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Morphological variation in three *Indigofera* spp.

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Abstract: A morphological investigation was carried out in three *Indigofera* species (*Indigofera tinctoria, I. suffruticosa* and *I. amblyantha*) with a view to identifying a set of characteristics for the individual indigo species. Distinct variation exists in stem, leaf, inflorescence, flower, pod and seed character. The species *I. tinctoria* possesses 5-11 obovate leaflets per leaf, pink flower with larger standard and wings, narrow cylindrical pod (average pod volume 161.1 mm^3) with slightly curved tip and cylindrical deep brown large seed (average 1000-seed weight, 5.79 g); *I. suffruticosa* possesses green stem with patches of reddish colour, 9-17 narrowly oblong leaflets per leaf, crimson flower with large standard and wings, short strongly curved pod (average pod volume 91.1 mm^3) and cylindrical dark green small seed (average 1000-seed weight, 3.75 g); and *I. amblyantha* possesses 13-15 ovate to lanceolate large leaflets per leaf, cerise-violet flower with largest standard and wings, long, thick, straight cylindrical pod (average pod volume 498.0 mm^3) and globose greenish yellow medium seed (average 1000-seed weight, 4.72 g).
Key words: Morphology, Indigo species, variation

Introduction

Indigofera is an important genus of the family Leguminosae and sub-family Papilionaceae. The latin word *Indigofera* means indigo (blue colour) bearing (Bailey, 1961). *Indigofera* spp. are shrubs to small trees. Many countries of the world from cool temperate to tropical climate, it is grown as ornamental, for production of indigo dye and also as herbal medicine (Ellison, 1999; Healthlink, 2001).

Indigo is the most important ancient blue dye used by man for textiles. Synthetic chemicals used as dyes in the many textile industry which are known to be a principal source of environmental pollution. Synthetic dye substances also have several carcinogenic properties and cause allergies to human. During the recent years there has been a resurgence of interest in natural dyes to replace synthetic ones because natural dyes are environment friendly and distinctive washing and sunlight fade-resistant (Angelini *et al*., 1997). Indigo dye is a derivative of Indican, a natural constituent of several *Indigofera* species (Simon *et al*., 1984). The indigo dyestuff is not present in the green leaves and stems but is produced post-harvest by hydrolysis. The revival of indigo plantation has also started in Bangladesh.

In Bangladesh, *I. tinctoria* has been grown as green manuring and fuel crops in some pocket areas of Nilphamari District (Anonymous, 2007). Indigo plants possess a repelling unpleasant smell and are not liked by grazing animals and therefore, may be used as live fence. Considering all the properties, indigo plants could be an excellent multipurpose shrub or small tree species in crop land Agroforestry in Bangladesh.

Some researches on indigo have been carried out in Belgium, India, Nepal and Philippines (Agustin *et al*., 1999; Kondo *et al*., 2000). Although literature on qualitative characters *viz*., type, colour, shape of leaflet, inflorescence, flower and pod is available (Bailey, 1961; Howard, 1988), there is very little information on comparative morphological characters especially quantitative characters *viz*., length, breadth and area of leaf, leaflet, flower and pod in different *Indigofera* species (Khan, 2007; 2008). A number of other researches were conducted to investigate the means of increasing biomass production in *Indigofera tinctoria* at the Department of Crop Botany, Bangladesh Agricultural University campus (Kabir, 2003; Rokonuzzaman, 2003; Pervin 2004; Hasan 2005; Rahman, 2007). This study investigates the variation in vegetative and reproductive characteristics among three cultivated Indigo species *viz*., *Indigofera tinctoria* L., *I. suffruticosa* Mill. and *I. amblyantha* Craib. The overall objective is to provide a set of diagnostic characters for individual *Indigofera* species.

Materials and Methods

This study was conducted at Botanical Garden, Department of Crop Botany, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh during the period of September 2006 to November 2007. The seeds of *I. tinctoria* and *I. suffruticosa* were sown in line in plot following Randomised Complete Block Design (RCBD) with four replications. The plot size was 4 m^2 (2 m × 2 m) and the spacing was 30 cm × 30 cm. All necessary intercultural operations were done following recommended practices.

Detailed morphological features of the three species were studied, both qualitative and quantitative measurements were recorded from randomly selected at least 5 individual samples of each species. The observed data were: i) stem colour, ii) length of rachis of leaf, iii) colour, size (length × breadth i.e., L × B), shape, surface of leaflets, iv) chlorophyll content of 2^{nd} pair leaflets, v) number of leaflets per leaf, vi) colour, size and shape of different floral parts (corolla, androecium, gynoecium), vii) colour, size and shape of pod and seed. Volume of dry matured pod was measured by $\pi r^2 h$ where, π = 3.1415, r = radius of the pod and h = length of the pod. The radius of the pod was calculated from mean value of diameter taken from each of base, middle and top of the pod. Length of pod was measured from base of pod to tip of matured ovary. Length of rachis of leaf was measured from base of the leaf to the tip of terminal leaflet. Chlorophyll content of the 2^{nd} pair of leaflets was estimated by chlorophyll meter, SPAD-502 (Konica Minolta Sensing, Inc. Japan) between 10^{th} and 12^{th} fully expanded leaf descending from top of the plant. All the specimens were photographed with a digital camera (Nikon Coolpix E 7600, Nikon Corp., Japan).

Results and Discussion

Comparative morphological study of vegetative and reproductive parts of three indigo plant species are described under the following heads.

Vegetative part

Stem: The stem of three species were commonly erect, round, solid (Fig. 1. ii). Stem colour was generally green in the *I. tinctoria* and *I. amblyantha* while in *I. suffruticosa* with patches of reddish colour covering the tender part of stem (Table 1, Fig. 1.ii). In mature stage bark colour was reddish brown in *I. tinctoria*; grey-brown in *I. suffruticosa* and grey in *I. amblyantha* (Table 1).

Leaf: Generally, leaf of all three species was pinnately compound, unipinnate, imparipinnate, petiolate having pulvinus leaf base, stipulate, entire margin with alternate phyllotaxy (Fig. 1.i). The length of the rachis of compound leaf varied between 9.71 to 25.98 cm with a mean value being 15.78 ± 5.13 cm (Table 2). Rachis of leaf was longer in *I. amblyantha* (25.98 cm) than in *I. tinctoria* (9.71 cm) and *I. suffruticosa* (11.66 cm) (Table 2, Fig. 1.iii). Colour of leaflet was deep green in *I. suffruticosa* with the highest total chlorophyll content (54.62) whereas the *I. amblyantha* had light green leaflet with the lowest total chlorophyll content (45.50) with the *I. tinctoria* being intermediate (49.90) (Table 1-2, Fig. 1.iv).

The upper surface of the leaflet was glabrous in *I. tinctoria* whereas both the surface of *I. suffruticosa* was densely hairy and that of *I. amblyantha* was slightly hairy (Table 1). Number of leaflets was greater in *I. suffruticosa* (9 to 17 per leaf) and *I. amblyantha* (13 to 15 per leaf) than in *I. tinctoria* (5 to 11 per leaf) (Table 2, Fig. 1.iii). Leaflets also varied in shape and size. The leaflets of *I. tinctoria* were obovate with rounded tip and that of *I. suffruticosa* were narrowly oblong with obtuse tip and that of *I. amblyantha* were ovate to lanceolate with acute tip (Table 1, Fig. 1.iv). Simon *et al.* (1984) also observed similar shape and number of leaflets per plant in indigo plants. The length and breadth of the leaflet was greater in *I. amblyantha* than in the other two species with the leaflet area was again greater in *I. amblyantha* (7.67 cm^2) than tinctoria (1.60 cm^2) and *I. suffruticosa* (1.18 cm^2) (Table 2). Reddish pigmentation was found on the upper surface of petiolule, rachis and pulvinus of leaves in *I. suffruticosa* which was absent in *I. tinctoria* and *I. amblyantha* (Table 1, Fig. 1.v-vi).

Table 1. Variation in morphological features (qualitative) of three *Indigofera* spp.

Species / Characters	*I. tinctoria*	*I. suffruticosa*	*I. amblyantha*
Leaflet colour	Green	Deep green	Light green
Leaflet surface	Upper surface glabrous	Densely hairy on both the surface	Slightly hairy on both the surface
Leaflet shape	Obovate	Narrowly oblong	Ovate to lanceolate
Stem colour	Green	Patches of reddish colour on green stem	Green
Bark colour	Reddish brown	Grey-brown	Grey
Pigmentation on rachis of leaves	None	Reddish colour on upper portion	None
Pigmentation on petiolule	None	Reddish colour on upper portion	None
Colour of corolla	Pink	Crimson	Cerise-violet
Colour of pod	Brown	Deep brown	Dark brown
Surface of pod at maturity	Smooth	Densely hairy	Crack
Shape of pod	Narrow cylindrical, slightly curved at the tip	Strongly curved	Thick, cylindrical straight
Shape of seed	Small, solid cylindrical	Small, solid cylindrical	Flattened, globose
Colour of seed	Mostly brown mixed with some black seed	Dark green mixed with some brown and black seed	Greenish yellow mixed with some black seed

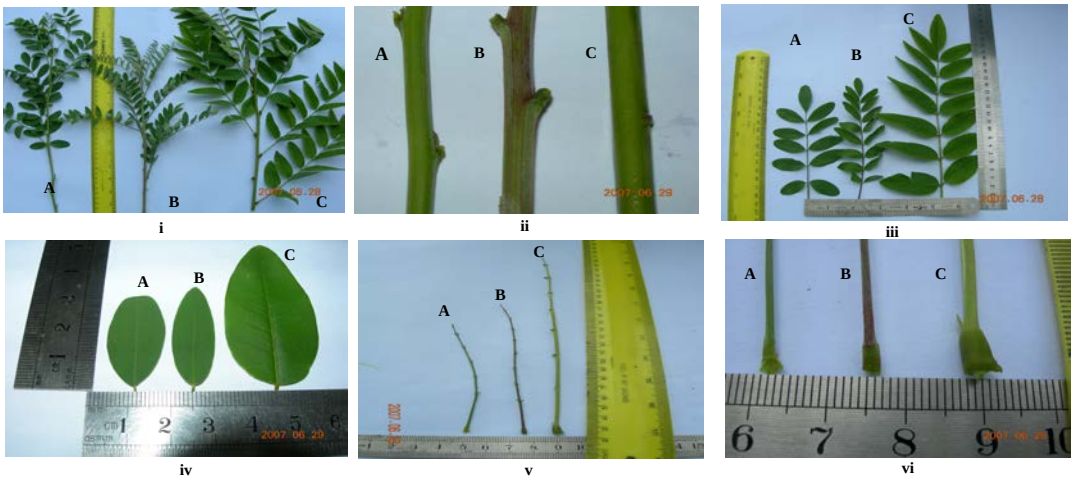

Fig. 1. Variation in vegetative parts in three *Indigofera* spp., *I. tinctoria* (A), *I. suffruticosa* (B), *I. amblyantha* (C). Plant parts are: twig (i); stem (ii); leaves (iii), leaflets (iv); rachis of leaves (v); pulvinus of leaves (vi).

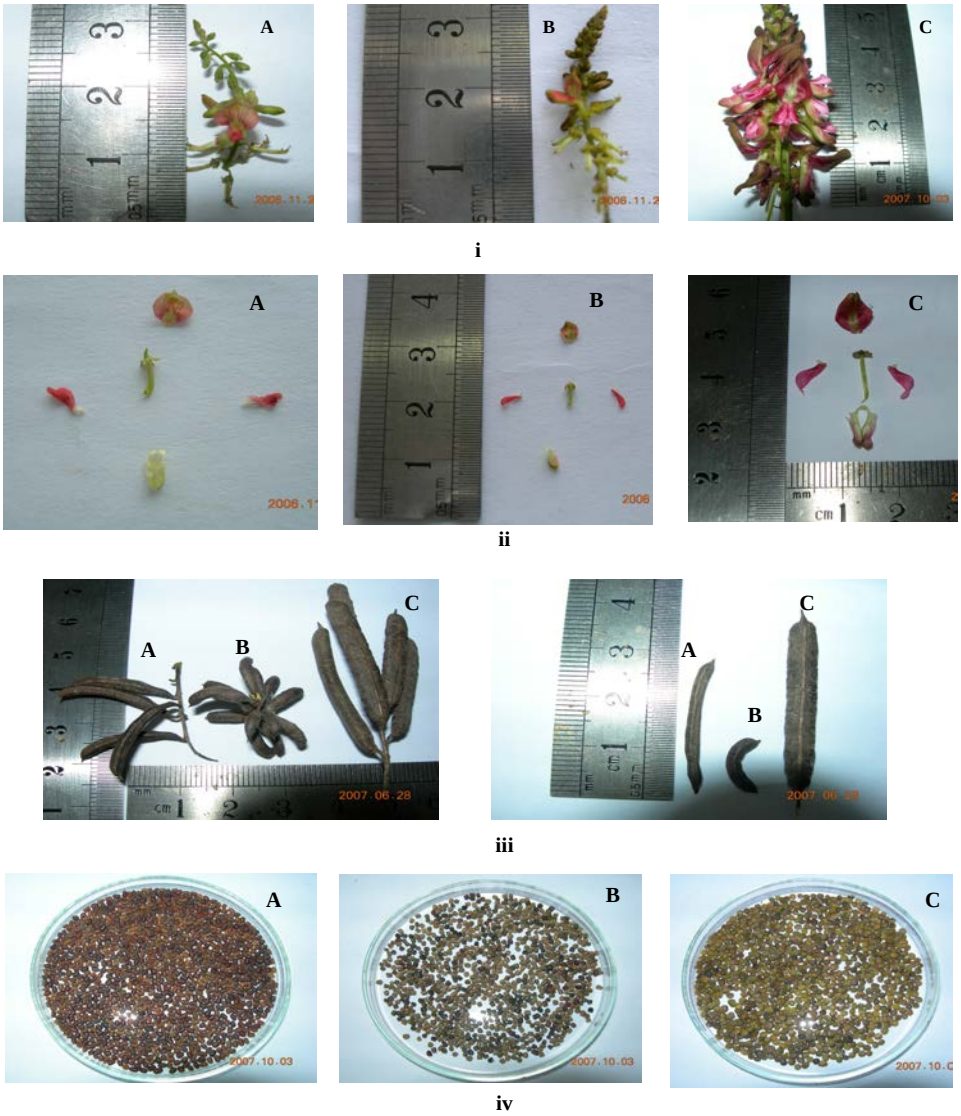

Fig. 2. Variation in reproductive parts in three *Indigofera* spp. *I. tinctoria* (A), *I. suffruticosa* (B), *I. amblyantha* (C). Plant parts are: inflorescence (i); separated parts of flowers (ii), pods (iii), seeds (iv).

Table 2. Variation in morphological features (quantitative) of leaf in three *Indigofera* spp.

Species	Length of rachis of leaf (cm)	Number of leaflets leaf^{-1} (No.)	Leaflet size			Chlorophyll content (SPAD Value)
			Length (L) (cm)	Breadth (B) (cm)	L × B (cm^2)	
I. tinctoria	9.71 †	5-11 (8)	1.57	1.02	1.60	49.90
I. suffruticosa	11.66	9-17 (13)	1.79	0.66	1.18	54.62
I. amblyantha	25.98	13-15 (14)	4.06	1.89	7.67	45.50
Mean	15.78	9-14 (11.66)	2.47	1.19	3.48	50.00
Range	9.71-25.98	5-17 (8-14)	1.57 - 4.06	0.66 - 1.89	1.18 - 7.67	45.50 - 54.64
SEM ±	5.13	1.85	0.79	0.36	2.09	2.63

Figures within parentheses are mean, †: Each figure is the mean of at least 20 samples.

Reproductive part

Inflorescence and flower: The *I. tinctoria* flowered in 2 to 3 months after planting throughout the year whereas *I. suffruticosa* flowered between September and March and *I. amblyantha* flowered for a short period of time i.e. between September and November (Table 3). It seems that *I. tinctoria* appear to be photoinsensitive while *I. suffruticosa* and *I. amblyantha* could be a short day plant. Further investigation in this regard is necessary.

Generally, inflorescence of the three species was axillary upright raceme (data not shown). Ellison (1999), however, mentioned that inflorescence of *I. tinctoria* was a spike and that contradicts the current finding. The length of inflorescence varied between 4.56 and 15.25 cm with a mean value being 8.94 ± 3.23 cm in three species (Table 3). Inflorescence was much longer in *I. amblyantha* (15.25 cm) than in *I. tinctoria* (7.00 cm) and *I. suffruticosa* (4.56 cm) (Table 3, Fig. 2i).

Flowers are complete, zygomorphic, irregular, bisexual, hypogynous and pedicellate, sepals 5, gamosepalous in all the three species (Fig. 2.i-ii).

Corolla characteristically papilionaceous, petals 5 but the colour varied in three species. The colour of wing was pink, crimson and cerise-violet in *I. tinctoria*, *I. suffruticosa* and *I. amblyantha*, respectively (Table 1, Fig. 2.ii).

Different species varied also in size of the petals of corolla *viz*., standard, wing and keel. Standard area was much larger in *I. amblyantha* (70.3 mm^2) than in *I. tinctoria* (22.4 mm^2) and *I. suffruticosa* (13.6 mm^2) (Table 3, Fig. 2.ii). Wing area was also larger in *I. amblyantha* (15.8 mm^2) than in *I. tinctoria* (9.6 mm^2) and *I. suffruticosa* (4.5 mm^2) (Table 3, Fig. 2.ii). Like standard and wing, the keel area was also greater in *I. amblyantha* (62.8 mm^2) than *I. tinctoria* (19.8 mm^2) and *I. suffruticosa* (17.7 mm^2) (Table 3, Fig. 2.ii). The tip of keel was coloured only in *I. amblyantha* (Fig. 2.ii).

Stamens are diadelphous in all the species but length of stamen varied between 3.67 and 9.62 mm with a mean value being 6.25 ± 1.76 mm (Table 3). Stamen length was greater in *I. amblyantha* (9.62 mm) than in *I. tinctoria* (5.47 mm) and *I. suffruticosa* (3.67 mm) (Table 3).

Ovary was superior, narrow and tubular with marginal placentation, 1-celled and many ovules in all the species but length of ovary varied between 2.96 and 8.00 mm with a mean value being 4.97 ± 1.53 mm (Table 3). The length of ovary was greater in *I. amblyantha* (8.00 mm) than *I. tinctoria* (3.97 mm) and *I. suffruticosa* (2.96 mm) (Table 3, Fig. 2.ii).

Types of inflorescence and colour of corolla in the current study were almost similar observation described by Bailey (1961), Howard (1988) and Ellison (1999). However, besides the qualitative description of the flowers, the detailed quantitative measurement of floral parts especially the area of standard and wings are made in the current study and are distinctly different between the three species. Such measurements would certainly be useful for species identification.

Pod and seed: Pods were narrow cylindrical, brown coloured with smooth surface and slightly curved at the tip in *I. tinctoria*; deep brown coloured with densely hairy surface, dehiscent in the suture and strongly curved in *I. suffruticosa*; thick, cylindrical, straight, dark brown coloured with crack surface in *I. amblyantha* at maturity (Table 1, Fig. 2.iii). Number of pods per inflorescence was greater in *I. suffruticosa* (29) than in *I. amblyantha* (16.4) and *I. tinctoria* (11.5) (Table 4). Variation in pod length was greater (14.5-32.8 mm) than its diameter (2.72-4.38 mm) with the volume of pod varied between 91.1 and 498 mm^3 (Table 4). Pod volume was three times larger in *I. amblyantha* (498 mm^3) than in the *I. tinctoria* (166.1 mm^3) and about five and half

times larger than *I. suffruticosa* (91.1 mm^3) (Table 4, Fig. 2.iii).

The current morphological features on length and curvature of pod were also similar with others (Simon *et al*., 1984; Howard, 1988). But the comparative length, breadth and especially the volume of pods, in the current study, in the three species would provide an index of species identification.

Different species varied also in colour, shape and size of seed. Seeds were deep brown, small, solid, cylindrical in *I. tinctoria*; dark green, small, solid cylindrical in *I. suffruticosa*; and greenish yellow, flattened and globose in *I. amblyantha* (Table 1, Fig. 2.iv). Number of seeds per pod was greater in *I. amblyantha* (12.3) than in *I. tinctoria* (7.5) and *I. suffruticosa* (4.0) but seed size (weight of 1000-seed) was greater in *I. tinctoria* (5.79 g) than in *I. amblyantha* (4.72 g) and *I. suffruticosa* (3.75 g) (Table 4).

Table 3. Variation in morphological features (quantitative) of inflorescence and flower in three *Indigofera* spp.

Species	Time of flowering	Length of inflorescence (cm)	Size of standard	Size of wing	Size of keel	Length of stamen (filament + anther) (mm)		Length of carpel (mm)	Length of ovary (mm)
			L × B (mm^2)	L × B (mm^2)	L × B (mm^2)	(9) + 1			
I. tinctoria	2 to 3 months after planting	7.00 †	22.4	9.6	19.8	5.47	3.88	5.46	3.97
I. suffruticosa	September to March	4.56	13.6	4.5	17.7	3.67	3.07	3.93	2.96
I. amblyantha	September to November	15.25	70.3	15.8	62.8	9.62	7.75	10.62	8.00
Mean	-	8.94	35.43	9.96	33.43	6.25	4.90	6.67	4.97
Range	-	4.56-15.25	13.6-70.3	4.5-15.8	17.7-62.8	3.67-9.62	3.07-7.75	3.93-10.62	2.96-8.00
SEM ±	-	3.23	17.61	3.26	14.69	1.76	1.44	2.02	1.53

†: Each figure is the mean of at least 20 samples.

Table 4. Variation in morphological features (quantitative) of pod and seeds in three *Indigofera* spp.

Species	Pod inflorescence^{-1} (No.)	Length of pod (mm)	Diameter of pod (mm)	Volume of pod (mm^3)	Seed pod^{-1} (No.)	1000-seed/g
I. tinctoria	11.5†	28.9	2.72	166.1	7.5	5.79
I. suffruticosa	29.0	14.5	2.82	91.1	4.0	3.75
I. amblyantha	16.4	32.8	4.38	498.0	12.3	4.72
Mean	18.96	25.4	3.30	251.73	7.93	4.75
Range	11.5-29	14.5-32.8	2.72-4.38	91.1-498	4-12.3	3.75-5.79
SEM ±	5.21	5.56	0.53	125	2.4	0.55

†: Each figure is the mean of at least 20 samples.

Several authors (Bailey, 1961; Simon *et al*., 1984 and Howard 1988) described only the size and shape of the pods of three Indigo species. However, in the present investigation, distinct colour and quantitative measurement of pod and seed, and seed size of the three species are provided. This would again be useful for identifying characters of the three species of indigo. Results conclude that *Indigofera tinctoria* is identified by obovate leaflets, pink coloured flower, narrowly cylindrical medium pod (average volume 166.1 mm^3) with slightly curved tip and brown large seed (average 5.79 g/1000-seed); *I suffruticosa* is recognised by patches of reddish colour on green stem, narrowly oblong leaflet, crimson coloured flower, strongly curved short pod (average volume 91.1 mm^3), and dark green small seed (average 3.75 g/1000-seed); *I amblyantha* is diagnosed by ovate to lanceolate leaflet, cerise-violet coloured flower, thick cylindrical long pod (average volume 498 mm^3) and greenish yellow medium seed (average 4.72 g/1000-seed).

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