera* accessions. Principal component analysis indicated that the first 2 components accounted for 90.72 % and 88.96 % of the total variation for morphological and anatomical data sets, respectively. Cluster analysis using morphological characters data revealed three main clusters. Four main clusters were then highlighted in cluster analysis of the anatomical character data. The grouping of the accession into clusters showed that there was no correlation between ecological habitat origin and the character diversity expressed among *Indigofera* accession. However, accessions that came from the same species had the tendency to cluster together.

Keywords: Anatomy, *Indigofera* accessions, Java, morphology, variation

INTRODUCTION

Indigofera is one of the largest genera in Fabaceae, including about 700 species (Airy Shaw 1985; Lewis et al. 2005; Mabberley 2008). The genus is widespread in the tropical and subtropical regions of the Old and New World (Mabberley 2008). The center of genetic diversity of this genus is in Africa and Madagascar, with up to 520 species (Gao et al. 2010). Other centers of diversity are found in India, South East Asia, and the New World (Schrire 1998; Schrire 2005; Schrire et al. 2009).

Several species in this genus have economic uses ranging from fodder, human food, ornamental plants, cover crops and green manures (Schrire 2005; Tamilselvi et al. 2011). *Indigofera* species is also known as a medicinal plant. For example, *Indigofera tinctoria* is used in constipation, liver disease, heart palpitation and gout treatment (Motamarri et al. 2012), anti-hyperglycaemic (Bangar and Saralaya 2011), antimicrobial activity on *Staphylococcus aureus, Bacillus pumilus* and *Streptococcus pyrogens* (Renukadevi and Sultana 2011), anti-inflammatory (Tyagi et al. 2010), antihepatotoxic (Felicia and Muthulingam 2012), and anthelminthic (Balamurugan and Selvarajan 2009). *Indigofera* is one of the oldest coloring agents known to man and is among the most widely used natural dye in the world (Howard 1988; Burkill 1995) such as *I. suffruticosa* and *I. tinctoria* which have been widely used as natural dye in the traditional textile manufacturing (for example Batik in Indonesia, especially in Java) (Muzzazinah et al. 2016).

In order to ascertain the level of variation among and within species, a variety of markers such as morphology, anatomy, biochemistry, and molecular are used (Suratman et al. 2016; Pitoyo et al. 2018; Restykania et al. 2019). Although morphological characters have been widely used in germplasm characterization, but they have a number of limitations, including low polymorphism, low heritability, late expression, and vulnerability to environmental influences (Smith and Smith 1992). However, besides their limitation, morphological character is still routinely used for preliminary evaluation of plant variations because it is fast, simple, inexpensive (Beyene et al. 2005; Jingura and Kamusoko 2015) and can be used as a general approach for assessing genetic diversity among morphologically distinguishable accessions (Said and Ehsan 2010; Suratman et al. 2016). In the characterization and identification of

plant groups, the use of anatomical features is also valuable (Nwachukwu and Mbagwu 2006; Munir et al. 2011; Rahayu et al. 2012; Chikmawati 2013), especially in distinguishing two or more taxa that have high morphological character similarities by showing the pattern of relationships of superficial convergence in morphological studies (Nwachukwu et al. 2016).

The morphological and anatomical character variation among *Indigofera* accessions from Java has not been investigated in detail. Information on genetic diversity and relationship among accessions of this plant will provide important input into determining resourceful management strategies and guiding the improvement of plants through plant breeding programs, especially in facilitating breeding material selection (Restykania et al. 2019). Therefore, this study aimed to evaluate morphological and anatomical character variation of *Indigofera* accessions from Java (Indonesia).

MATERIALS AND METHODS

Plant material

A total of 15 accessions from three species of *Indigofera* used in this study were collected from centers of Batik manufacturing in Java located in the following locations: West Java (Pangandaran), Yogyakarta (Bantul, Kulonprogo), Central Java (Sragen, Sukoharjo, Karanganyar) and East Java (Tuban) (Table 1, Figure 1).

Field experiment

The seeds of 15 *Indigofera* accessions were carefully removed from the pods without scarification and selected after floatation test (Osawaru et al. 2013). Seeds were then sown in trays in a nursery (Hassen et al. 2006). After establishment, seedlings were then transplanted to experimental garden in Magelang District, Central Java (80 m asl). The study was laid out as a Randomised Complete Block Design (RCBD) with five replications. The plot size was 4.5 m^2 (1.5 m × 3 m) and the spacing was 40 cm between plants. Five plants for each accession then used in this study.

Table 1. The location of *Indigofera* accessions collected from centers of Batik manufacturing in Java (Indonesia) with climatic data or each locality

No.	Species	Acc. code	Location	Latitude Longitude	Alt. (m asl.)	Temp. (ºC)	Hum. (%)	Light intensity (lux)
1	*I. tinctoria*	A1PNG	Pangandaran, Pangandaran, West Java	S 07^{0}38'10"; E 108^{0}39'11"	24	30	81	22,500
2	*I. tinctoria*	A1KPG	Trisik, Galur, Kulonprogo, Yogyakarta	S 07^{0}57'18"; E 110^{0}11'06"	28	31	74	39,400
3	*I. suffruticosa*	A2KPG	Trisik, Galur, Kulonprogo, Yogyakarta	S 07^{0}57'18"; E 110^{0}11'06"	28	31	74	39,400
4	*I. tinctoria*	A1BTL1	Pacar, Sewon, Bantul, Yogyakarta	S 07^{0}52'36"; E 110^{0}22'49"	81	28.5	84	8,090
5	*I. suffruticosa*	A2BTL1	Pacar, Sewon, Bantul, Yogyakarta	S 07^{0}52'36"; E 110^{0}22'49"	81	28.5	84	8,090
6	*I. galegoides*	A3BTL1	Pacar, Sewon, Bantul, Yogyakarta	S 07^{0}52'36"; E 110^{0}22'49"	81	28.5	84	8,090
7	*I. tinctoria*	A1BTL2	Giriloyo, Imogiri, Bantul, Yogyakarta	S 07^{0}54'56"; E 110^{0}23'54"	71	30	74	9,580
8	*I. suffruticosa*	A2BTL2	Giriloyo, Imogiri, Bantul, Yogyakarta	S 07^{0}54'56"; E 110^{0}23'54"	71	30	74	9,580
9	*I. tinctoria*	A1SKH	Telukan, Grogol, Sukoharjo, Central Java	S 07^{0}37'23"; E 110^{0}49'18"	100	35	75	7,350
10	*I. tinctoria*	A1KRA	Ngringo, Jaten, Karanganyar, Central Java	S 07^{0}33'30"; E 110^{0}52'36"	179	27.5	80.5	3,500
11	*I. suffruticosa*	A2KRA	Ngringo, Jaten, Karanganyar, Central Java	S 07^{0}33'30"; E 110^{0}52'36"	179	27.5	80.5	3,500
12	*I. tinctoria*	A1SRA	Pilang, Masaran, Sragen, Central Java	S 07^{0}27'06"; E 110^{0}54'22"	125	39	65	16,350
13	*I. tinctoria*	A1TBN1	Margorejo, Kerek, Tuban, East Java	S 06°53'06"; E 111°52'38"	27	28	99	2,000
14	*I. tinctoria*	A1TBN2	Jarorejo, Kerek, Tuban, East Java	S 06°53'12"; E 111°52'40"	27	27	99.5	12,900
15	*I. tinctoria*	A1TBN3	Kedungrejo, Kerek, Tuban, East Java	S 06°53'43"; E 111°52'35"	27	27.5	99.5	13,900

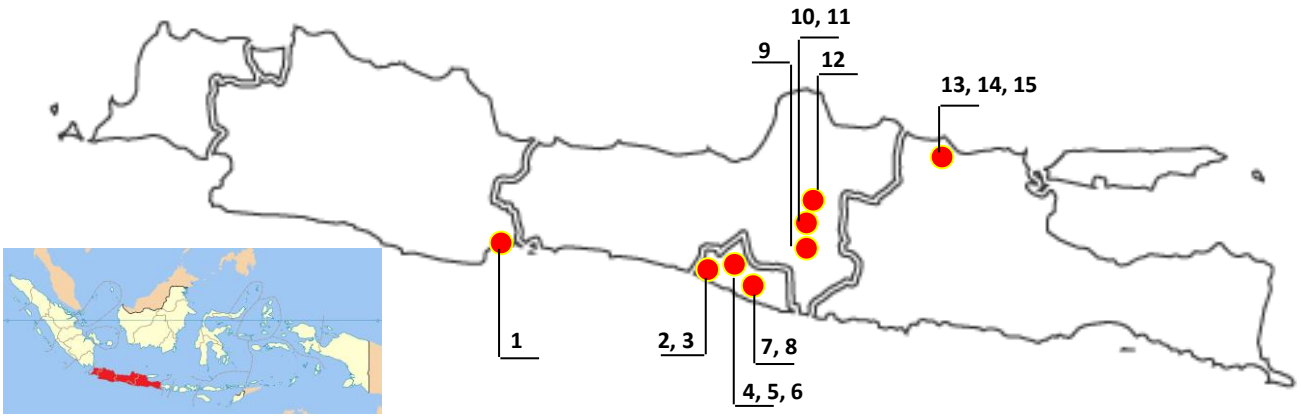

Figure 1. Map of the collection areas for *Indigofera* accessions from centers of Batik manufacturing in Java (Indonesia). Note: The number (1 to 15) indicated location of each collected accession as presented in Table 1.

Data collection

Data was gathered from the evaluated morphological and anatomical characters of *Indigofera* accession plants. Morphological characters were characterized based on measurements of vegetative and generative structures of the *Indigofera* plant such as stems, leaves, flowers, fruits and seeds. There were 14 measured morphological characters included plant height, canopy diameter, stem diameter, number of branches, length of leaf rachis, leaflets number per leaf rachis, leaflet length, leaflet width, inflorescence length, number of flowers in an inflorescence, number of fruits in a raceme, fruits length, fruits width and number of seeds in a pod.

Leaf anatomy characters were observed from paradermal sections. The preparation of microscope slides of the leaf paradermal section was carried out according to Munir et al. (2011), Arzani et al. (2013), Chikmawati (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 (Pitoyo et al. 2018). There were 12 measured anatomical characters included stomatal density, stomatal length, stomatal width, trichome density, trichome length, and trichome width on both surfaces (abaxial and adaxial).

Data analysis

Analysis of variance was performed for observed quantitative morphological and anatomical characters data in order to test the significance of variation among accessions. Analysis of variance was performed by Duncan's multiple range test ($p < 0.05$) using SPSS 20.0 version software for all measured characters. The characters' mean values then were used to perform principal component analysis and cluster analysis using PAST 4.03 version software. A cluster analysis was conducted by applying an Unweighted Pair Group Method with Arithmetic Averages (UPGMA) in order to group the accessions based on morphological and anatomical character similarity.

RESULTS AND DISCUSSION

Analysis of morphological characters

The analysis of variance for the evaluated morphological characters is presented in Table 2 and Table 3. The results show that the accessions are significantly different at 5% probability with respect to the 14 morphological characters studied. The analysis of variance revealed highly significant differences among accessions for all of the tested characters. Canopy diameter (51.4-120.4 cm) and plant height (46.4-114.8 cm) showed wide variation while inflorescence length (20.2-76.4 mm), length of leaf rachis (23.6-48.6 cm), fruits length (11.8-65.8 mm), number of flowers in an inflorescence (19.4-30.4), stem diameter (10.8-18.6 mm), number of fruits in a raceme (9.8-20.2), leaflet length (11.4-20.8 mm), number of branch (8.4-17.8), leaflet width (6.2-11.6 mm), leaflets number per leaf rachis (7.4-12.6), number of seeds in a pod (4-12.4) and fruits width (0.88-2.36 mm) showed a narrower range of phenotypic variation. The fruit length had the highest coefficient of variance (45.2%) then followed by plant height (38.29%), inflorescence length (32.5 %), number of seeds in a pod (31.14%), length of leaf rachis (25.88 %), canopy diameter (25.88 %), leaflet length (24.45 %), stem diameter (22.46%), number of fruits in a raceme (21.59%), number of branch (21.38%), leaflet width (21.15%), fruits width (19.99%), leaflets number per leaf rachis (16.87%) and number of flowers in an inflorescence (11.83%).

Table 2. Estimates of variability of morphological characters among *Indigofera* accessions in Java

Morphological characters	Mean	Min	Max	Standard deviation	Coefficient of variance (%)	P*(F-test)
Plant height (cm)	71.79	46.4	114.8	27.48	38.29	< 0.01
Canopy diameter (cm)	75.17	51.4	120.4	19.45	25.88	< 0.01
Stem diameter (mm)	13.67	10.8	18.6	3.07	22.46	< 0.01
Number of branches	11.97	8.4	17.8	2.56	21.38	< 0.01
Length of leaf rachis (cm)	30.08	23.6	48.6	7.79	25.88	< 0.01
Leaflets number per leaf rachis	9.4	7.4	12.6	1.58	16.87	< 0.01
Leaflet length (mm)	14.94	11.4	20.8	3.65	24.45	< 0.01
Leaflet width (mm)	9.4	6.2	11.6	1.99	21.15	< 0.01
Inflorescence length (mm)	42.87	20.2	76.4	13.93	32.5	< 0.01
Number of flowers in an inflorescence	27.36	19.4	30.4	3.23	11.83	< 0.01
Number of fruits in a raceme	13.10	9.8	20.2	2.83	21.59	< 0.01
Fruits length (mm)	27.59	11.8	65.8	12.47	45.2	< 0.01
Fruits width (mm)	1.68	0.88	2.36	0.33	19.99	< 0.01
Number of seed in a pod	7.89	4	12.4	2.45	31.14	< 0.01

Note: *p < 0.01= highly significant

Table 3. Morphological characters variation among *Indigofera* accessions in Java

Accessions	PlH	CaD	StD	NBr	LrL	NLf	LfL	LfW	IfL	NFl	NFr	FrL	FrW	NuS
A1PNG	55.2±0.44e	71.4±1.51g	11.6±0.89abcd	8.4±1.14a	25.6±1.67ab	9.8±1.78bcd	13.6±1.14d	10.4±0.89cdef	44.6±3.2cd	30.4±1.51d	11.6±1.51ab	28.2±1.3c	1.76±0.23de	8.6±2.6cd
A1KPG	46.8±2.59ab	56.2±0.83b	10.8±1.30a	9.6±1.14ab	23.6±0.89a	9.4±1.67abcd	13.2±0.83d	10.8±0.83cdefg	41.2±1.3b	28.4±1.51cd	10.2±1.3a	26.4±0.89c	1.70±0.15de	9.0±3.16cd
A2KPG	108.6±2.07i	91.2±4.81h	18.4±0.54f	14.2±0.83e	37.4±2.07c	10.6±0.89de	20.2±1.09f	6.2±0.83a	21.8±1.92a	24.6±1.14b	14.2±1.3bc	21.6±1.51b	1.44±0.08bc	4.0±1a
A1BTL1	53.2±0.44e	64.2±1.92de	11.6±1.14abcd	10.8±1.78bc	24.4±1.51ab	8.2±2.28abc	13.4±0.54d	11.0±0.7defg	45.8±3.56cde	30.2±1.92cd	11.6±1.14ab	28.8±0.83cd	1.88±0.13de	8.0±3.6cd
A2BTL1	114.8±1.09j	103.2±0.83i	18.2±0.44f	14.8±1.78e	37.6±1.67c	10.2±1.09cde	20.4±1.14f	6.8±0.83a	20.6±1.81a	24.8±0.83b	17.8±1.09e	14.4±1.51a	1.26±0.11b	4.4±0.89ab
A3BTL1	99.4±2.07h	120.4±1.14j	15.2±0.44e	17.8±1.3f	48.6±1.14d	12.2±1.09ef	16.4±1.14e	8.2±0.44b	76.4±2.3f	19.4±1.14a	20.2±4.14f	65.8±5.26f	2.36±0.15f	12.4±0.54e
A1BTL2	49.2±4.2abc	61.8±0.44d	12.4±0.54cd	9.8±1.09ab	26.2±1.78b	9.4±0.89abcd	11.4±0.54a	11.2±0.83efg	43.8±1.92bc	28.2±0.83c	9.8±1.3a	27.2±1.3c	1.88±0.13de	7.0±2.44bc
A2BTL2	109.6±2.07i	89.6±1.67h	18.0±0.7f	13.8±1.92de	38.6±1.14c	10.2±1.09cde	20.6±1.14f	6.4±1.14a	20.2±1.92a	24.4±1.14b	14.2±3.19bc	13.2±1.48a	1.42±0.29bc	4.6±0.54ab
A1SKH	49.6±2.4bcd	58.8±1.92c	11.0±0.7a	12.2±1.09cd	24.6±1.14ab	7.4±1.67a	12.4±0.54abcd	10.2±0.44cde	45.4±2.3cde	30.0±1.58cd	11.4±1.14ab	29.2±1.3cd	1.82±0.08de	8.8±2.16cd
A1KRA	69.6±4.03g	66.2±1.92e	12.2±0.44bcd	11.8±1.3c	24.2±1.3ab	7.8±1.09ab	12.6±0.54bcd	10.6±0.89cdefg	45.8±2.58cde	29.0±1.58cd	11.8±0.83ab	28.4±0.89c	1.80±0.14de	9.4±0.54cd
A2KRA	109.2±1.92i	90.5±0.5h	18.6±0.54f	14.2±1.09e	38.2±0.83c	12.6±1.67f	20.8±0.83f	6.6±1.14a	47.2±3.27de	23.6±1.14b	15.2±3.03d	11.8±1.64a	0.88±0.13a	4.8±1.3ab
A1SRA	46.4±1.14a	51.4±1.51a	11.2±0.83ab	9.8±1.09ab	24.8±1.48ab	8.6±1.67abcd	11.6±0.54ab	10.0±0.7cd	47.8±0.83de	29.8±1.92cd	11.4±1.14ab	31.8±4.54de	1.74±0.15de	10.6±1.51de
A1TBN1	60.4±0.54f	66.6±2.07e	12.6±1.14d	9.4±0.89ab	26.2±0.83b	7.4±1.67a	12.8±0.44cd	11.4±0.54fg	46.2±2.28cde	28.6±1.94cd	12.2±0.83ab	32.6±2.3e	1.76±0.34de	8.6±2.7cd
A1TBN2	52.2±2.38cde	69.2±1.78fg	11.8±0.44abcd	11.2±1.09bc	25.8±1.48b	9.0±1.41abcd	13.0±1.0d	11.6±0.54g	48.4±1.67e	30.2±1.3cd	12.4±1.14ab	28.2±2.16c	1.64±0.11cd	8.8±1.04cd
A1TBN3	52.6±2.70de	66.8±1.78ef	11.4±1.14abc	11.8±1.09c	25.4±1.14ab	8.2±1.09abc	11.8±0.44abc	9.8±0.83c	47.8±1.92de	28.8±1.3cd	12.6±1.14ab	26.2±2.58c	1.90±0.07e	9.4±1.14cd

Note: * PlH: plant height (cm); CaD: canopy diameter (cm); StD: stem diameter (mm); NBr: number of branch; LrL: length of leaf rachis (cm); NLf: leaflets number per leaf rachis; LfL= leaflet length (mm); LfW=leaflet width (mm); IfL: inflorescence length (mm); NFl: number of flowers in an inflorescence; NFr: number of fruits in a raceme; FrL: fruits length (mm); FrW: fruits width (mm); NuS: number of seed in a pod. ** Values are means ± standard deviation. Different letters in the same column indicate significant differences at Duncan Multiple Range Test ($p < 0.05$)

Table 4. Eigenvectors, eigenvalues, individual and cumulative percentage of variation explained by the first two principal components after assessing 14 morphological characters of *Indigofera* accessions in Java

Morphological characters	Principal Components (PC)	
	PC 1	PC 2
Plant height	0.983	-0.053
Canopy diameter	0.901	0.364
Stem diameter	0.968	-0.201
Number of branches	0.844	0.385
Length of leaf rachis	0.924	0.359
Leaflets number per leaf rachis	0.813	0.208
Leaflet length	0.958	-0.238
Leaflet width	-0.941	0.157
Inflorescence length	-0.310	0.850
Number of flowers in an inflorescence	-0.886	-0.393
Number of fruits in a raceme	0.830	0.463
Fruits length	-0.178	0.961
Fruits width	-0.503	0.755
Number of seed in a pod	-0.585	0.767
Eigenvalue	8.948	3.754
Individual percentage	63.91	26.81
Cumulative percentage	63.91	90.72

Principal component (PC) analysis for the morphological characters data set revealed 2 components with Eigenvalues greater than 1 (Table 4). The first 2 principal components (PC) explained 90.72 % of the total variation. The first principal component (PC1), which accounted for 63.91 % of the total variation, was strongly and positively associated with plant height, canopy diameter, stem diameter, number of branch, length of leaf rachis, leaflets number per leaf rachis, leaflet length and number of fruits in a raceme but negatively associated with leaflet width, inflorescence length, number of flowers in an inflorescence, fruits length, fruits width and number of seed in a pod. The second principal component (PC2) explained only 26.81 % of the total variation, was strongly and positively associated with inflorescence length, fruits length, fruits width, and number of seed in a pod but negatively associated with plant height, stem diameter, leaflet length and number of flowers in an inflorescence.

Plotting of the accessions based on morphological characters across the first 2 PCs (PC1 and PC2), revealed a slight separation of groups across the PC1 axis (Figure 2). Accessions with higher values for PC1 (A2KRA, A2BTL1, A2KPG, A2BTL2) had a higher plant, larger stem diameter, and elongated leaflet while A3BTL1 had a longer leaf rachis, larger canopy diameter, more leaflets, branch and fruits than accessions with lower PC1 values such as A1SRA, A1SKH, A1KPG and A1TBN1. Similarly, accessions with higher values for PC2 (A1TBN3, A1SRA, A1KRA, A1TBN2, A1TBN1) had longer fruit and inflorescence, more seeds in a pod, and wider leaflet than accessions with lower PC2 values such as A2KRA, A2BTL1, A2KPG, A2BTL2.

A dendrogram of similarity among *Indigofera* accessions generated from the morphological character data is presented in Figure 3. The UPGMA cluster analysis revealed three clusters of *Indigofera* accessions. Cluster I only contained one accession from *I. galegoides* (A3BTL1) which characterized by longer leaf rachis, larger canopy diameter, more leaflets, branch and fruit. Cluster II included four accessions of *I. suffruticosa* (A2KPG, A2BTL2, A2BTL1, A2KRA) which differed from accessions in Cluster I and Cluster III, mainly due to their higher plant, larger stem diameter, and elongated leaflet. Cluster III included ten accessions of *I. tinctoria* (A1PNG, A1TBN2, A1TBN3, A1BTL1, A1SKH, A1KRA, A1TBN1, A1KPG, A1BTL2, A1SRA) which had longer fruit and inflorescence, more seeds in a pod, and wider leaflet than accessions in Cluster I and Cluster II. Therefore, cluster analysis based on measured morphological characters in this study classified accessions into three groups not completely match to their geographic origin. Accessions came from the same species had the tendency to cluster together.

Analysis of anatomical characters

The anatomical features of leaves of *Indigofera* accessions studied in Java are summarized in Table 5 and Table 6. The analysis of variance for anatomical characters revealed that there was highly significant variation ($p < 0.05$) for all the tested characters among *Indigofera* accessions. Stomatal density abaxial (329.9-625.46/mm$^{2)}$, stomatal density adaxial (299.76-599.98/mm^2), trichome length adaxial (231.9-335.8 µm) and trichome length abaxial (209.2-317.6 µm) showed wide variation while stomatal length abaxial (20.4-28.06 µm), trichome width adaxial (18.8-29.4 µm), trichome width abaxial (18.3-28.2 µm), stomatal length adaxial (16.05-23.52 µm), stomatal width abaxial (12.3-16.28 µm), stomatal width adaxial (11.1-16.04 µm), trichome density adaxial (7.14-15.18 /mm^2) and trichome density abaxial (7.18-13.96/mm$^{2)}$ showed a narrower range of phenotypic variation. The trichome density adaxial had the highest coefficient of variance (22.62%) then followed by stomatal density adaxial (22.28%), trichome density abaxial (21.37%), stomatal density abaxial (20.83%), stomatal length adaxial (12.38%), trichome length abaxial (12.34%), trichome width abaxial (11.37%), trichome width adaxial (11.26%), stomatal width adaxial (10.78%), trichome length adaxial (10.02%), stomatal length abaxial (8.58%) and stomatal width abaxial (8.46%).

Principal component (PC) analysis for the anatomical characters data set revealed 2 components with Eigenvalues greater than 1 (Table 7). The first 2 principal components (PC) explained 88.96 % of the total variation. In particular, the first principal component (PC1), which explained 61.07 % of the total variation, was strongly and positively associated with stomatal length abaxial, stomatal width abaxial, stomatal length adaxial, and stomatal width adaxial but negatively associated with stomatal density abaxial, trichomes density abaxial, stomatal density adaxial and trichomes density adaxial. The second PC (PC2), which explained 27.49% of the total variation, was strongly and positively associated with trichomes length abaxial, trichomes width abaxial, trichomes length adaxial, and trichomes width adaxial but negatively associated with

stomatal length abaxial, stomatal width abaxial, stomatal length adaxial, and stomatal width adaxial.

Plotting of the accessions based on anatomical characters across the first 2 PCs (PC1 and PC2), revealed a slight separation of groups across the PC1 axis (Figure 4). Accessions with higher values for PC1 (A3BTL1) had larger size of stomata (length and width) on both surfaces (abaxial and adaxial) than accessions with lower PC1 values such as A1BTL2, A1TBN2, A1TBN1 and A1KPG. Similarly, accessions with higher values for PC2 (A2KPG, A2BTL1, A2KRA, A2BTL2) had larger size of trichomes (length and width) on both surfaces (abaxial and adaxial) than accessions with lower PC2 values such as A2KPG, A2BTL2, A2BTL1, A2KRA, A3BTL1.

A dendrogram of similarity among *Indigofera* accessions generated from the anatomical character data is presented in Figure 5. The UPGMA cluster analysis revealed four clusters of *Indigofera* accessions. Cluster I included six accessions of *I. tinctoria* (A1KPG, A1TBN1, A1BTL1, A1TBN2, A1TBN3, A1BTL2) which differed from accessions in Cluster II, Cluster III and Cluster IV, mainly due to their lower density of stomata and trichome on both surfaces (abaxial and adaxial). Cluster II only contained one accession from *I. galegoides* (A3BTL1) which characterized by large size of stomata (length and width) on both surfaces (abaxial and adaxial). Cluster III included four accessions of *I. suffruticosa* (A2KPG, A2BTL2, A2BTL1, A2KRA) which had larger size of trichomes (length and width) on both surfaces (abaxial and adaxial) than Cluster IV.

Cluster IV included four accessions of *I. tinctoria* (A1PNG, A1SRA, A1SKH, A1KRA) which characterized by small size of trichomes (length and width) on both surfaces (abaxial and adaxial). Therefore, cluster analysis based on measured anatomical characters in this study classified accessions into four groups not completely match their geographic origin. The grouping of the accession into four clusters shows that there was no correlation between ecological habitat origin and the anatomical character diversity expressed among *Indigofera* accession. However, accessions that came from the same species had the tendency to cluster together.

Table 7. Eigenvectors, eigenvalues, individual and cumulative percentage of variation explained by the first two principal components after assessing 12 anatomical characters of *Indigofera* accessions in Java, Indonesia

Anatomical characters	Principal Components (PC)	
	PC 1	PC 2
Stomatal density abaxial	-0.841	0.158
Stomatal length abaxial	0.792	-0.549
Stomatal width abaxial	0.884	-0.338
Trichomes density abaxial	-0.968	0.134
Trichomes length abaxial	0.609	0.761
Trichomes width abaxial	0.524	0.838
Stomatal density adaxial	-0.805	0.204
Stomatal length adaxial	0.902	-0.183
Stomatal width adaxial	0.834	-0.309
Trichomes density adaxial	-0.965	0.177
Trichomes length adaxial	0.567	0.803
Trichomes width adaxial	0.469	0.843
Eigenvalue	7.328	3.298
Individual percentage	61.07	27.49
Cumulative percentage	61.07	88.96

Table 5. Estimates of variability of anatomical characters among *Indigofera* accessions in Java, Indonesia

Morphological characters	Mean	Min	Max	Standard deviation	Coefficient of variance (%)	P*(F-test)
Stomatal density abaxial (pore/mm^2)	469.23	329.9	625.46	97.76	20.83	< 0.01
Stomatal length abaxial (µm)	22.47	20.4	28.06	1.93	8.58	< 0.01
Stomatal width abaxial (µm)	13.56	12.3	16.28	1.14	8.46	< 0.01
Trichomes density abaxial (unit/mm^2)	11.63	7.18	13.96	2.48	21.37	< 0.01
Trichomes length abaxial (µm)	265.52	209.2	317.6	32.77	12.34	< 0.01
Trichomes width abaxial (µm)	23.93	18.3	28.2	2.72	11.37	< 0.01
Stomatal density adaxial (pore/mm^2)	499.78	299.76	599.98	100.21	22.28	< 0.01
Stomatal length adaxial (µm)	19.18	16.05	23.52	2.37	12.38	< 0.01
Stomatal width adaxial (µm)	12.98	11.1	16.04	1.39	10.78	< 0.01
Trichomes density adaxial (unit/mm^2)	12.65	7.14	15.18	2.86	22.62	< 0.01
Trichomes length adaxial (µm)	289.47	231.9	335.8	29.01	10.02	< 0.01
Trichomes width adaxial (µm)	24.66	18.8	29.4	2.77	11.26	< 0.01

Note: *p < 0.01= highly significant

Table 6. Anatomical character variation among *Indigofera* accessions in Java, Indonesia

Accessions	ABStd	ABStl	ABStw	ABTrd	ABTrl	ABTrw	ADStd	ADStl	ADStw	ADTrd	ADTrl	ADTrw
A1PNG	440.08±23.17b	21.74±0.73abcd	12.9±0.40abc	13.38±0.53cd	251.4±4.21d	22.75±1.25b	415.18±2.92bc	17.75±1.36abc	12.02±1.43abcd	14.14±0.61cd	289.9±1.34e	24.8±1.2cd
A1KPG	602.75±16.57de	20.98±1.58a	13.48±0.72bc	13.32±0.62cd	244.6±4.15bc	23.2±1.44b	594.56±51.63e	16.75±3.01ab	13.25±0.60bcde	14.16±0.61cd	278.2±2.58bcd	25.4±1.71d
A2KPG	374.26±2.40a	23.2±0.58bcde	14.54±0.45de	8.4±0.51b	312.2±1.48h	28.2±0.93c	367.28±68.83b	21.25±1.25d	14.02±1.70cde	9.04±0.75b	328.2±2.25fg	29.4±0.97e
A1BTL1	566.14±22.85cd	21.26±1.27abc	13.14±0.47abc	13.04±1.00cd	251.8±4.2d	23.9±1.09b	560.08±10.21de	18.25±0.68abc	13.02±0.56abcde	14.88±0.67cd	282.1±1.43d	24.0±1.58bcd
A2BTL1	369.40±7.27a	23.8±1.27de	14.72±0.78e	8.26±0.62b	315.1±1.74hi	27.8±1.71c	347.28±16.22ab	21.98±1.06def	14.02±2.15cde	9.5±0.79b	332.7±2.19gh	28.3±0.67e
A3BTL1	329.90±47.56a	28.06±0.84f	16.28±0.60f	7.18±0.73a	209.2±1.68a	18.3±0.97a	299.76±2.54a	23.52±1.37f	16.04±0.84f	7.14±0.56a	231.9±3.79a	18.8±1.30a
A1BTL2	523.92±33.84c	20.4±1.75a	12.46±0.45a	13.18±0.43cd	252.2±4.1de	22.2±1.14b	512.76±37.8d	16.05±1.38a	11.27±0.89ab	14.52±0.91cd	273.2±3.27b	22.3±1.39bc
A2BTL2	371.60±5.66a	24.42±2.13e	14.62±0.73de	8.7±0.48b	312.2±2.65h	27.5±1.56c	363.64±39.09ab	22.5±1.76def	14.52±3.36ef	9.4±0.57b	335.8±3.68h	27.6±1.14e
A1SKH	446.52±29.23b	21.98±1.62abcd	12.52±0.59ab	12.64±0.91c	246.2±4.43c	22.5±1.29b	415.18±33.75c	19.02±1.04bc	12.06±0.95abcd	13.92±0.90c	287.4±1.81e	22.0±1.0b
A1KRA	459.22±2.22b	20.6±1.67a	13.74±0.85cd	13.6±0.68cd	240.4±4.87b	23.8±1.14b	399.98±32.1bc	16.25±1.53a	13.28±1.38bcde	14.76±0.95cd	273.6±8.96b	24.6±2.3cd
A2KRA	374.44±2.46a	23.3±0.76cde	14.54±0.95de	8.96±0.32b	317.6±2.07i	27.5±1.55c	363.3±1.57ab	21.25±3.42d	14.1±0.61de	9.08±0.85b	327.3±1.68f	27.9±1.94e
A1SRA	423.36±24.43b	21.9±1.29abcd	12.54±0.78ab	13.58±0.89cd	263.2±4.1g	21.8±1.2b	409.54±1.53bc	17.2±0.75abc	11.28±0.89ab	14.86±0.50cd	275.1±7.82b	23.0±1.22bcd
A1TBN1	625.46±30.42e	21.92±1.59b	12.84±0.59abc	13.32±0.77abc	256.8±3.11ef	23.2±1.59b	599.98±85.04e	19.1±0.51c	12.02±0.60abc	14.26±0.39cd	272.8±5.06b	23.9±2.7bcd
A1TBN2	559.56±68.94cd	21.58±0.93abc	12.3±0.25a	13.02±0.68cd	251.2±4.48d	23.2±2.07b	549.08±54.01de	18.02±0.71abc	11.1±1.06a	14.92±0.58cd	280.2±1.92cd	23.3±2.68bcd
A1TBN3	571.92±89.39cd	21.88±1.17abcd	12.82±0.43abc	13.96±0.49d	258.8±5.35fg	23.1±2.67b	549.1±10.32de	18.78±0.88bc	12.7±0.66abcde	15.18±0.77d	273.6±2.07b	24.6±2.3cd

Note: * ABStd: stomatal density abaxial (pore/mm^2); ABStl: stomatal length abaxial (µm); ABStw: stomatal width abaxial (µm); ABTrd: trichomes density abaxial (unit/mm^2); ABTrl: trichomes length abaxial (µm); ABTrw: trichomes width abaxial (µm);ADStd: stomatal density adaxial (pore/mm^2); ADStl: stomatal length adaxial (µm); ADStw: stomatal width adaxial (µm); ADTrd: trichomes density adaxial (unit/mm^2); ADTrl: trichomes length adaxial (µm); ADTrw: trichomes width adaxial (µm). ** Leaf anatomy comparison based on five samples for each *Indigofera* accessions. *** Values are means ± standard deviation. Different letters in the same column indicate significant differences at Duncan Multiple Range Test ($p < 0.05$)

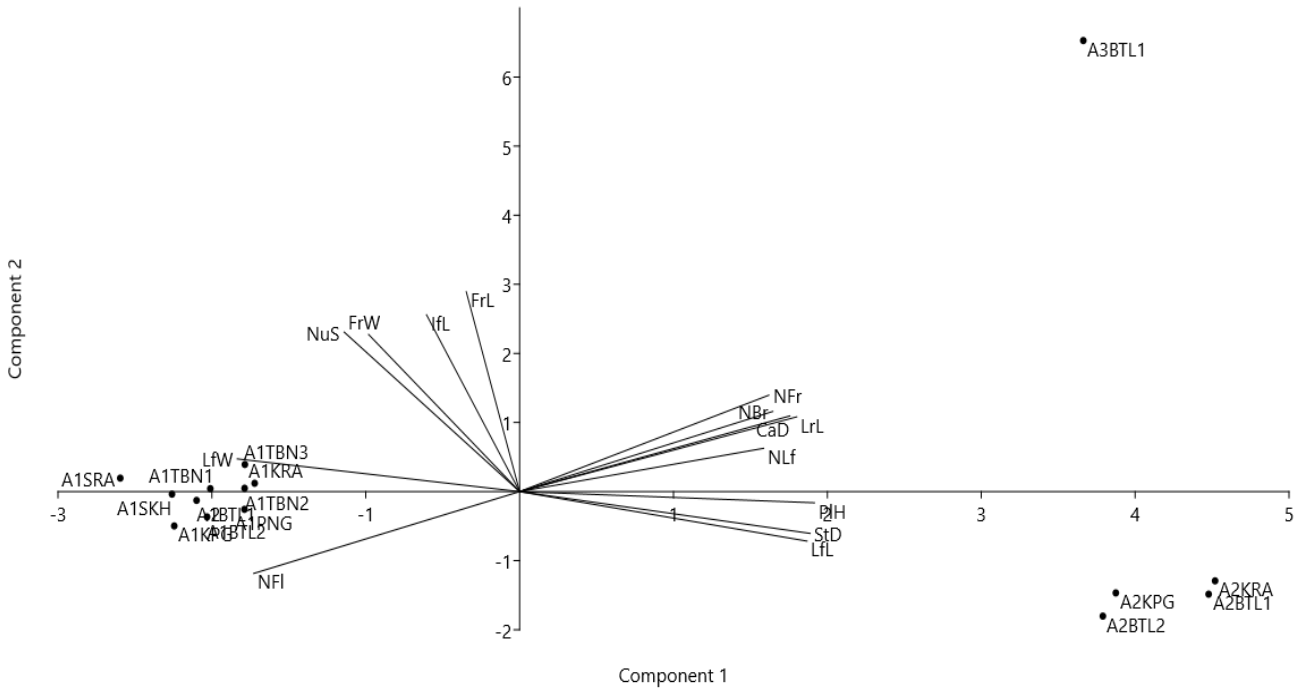

Figure 2. Scatter diagram of 15 *Indigofera* accessions from Java, Indonesia and 14 morphological characteristics when plotted against the first two principal components of the correlation matrix (explaining 90.72 % of the total variation). PlH: plant height, CaD: canopy diameter; StD: stem diameter; NBr: number of branch; LrL: length of leaf rachis; NLf: leaflets number per leaf rachis; LfL: leaflet length; LfW:leaflet width; IfL: inflorescence length; NFl: number of flowers in an inflorescence; NFr: number of fruits in a raceme; FrL: fruits length; FrW: fruits width; NuS: number of seed in a pod.

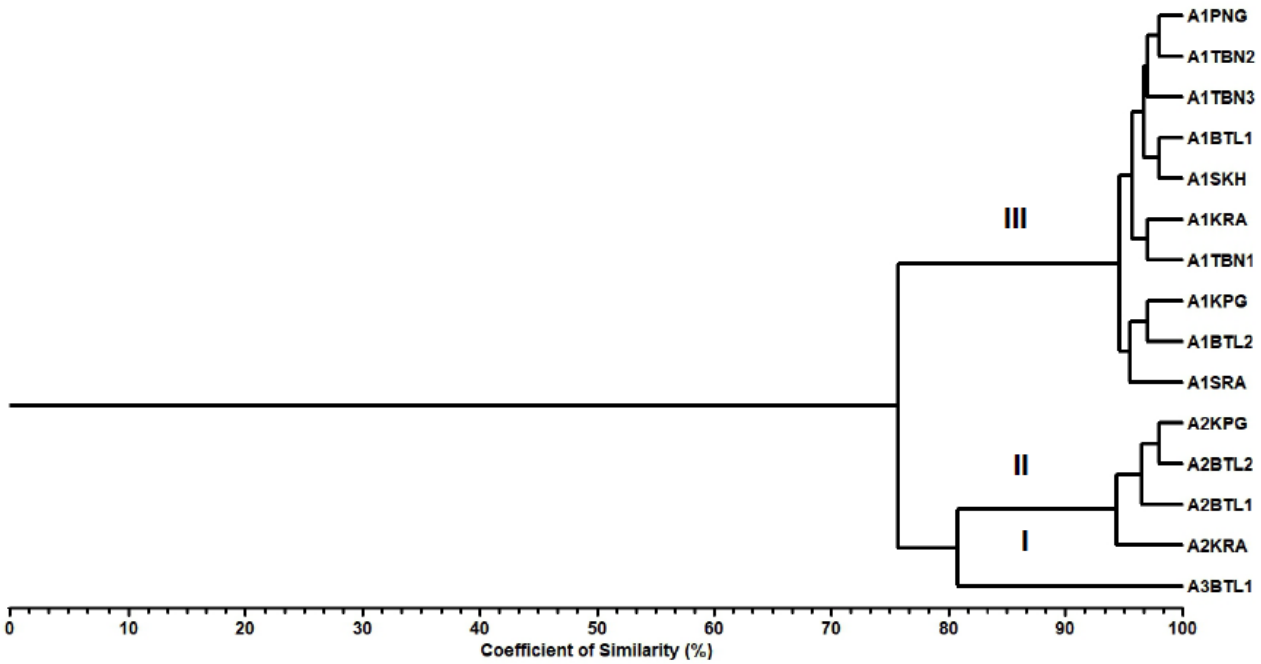

Figure 3. Dendrogram of *Indigofera* accessions from Java, Indonesia derived by UPGMA from the similarity matrix of the morphological characters data.

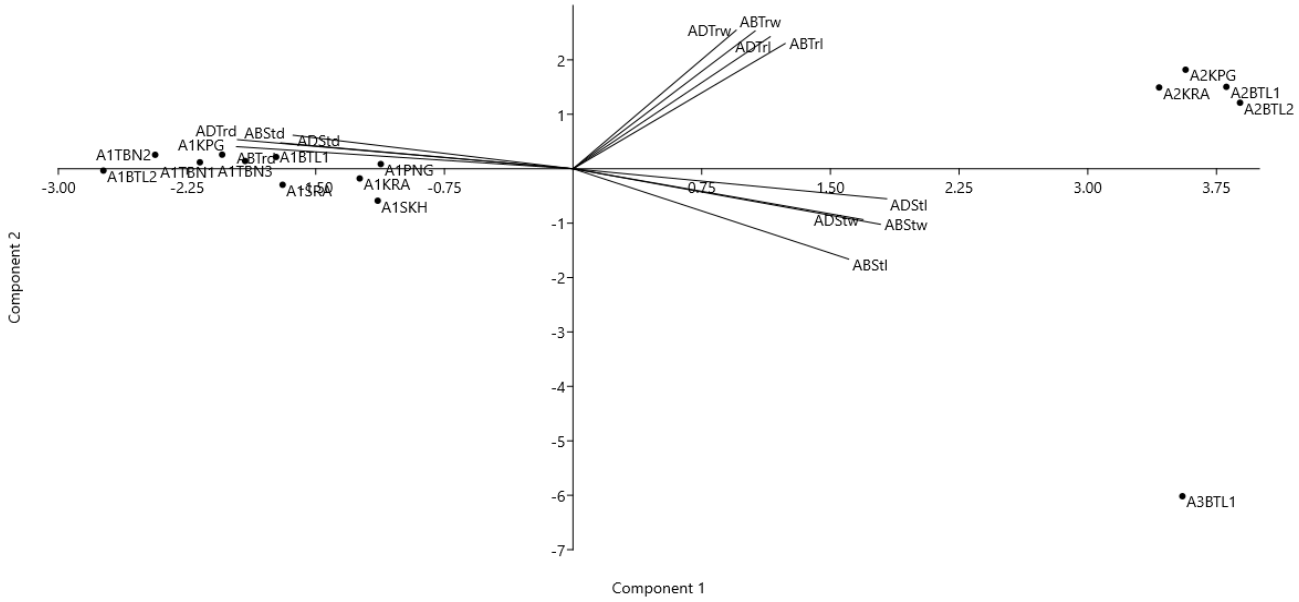

Figure 4. Scatter diagram of 15 *Indigofera* accessions from Java, Indonesia and 12 anatomical characteristics when plotted against the first two principal components of the correlation matrix (explaining 88.96 of the total variation). ABStd: stomatal density abaxial; ABStl: stomatal length abaxial; ABStw: stomatal width abaxial; ABTrd: trichomes density abaxial; ABTrl: trichomes length abaxial; ABTrw: trichomes width abaxial;ADStd: stomatal density adaxial; ADStl: stomatal length adaxial; ADStw: stomatal width adaxial; ADTrd: trichomes density adaxial; ADTrl: trichomes length adaxial; ADTrw: trichomes width adaxial.

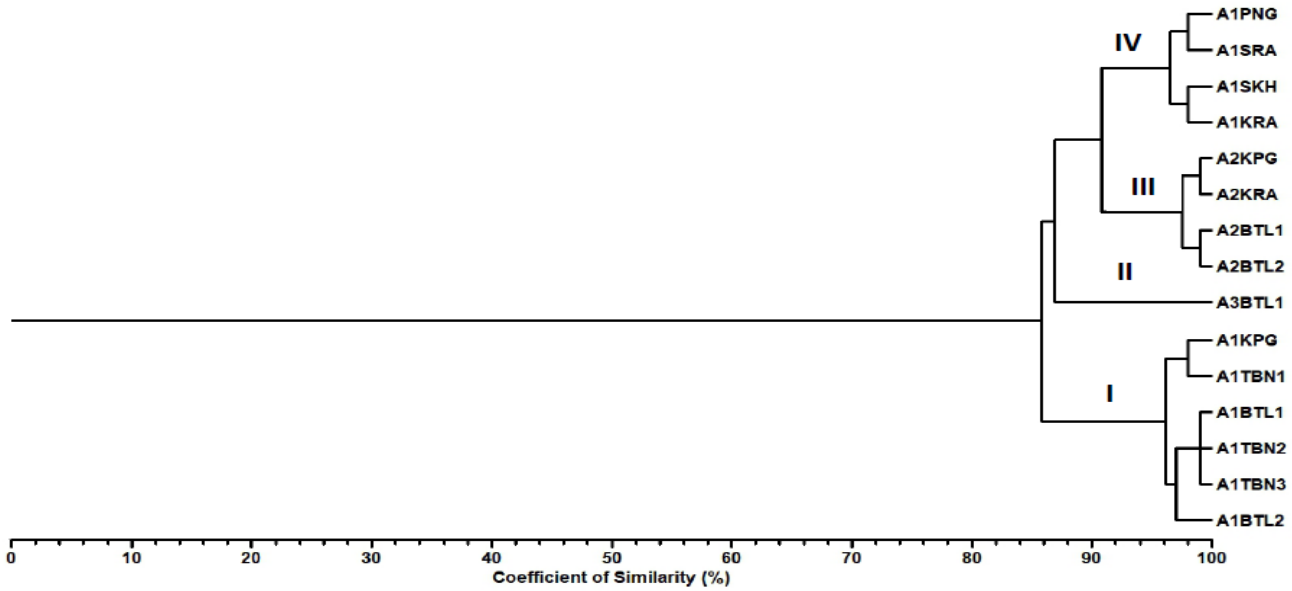

Figure 5. Dendrogram of *Indigofera* accessions from Java, Indonesia derived by UPGMA from the similarity matrix of the anatomical characters data

Discussion

The variations of 15 *Indigofera* accessions from Java were studied using morphological and anatomical characters for description. Morphological characters include the external features of the plant parts used, including the particulars of their size, shape, and color. Most plants are classified based on external morphological structures, such as flowers and fruits (Awomukwu et al. 2015). Therefore, morphological character analysis can be used to measure phenotypic diversity (Sabaghnia et al. 2014; Suratman et al. 2016; Pitoyo et al. 2018). Leaf characters are the most varied anatomical features in Angiosperms. These features can be employed as useful taxonomic characters. Foliar micromorphological characters are considered as some of the primary diagnostic features in segregating the major groups of plants. In fact, the leaf characters are considered as second to those of flowers and fruits in taxonomic studies. Foliar epidermal characters are used successfully in the delimitation of a number of taxa (Prabhakar et al. 1985; Vijay-Kumar and Ramayya et al. 1987; Vijay-Kumar 1988; Chauhan and Daniel 2011).

The analysis of variance for the evaluated morphological and anatomical characters revealed highly significant differences among accessions for all of the tested characters suggesting that there was a high degree of phenotypic diversity among *Indigofera* accessions. Some morphological characters such as canopy diameter and plant height showed wide variation while the remained characters showed a narrower range of phenotypic variation. *Indigofera* accessions also showed wide variation of anatomical characters such as stomatal density and trichome length on both surfaces (abaxial and adaxial). This implies that these characters are indicative for distinguishing variability in the accessions could be employed in distinguishing the accessions (Osawaru et al. 2013).

Stomata of *Indigofera* accessions are generally paracytic and can be found on adaxial and abaxial surfaces although more abundant in lower (abaxial) surfaces. The highest stomatal density of abaxial surface was found in *I. tinctoria* accessions then followed by *I. suffruticosa* accessions and *I. galegoides* accession. The stomatal density or stomatal index has been found useful in diagnosis of some species (Aworinde et al. 2013). High values of stomata density generally on the abaxial surface of all the taxa are greater than that of the adaxial surface may be a strategy for water conservation for these species (Adegbite, 2008; Mbagwu et al., 2008). The diversity of stomata characteristics even on the same foliar surface has been reported for *Indigofera* species (Quesada 1997). Stomata characteristics such as frequency and dimensions can be affected by type of species and environmental factors. 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; Munir et al. 2011; Suratman et al. 2016; Pitoyo et al. 2018).

The trichomes are present on both surfaces but more abundant in upper (adaxial) surface. The longest trichome of adaxial surface was found in *I. suffruticosa* accessions then followed by *I. tinctoria* accessions and *I. galegoides* accession. However, trichome cells vary in size with taxonomic value. Therefore, trichomes can be used as a diagnostic character to distinguish each plant taxa because it can show differences qualitatively and quantitatively, besides its dispersion varies from one taxon to another, from one organ to another as well as from one surface to another from a particular organ (Leelavathi and Ramaya 1983). Since, trichomes have long been reported to play a significant role not only in plant biology including defense, pollination, dispersal of seeds, fruits, and propagules, but also in taxonomy (Prabhakar et al.1985). The morphological details found from this structure as diagnostic characters to delimitate species have been previously used within Neotropical *Indigofera* (Marquiafável et al. 2009).

Information of existing genetic variation between various morphological and anatomical characters, which is vital for any breeding program, will be obtained through multivariate analysis techniques (Hassen et al. 2006). Multivariate analysis is useful for characterization, evaluation, and classification of plant genetic resources when a number of accessions are assessed (Peeters and Martinelli 1989). Multivariate analysis such as principal component analysis and cluster analysis may assist plant breeders in the characterization of germplasm (Hassen et al. 2006). This multivariate analysis can be used to explore the presence of genetic variation (van de Wouw 1999), to identify valuable characteristics, which account for genetic variation (Veasey et al. 2001; Nunes and Smith 2003) and to find a limited number of highly differentiated populations for use in programs of crossing and selection (Veronesi and Falcinelli 1988). Multivariate analysis combines the capacity to provide synthetic summary of the most relevant traits and assessment of the relative importance of the different characters to the total difference for the study of morphologically complex samples (Camussi 1979; Abadie et al. 1998; Brandolini and Brandolini 2001; Osawaru et al. 2013). The use of statistical model and tests provide informative conclusion about a very large group of occurrences by observing a small representative (Osawaru et al. 2013). Therefore, multivariate analysis based on principal component analysis and hierarchical cluster analysis was also used in this study.

Principal Component Analysis (PCA) can be used to obtain ideas about how to identify groups of accessions that have desirable traits for breeding and enlightening the pattern of variation in a germplasm collection, to identify relationships among accessions and possible gaps (Camussi et al. 1985; Cowen and Frey 1987; Peeters and Martinelli 1989; Osawaru et al. 2013). PCA has the advantage of showing how distant each accession is from others and the variable most responsible for giving that pattern of relationship among the accessions (Adeniji et al. 2012).

Only principal component (PC) with eigenvalues greater than 1.00 which used in this study and cumulative proportion of variation explained was used to identify number of principal components (Thattil and Samita 2007). In this study, the variance accounted for by the first two PC of evaluated characters data sets was relatively high (>80%). The first two PC explained 90.72 % of the total variation of morphological characters while the first two PC explained 88.96 % of the total variation of anatomical characters. This result satisfactorily explains the variability manifested between individuals of accessions (Veasey et al. 2001; Hassen et al. 2006).

By the principal component analysis, it is possible to determine the relative contribution of each character to the total variation in accessions and to identify the most informative to represent the variability of the germplasm available (Arriel et al. 2007). Therefore, the importance of the principal component for each character is also evaluated by means of the percentage of the total variation it explains. The PC1 accounted for 63.91 % of the total variation for morphological characters while PC 2 explained only 26.81 %. For anatomical characters, PC1 explained 61.07 % of the total variation while PC2 only accounted for 27.49% of the total variation. These first two PC can explain the greatest part of the total variation found in the original data. If the first two PC accumulate a relatively high percentage of the total variation, generally determined as over 80%, they satisfactorily explain the variability expressed in the evaluated plants (Cruz and Carneiro 2003; Arriel et al. 2007).

Morphological characters such as plant height, canopy diameter, stem diameter, number of branches, length of leaf rachis, leaflets number per leaf rachis, leaflet length and number of fruits in a raceme, inflorescence length, fruits length, fruits width and number of seed in a pod had shown a strong contribution to PC1 and PC2 axis. Similarly, anatomical characters such as stomatal size (length and width) and trichomes size (length and width) on both surfaces (abaxial and adaxial) also strongly associated with PC1 and PC2 axis. Thus, improvement of *Indigofera* species is possible by selecting valuable morphological characteristics with anatomical characters significance (Hassen et al. 2006)

Cluster analysis is the partitioning of a set of objects into groups so that objects within a group are similar and objects in different groups are dissimilar. It is efficient in grouping objects with similar characters (Hodgkin et al, 1995). Cluster analysis using morphological characters data revealed three main clusters while four main clusters were then highlighted in cluster analysis of the anatomical character data.PC analysis helps to understand how the accessions of similar categories group together compared to dissimilar ones. The results of PC analysis partly confirmed the findings of cluster analysis. In PC analysis, the accessions grouped into three main clusters based on morphological character (Figure 2), while the cluster analysis of the accessions derived by UPGMA from the similarity matrix exhibited a similar dendrogram topology and cluster membership (Figure 3). PC analysis based on anatomical character revealed three main clusters (Figure 4), with only few differences between Cluster III and IV compared to cluster analysis (Figure 5). This demonstrates that the data obtained from this experiment were accurate, precise, and reliable. The groupings of hierarchical cluster analysis exhibited a similar dendrogram topology and cluster membership to that produced using PC analysis thereby confirming the accuracy of the constructed dendrogram. Therefore, the results of PC analysis clarify or verify the cluster analysis, if the results of one analysis support those of another, this confirms that the data are more precise and accurate (Tuhina-Khatun et al. 2015).

The existence of variation among accessions for the majority of the studied morphological and characters is a sign of the presence of genetic variation implying great potency for future breeding programs through selection (Nkansah et al. 2013; Roy et al. 2013; Sabaghnia et al. 2014; Suratman et al. 2016; Pitoyo et al. 2018). Genetic variability as reflected from variation of morphological and anatomical characters is the raw material of crop breeding. 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; Pitoyo et al. 2018). Subsequently, the grouping of *Indigofera* accessions by phenotypic diversity in the present study can be used to classify the accessions into distinct morphological and anatomical levels, which could be used for various breeding, collection, and conservation programs (Hassen et al. 2006).

ACKNOWLEDGEMENTS

The authors would like to thank the Head of the Department of Biology Education, Faculty of Teaching Training and Education, Universitas Sebelas Maret (UNS), Surakarta, Indonesia for providing the infrastructural facilities for the research. The authors also acknowledge gratefully to General Directorate of Higher Education, Ministry of Education and Culture, Republic of Indonesia for financial supports of this research.

REFERENCES

Abadie T, Magallines JR, Cordeiro C, Parentoni SN, Andrade de RV.1998. A classification for Brazilian maize landraces. Pl Gen Res Newsl 114: 43-44.

Adegbite AE. 2008. Leaf anatomical studies in some species of the tribe *Cichorieae* (*Asteraceae*) in Nigeria. Comp Newsl 46: 49-58. DOI: 10.21829/abm126.2019.1515

Adeniji OT, Kusolwa PM, Reuben SOWM. 2012.Genetic diversity among accessions of *Solanum aethiopicum* L. groups based on morpho-agronomic traits. Plant Genet Resour: 10 (3): 177-185. DOI: 10.1017/S1479262112000226

Airy Shaw HK. 1985. A Dictionary of The Flowering Plants & Ferns. 8th Edition. Cambridge University Press, Cambridge.

Arriel NHC, Mauro AOD, Arriel EF, Unêda-Trevisoli SH, Costa MM, Bárbaro IM, Muniz FRS. 2007. Genetic divergence in sesame based on morphological and agronomic traits. Crop Breed Appl Biotechnology 7: 253-261.

Arzani K, Ghasemi M, Yadollahi A, Hokmabadi H.2013. Study of foliar epidermal anatomy of four pistachio rootstocks under water stress. Idesia (Chile) 31 (1): 101-107.

Awomukwu DA, Nyananyo BL, Uka CJ, Okeke CU. 2015. Identification of the genus *Phyllanthus* (Family *Phyllanthaceae*) in Southern Nigeria using comparative systematic morphological and anatomical studies of the vegetative organs. Glob J Sci Front Res (C) 15 (2): 79-93. DOI:10.11648/j.jps.20150303.15

Aworinde DO, Ogundairo BO, Erinoso SM, Sharaibi OJ. 2013. Foliar epidermal and petiole anatomical studies of some Nigerian species of *Ipomoea* (*Convolvulaceae*). Curr Bot 4 (3): 55-58.

Balamurugan G, Selvarajan S. 2009. Preliminary phytochemical screening and anthelmintic activity of *Indigofera tinctoria* Linn. Int J Drug Dev Res 1 (1): 157-160.

Bangar AV, Saralaya MG. 2011. Antihyperglycemic activity of ethanol extract and chloroform extract of *Indigofera tinctoria* leaves in streptozotocin-induced diabetic mice. Res J Pharm Biol Chem Sci 2 (1): 445-455.

Beyene P, Botha A, Myburg AA. 2005. A comparative study of molecular and morphological methods of describing genetic relationships in traditional Ethiopian highland maize. Afr J Biotechnol 4 (7) 586-595. DOI: 10.5897/AJB2005.000-3107

Brandolini A, Brandolini A. 2001. Classification of Italian maize (*Zea mays* L.) germplasm. Plant Genet Res Newsl 126: 1-11.

Burkill HM.1995. The Useful Plants of Tropical Africa. 2nd ed. Royal Botanic Gardens, Kew.

Camussi A. 1979. Numerical taxonomy of Italian populations of maize based on quantitative traits. Maydica 25: 161-174.

Camussi A, Ottaviano E, Calinski T, Kaczmarek Z. 1985. Genetic distances based on quantitative traits. Genetics 111 (4): 945-962. DOI: 10.1093/genetics/111.4.945

Chauhan D, Daniel M. 2011. Foliar micromorphological studies on some members of the family Fabaceae. Int J Pharm Bio Sci 2 (4): 603-611.

Chikmawati T. 2013. Anatomical and cytological features of *Spathoglottis plicata* from Java island. J Trop Life Sci 3 (2): 87-90.

Cowen NM, Frey KJ. 1987. Relationships between three measures of genetic distance and breeding methods in oat (*Avena sativa* L.). Genome 29 (1): 97-106. DOI: 10.1139/g87-017

Cruz CD, Carneiro PCS. 2003. Modelos Biométricos Aplicados ao Melhoramento Genético. vol. 2. Imprensa Universitária, Viçosa.

Felicia FA, Muthulingam M. 2012. Antihepatotoxic efficacy of methanolic extract of *Indigofera tinctoria* (Linn.) on paracetamol-induced liver damage in rats. J Nat Prod Plant Resour 2 (2): 244-250.

Gao XF, Sun H, Schrire BD. 2010. *Indigofera*. Flora of China 10: 137-164 .

Hassen A, Rethman NFG, Apostolides Z. 2006. Morphological and agronomic characterisation of *Indigofera* species using multivariate analysis.Trop Grassl 40 (1): 45-59.

Hetherington AM, Woodward FI. 2003. The role of stomata in sensing and driving environmental change. Nature 424 (6951): 901-908. DOI: 10.1038/nature01843

Hodgkin T, Brown AHD, Hintum TJL, Morales EAV. 1995. Core Collections of Plant Genetic Resources. John Wiley & Sons, UK.

Howard RA.1988. Flora of the Lesser Antilles. Leeward and Windward Islands. Dicotyledoneae.Harvard University Press, Harvard.

Jingura RM, Kamusoko R. 2015. Utility of markers for determination of genetic diversity in *Jatropha*: A Review. Open Renew Energ J 8 (1): 1-6. DOI: 10.2174/1876387101508010001

Kumar V, Singh PK, Dudhane AS, De DK, Satya P. 2014. Anatomical and morphological characteristics of nine jute genotypes. J Crop Weed 10 (2): 334-339.

Lewis G, Schrire B, Mackinder B, Lock M. 2005. Legumes of the World. Royal Botanic Gardens, Kew.

Leelavathi P, Ramaya N. 1983. Structure, distribution and classification of plant trichomes in relation to taxonomy III, Papilionoideae. Proc Indian Acad Sci 92 (5): 421-441. DOI: 10.1007/BF03052991

Mabberley DJ. 2008. The Plant-Book. A Portable Dictionary of The Vascular Plants. Edition 3. Cambridge University Press, Cambridge.

Marquiafável FS, Ferreira MDS, Teixeira SP. 2009. Novel reports of glands in Neotropical species of *Indigofera* L. (Leguminosae, Papilionoideae). Flora 204 (3): 189-197. DOI: 10.1016/j.flora.2008.01.012

Munir M, Khan MA, Ahmed M, Bano A, Ahmed SN, Tariq K, Tabassum S, Mukhtar T, Ambreen M, Bashir S. 2011.Foliar epidermal anatomy of some ethnobotanically important species of wild edible fruits of northern Pakistan. J Med Plants Res 5 (24): 5873-5880.

Mbagwu FN, Nwachukwu CU, Okoro OO. 2008. Comparative leaf epidermal studies on *Solanum marcocarpon* and *S. nigrum*. Res J Bot 3 (1): 45-48. DOI: 10.3923/rjb.2008.45.48

Motamarri SN, Karthikeyan M, Rajasekar S, Gopal V. 2012. *Indigofera tinctoria* - a phytopharmacological review. Int J Res Pharm Biomed Sci 3 (1): 164-169.

Muzzazinah, Chikmawati T, Ariyanti NS. 2016. Correlation of morphological characteristics with the presence of indicant in *Indigofera* sp. dyestuff. Sains Malay 45 (6): 883-890.

Nkansah GO, Ofosu-Budu KG, Ayarna AW. 2013 Genetic diversity among local and introduced avocado germplasm based on morphoagronomic traits. Intl J Plant Breed Genet 7 (2): 76-91. DOI: 10.3923/ijpbg.2013.76.91

Nunes MES, Smith GR.2003. Characterisation of rose clover germplasm for flowering traits. Crop Sci 43 (4): 1523-1527. DOI: 10.2135/cropsci2003.1523

Nwachukwu CU, Mbagwu FN. 2006. Anatomical studies on the petiole of some species of *Indigofera*. Agric J 1 (2): 55-58.

Nwachukwu CU, Edeoga HO, Kemka-Evans CI, Iwuanyanwu CJ. 2016. Stem anatomical studies of some species of *Indigofera* L. (Leguminosae-Papilionoideae). J Adv Biol 9: 1967-1971.

Osawaru ME, Ogwu MC, Dania-Ogbe FM. 2013. Morphological assessment of the genetic variability among 53 accessions of West African okra [*Abelmoschus caillei* (A. Chev.) Stevels] from South Western Nigeria. Nigerian J Basic Appl Sci 21 (3): 227-238. DOI: 10.4314/njbas.v21i3.8

Peeters JP, Martinelli JA. 1989. Hierarchical cluster analysis as a tool to manage variation in germplasm collections. Theor Appl Genet 78 (1): 42-48. DOI: 10.1007/BF00299751

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 (5): 1811-1819. DOI: 10.13057/biodiv/d190531

Prabhakar M, Vijay-Kumar BK, Ramayya N, Leelavathi P. 1985. Structure, distribution and taxonomic significance of trichomes in some *Indigofera* L. (Fabaceae). Proc Plant Sci 95 (5): 309-314. DOI: 10.1007/BF03053241

Quesada EM. 1997. Taxonomic significance of foliar dermotypes and floral trichomes in some Cuban taxa of *Indigofera* L. (Fabaceae - Faboideae). Polibotanica 6: 1-18.

Rahayu SE, Chikmawati T, Kartawinata K, Hartana A. 2012. Leaf anatomy of *Pandanus* species (Pandanaceae) from Java. Plant Soc Ecol 13 (4): 317-378. DOI: 10.14203/reinwardtia.v13i3.449

Renukadevi KP, Sultana SS.2011. Determination of antibacterial, antioxidant and cytotoxicity effect of *Indigofera tinctoria* on lung cancer cell line NCI-h69. Int J Pharmacol 7 (3): 356-362. DOI: 10.3923/ijp.2011.356.362

Restykania, Suratman, Pitoyo A, Suranto. 2019. Morphology and isozyme variation among Madeira vine (*Anredera cordifolia*) accessions from southeastern part of Central Java, Indonesia. Biodiversitas 20 (10): 3024-3032. DOI: 10.13057/biodiv/d201035

Roy S, Islam MA, Sarker A, Malek MA, Rafii MY, Ismail MR. 2013. Determination of genetic diversity in lentil germplasm based on quantitative traits. Aust J Crop Sci 7 (1): 14-21.

Sabaghnia N, Janmohammadi M, Bashiri A, Asghari-Shirghanr R. 2014. Genetic variation of several bread wheat (*Triticum aestivum* L.) genotypes based on some morphological traits. Ann Univ Mariae Curie-Sklodowska Lublin-Polonia 69 (1): 44-54.

Said WM, Ehsan NOM. 2010. Morphological and molecular evidence among four heteroforms of *Avicennia marina* (Forssk) Vierh. J Am Sci 6 (11): 843-856.

Schrire BD. 1998. Notes relating to the genus *Indigofera (Leguminosae-Papilionoideae)* for Flora Zambesiaca: 2. Section *Indigofera*. Kew Bull 53: 651-668. DOI: 10.2307/4110484

Schrire BD. 2005. Tribe Indigofereae. In Lewis G, Schrire B, Mackinder B, Lock M (eds) Legumes of the World. Royal Botanic Gardens, Kew.

Schrire BD, Lavin M, Nigel P, Félix F. 2009. Phylogeny of the tribe Indigofereae (Leguminosae-Papilionoideae): geographically structured more in succulent-rich and temperate settings than in grass-rich environments. Am J Bot 96 (4): 816-52. DOI: 10.3732/ajb.0800185.

Smith JSC, Smith OS. 1992. Fingerprinting crop varieties. Adv Agron 47: 85-140. DOI: 10.1016/S0065-2113(08)60489-7

Suratman, Pitoyo A, Kurniasari S, Suranto. 2016. Morphological, anatomical and isozyme variation among giant taro (*Alocasia macrorrhizos*) accessions from Central Java, Indonesia. Biodiversitas 17(2): 422-429. DOI: 10.13057/biodiv/d170204

Tamilselvi N, Dhamotharan R, Krishnamoorthy P, Shivakumar. 2011. Anatomical studies of *Indigofera aspalathoides* Vahl (Fabaceae). J Chem Pharm Res 3 (2): 738-746.

Thattil RO, Samita S. 2007. Multivariate Methods. University of Peradeniya, Peradeniya, Sri Lanka.

Tuhina-Khatun M, Hanafi MM, Yusop MR, Wong MY, Salleh FM, Ferdous J. 2015. Genetic variation, heritability, and diversity analysis of upland rice (*Oryza sativa* L.) genotypes based on quantitative traits. BioMed Res Int 290861: 1-7 http://dx.doi.org/10.1155/2015/290861

Tyagi PK, Rai VK, Pahria AK, Kumar SS, Singh Y, Sharma M, Goyal M. 2010. Preliminary phytochemical screening and evaluation of antiinflammatory activity of ethanolic extract of leaves of *Indigofera tinctoria* Linn. J Curr Pharm Res 3(1): 47-50.

Van de Wouw M, Hanson J, Lenthi S. 1999. Morphological and agronomic characterization of a collection of Napier grass (*Pennisetum purpureum)* and *P. purpureum x P. glaucum.* Trop Grassl 33: 150-158.

Veasey EA, Schammass EA, Vencovsky R, Martins PS, Bandel G. 2001 Germplasm characterization of *Sesbania* accessions based on multivariate analysis. Genet Resour Crop Evol 48 (1): 79-90. DOI: 10.1023/A:1011238320630

Veronesi F, Falcinelli M. 1988. Evaluation of an Italian germplasm collection of *Festuca arundinacea* Schreb. through a multivariate analysis. Euphytica 38 (3): 211-220. DOI: 10.1007/BF00023523

Vijay-Kumar BK. 1988. Structure, distribution and classification of plant trichomes in relation to taxonomy in *Indigofera* L. (Fabaceae). Indian J For 11 (2): 120-130.

Vijay-Kumar BK, Ramayya N. 1987. Foliar dermotypes of some endemic taxa of *Indigofera* (*Fabaceae*) and their taxonomic significance. Indian J Bot 10 (2): 113-120.