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Floral morphology and seed yield in two *Indigofera* spp. as affected by shoot clipping

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Abstract

An experiment was carried out to know the effect of shoot clipping on floral morphology and seed yield in two *Indigofera* spp. *viz. I. tinctoria* L. and *I. suffruticosa* Mill. All parts of shoot including the main stem were clipped at 60 cm height from the base at 120 days after sowing (DAS). At 120 DAS, *I. tinctoria* already exhibited 1st flowering while *I. suffruticosa* showed no flowering. Shoot clipping had significant effect on almost all floral morphological features in two *Indigofera* spp. Flower raceme^{-1} and pod raceme^{-1} were fewer in clipped plant than in unclipped one. Shoot clipping altered the size of different floral parts to a great extent. The seed yield was decreased in clipped plant than that of unclipped one. Shoot clipping decreased flower and pod size and seed yield in two *Indigofera* spp.

Keywords: *Indigofera* spp., Shoot clipping, Floral morphology, Seed yield

Introduction

Indigofera is the third largest genus in legumes comprising *ca*. 750 species (Schrire, 2005) under the family Leguminosae and 19th (Mabberley, 1997) to 26th (Stevens, 2001) in size of all angiosperm genera. These species are grown in many countries of the world as ornamental, for production of indigo dye and also as herbal medicine (Ellison, 1999; Puri *et al.*, 2007; Luiz-Ferreira *et al.*, 2011). *Indigofera tinctoria* L. and *I. suffruticosa* Mill. are the two most important species frequently used to produce the indigo dye. Natural dyes are environmentally friendly and distinctive washing and sunlight fade-resistant than synthetic ones (Angelini *et al*., 1997).

Although the tale of indigo in this region (northern Bangladesh and parts of West Bengal) dates back to ancient times, *Indigofera* has been cultivated now only in small patches of Nilphamari district in Bangladesh as green manure, fuel crop, hedge plant, etc. (Anon., 2007). The resurgence of interest for natural dye to replace synthetic ones revives the cultivation probabilities of *Indigofera* for commercial dye manufacture in Bangladesh. Some research works have been conducted on different aspects of *Indigofera* production *viz*. morphological variations (Khan *et al*., 2008; Jahan *et al*., 2012); or to find out ways to increase biomass production by manipulating planting density and shoot clipping height (Rokonuzzaman, 2003), or by shoot clipping and Gibberellic acid application (Kabir, 2003). Previously, we reported the variation in phenology, floral and pollen morphology, and seed yield between two *Indigofera* spp. (Jahan *et al*., 2013). Clipping a portion of the shoot is one of the most important means for getting additional vegetative biomass yield and means of increasing plant growth (Costa *et al.,* 1992; Rokonuzzaman, 2003; Howlader, 2013). The current research investigated the effect of shoot clipping on floral morphological features and seed yield in *Indigofera* spp.

Materials and Methods

The experiment was conducted at the Field, and Plant Morphology Laboratory of the Department of Crop Botany, Bangladesh Agricultural University, Mymensingh, during the period from April to December, 2011. The experiment was carried out following a randomized complete block design (RCBD) with three replications. The seeds of two *Indigofera* spp. *viz. I. tinctoria* and *I. suffruticosa*, were sown at 2–3 cm depth in the rows 30 cm apart. Emergence of seedling commenced at 4–5 days after sowing (DAS). Seedlings were thinned to maintain 15 cm distance between two adjacent seedlings. Different intercultural operations were done as and when necessary. It was found that shoot clipping at 60 cm height at 120 DAS resulted in higher biomass yield (Kabir, 2003; Rokonuzzaman, 2003). Therefore, the shoot clipping was done at 120 DAS where all the shoots of plants were clipped at 60 cm height from the base to maximize biomass yield for dye production. Final harvesting was done at 240 DAS.

Morphological features (colour, size and shape) of different floral parts (corolla, androecium and gynoecium), pod and seed were studied. The general morphological features of reproductive parts in two species are presented in Table 1. Quantitative data were recorded from randomly selected at least 5 individual plants of two species. Volume of the pod was determined following Khan *et al*. (2008). Collected data were statistically analyzed by using the computer software program MSTAT-C and the difference between means was adjudged by Duncan's New Multiple Range Test (DMRT).

Table 1. Variation in morphological (reproductive) features in two *Indigofera* spp.

Characters	Species	
	Indigofera tinctoria	*Indigofera suffruticosa*
Inflorescence	Axillary raceme	Axillary raceme
Flower colour	Pink	Crimson
Pod colour	Green, it turns to brown when ripen	Reddish green, it turns to brown when ripen
Pod at maturity	Smooth surface, indehiscent	Densely hairy surface, dehiscent
Pod shape	Narrow cylindrical, slightly curved at the tip	Strongly curved
Seed shape	Small, solid cylindrical	Smaller than *I. tinctoria*, solid cylindrical
Seed colour	Brown (mixed with some light brown and black seed)	Dark green (mixed with some brown and black seed)

Results

Effect of shoot clipping on morphological features of flower in two *Indigofera* spp.

Flower per raceme: Flower raceme^{-1} significantly varied with clipping treatment and the species studied. It was greater in unclipped plant (41.68) than in clipped plants (32.75) and in *I. suffruticosa* (39.40) than in *I. tinctoria* (35.03) (Table 2). Flower raceme^{-1} was the highest in both unclipped *I. tinctoria* and *I. suffruticosa* (av. 41.69) and the lowest in clipped *I. tinctoria* (28.00) with the intermediate in clipped *I. suffruticosa* (37.50) (Table 3).

Table 2. Effects of shoot clipping and species on morphological features of flower in two *Indigofera* spp. at 145 DAS. L: Length, B: Breadth

Treatments	Flower Raceme^{-1} (No.)	Standard Size (mm^2) L×B	Wing Size (mm^2) L×B	Keel Size (mm^2) L×B	Stamen Length (mm) (9) +	Stamen Length (mm) 1	Carpel Length (mm)	Ovary Length (mm)
Shoot clipping								
Unclipped	41.68 a	15.89	5.83 b	16.31 a	4.60	3.64	4.73 a	3.60
Clipped	32.75 b	16.09	6.14 a	16.09 b	4.55	3.63	4.56 b	3.52
Species								
I. tinctoria	35.03 b	18.82 a	7.67 a	17.11 a	5.33 a	4.07 a	5.32 a	4.02 a
I. suffruticosa	39.40 a	13.16 b	4.30 b	15.29 b	3.82 b	3.20 b	3.97 b	3.10 b
$LSD_{0.05}$	2.24	0.70	0.25	0.19	0.06	0.14	0.11	0.09

In a column, figures bearing different letters differ significantly at $P \leq 0.05$ by DMRT.

Size of standard: The size of standard significantly varied with species. It did not vary with shoot clipping. Interaction effect of species and shoot clipping for size of standard was significant. The standard was larger in *I. tinctoria* (18.82 mm^2) than in *I. suffruticosa* (13.16 mm^2) (Table 2). Size of standard was the largest in clipped *I. tinctoria* (19.49 mm^2) and the lowest value was recorded in clipped *I. suffruticosa* (12.69 mm^2) (Table 3).

Table 3. Interaction effects of shoot clipping and species on morphological features of flower in two *Indigofera* spp. at 145 DAS. L: Length, B: Breadth

Treatments	Species	Flower Raceme^{-1} (No.)	Standard Size (mm^2) L×B	Wing Size (mm^2) L×B	Keel Size (mm^2) L×B	Stamen Length (mm) (9) +	Stamen Length (mm) 1	Carpel Length (mm)	Ovary Length (mm)
Control	*I. tinctoria*	42.07 a	18.16 b	7.27 b	17.08 a	5.29 a	4.00 a	5.47 a	4.00 a
	I. suffruticosa	41.30 a	13.63 c	4.40 c	15.55 b	3.92 b	3.28 b	4.00 c	3.22 b
Clipped	*I. tinctoria*	28.00 c	19.49 a	8.07 a	17.15 a	5.37 a	4.15 a	5.17 b	4.05 a
	I. suffruticosa	37.50 b	12.69 c	4.21 c	15.04 c	3.73 c	3.13 b	3.95 c	3.00 c
$LSD_{0.05}$		3.17	0.99	0.36	0.27	0.09	0.20	0.15	0.13

In a column, figures bearing different letters differ significantly at $P \leq 0.05$ by DMRT

Size of wing: Size of wing significantly varied with shoot clipping and with species. The wing was larger in clipped plants (6.14 mm^2) than in unclipped plants (5.83 mm^2). The wing was larger in *I. tinctoria* (7.67 mm^2) than in *I. suffruticosa* (4.30 mm^2) (Table 2). It was largest in clipped *I. tinctoria* (8.07 mm^2) and smallest in both unclipped and clipped *I. suffruticosa* (av. 4.30 mm^2) (Table 3).

Size of keel: Size of keel was greater in unclipped plant (16.31 mm^2) than in clipped plant (16.09 mm^2). Size of keel was greater in *I. tinctoria* (17.11 mm^2) than in *I. suffruticosa* (15.29 mm^2). Size of keel was greatest in both unclipped and clipped *I. tinctoria* (17.08 and 15.17 mm^2, respectively) and lowest in clipped *I. suffruticosa* (15.04 mm^2) (Table 3).

Length of stamen: Shoot clipping had no significant effect on length of stamen. There was significant difference in length of stamen between the two species. Stamen was longer in *I. tinctoria* (5.33 mm) than in *I. suffruticosa* (3.20 mm) (Table 2). Interaction effect of clipping and species for length of stamen was significant. The stamen was the longest in both unclipped and clipped *I. tinctoria* (av. 5.33 mm) than in the other treatments (Table 3).

Length of carpel: There was significant difference in length of carpel between shoot clipping treatments, and between the two *Indigofera* spp. The interaction effects of clipping and species are significant. Carpel had greater length in unclipped plant (4.73 mm) than in clipped plant (4.56 mm); and in *I. tinctoria* (5.32 mm) than in *I. suffruticosa* (3.97 mm) (Table 2). The length of carpel was the greatest in unclipped *I. tinctoria* (5.47 mm) and the lowest in both unclipped and clipped *I. suffruticosa* (av. 3.98 mm) (Table 3).

Length of ovary: There was no significant difference in length of ovary between the two shoot clipping treatments. Length of ovary varied significantly with the species. Ovary was greater in *I. tinctoria* (4.02 mm) than in *I. suffruticosa* (3.10 mm) (Table 2). Interaction effect of shoot clipping and species on length of ovary was significant. Ovary was the longest in both unclipped and clipped *I. tinctoria* (av. 4.02 mm) and the smallest in clipped *I. suffruticosa* (3.00 mm) (Table 3).

Effect of shoot clipping on morphological features of pod and seed in two *Indigofera* spp.

Pod per raceme: Effects of shoot clipping and species on number of pod raceme^{-1} were significant. Pod raceme^{-1} was greater in unclipped one (18.16) than in clipped one (16.98) and in *I. suffruticosa* (24.43) than in *I. tinctoria* (10.71) (Table 4). The interaction effect of shoot clipping and species on pod raceme^{-1} was significant. The pod raceme^{-1} was the greatest in unclipped *I. suffruticosa* (24.90) and the lowest in clipped *I. tinctoria* (10.00) (Table 5).

Pod length: Pod length was greater in unclipped plant (21.30 mm) than in clipped plant (20.77 mm). Pod length was greater in *I. tinctoria* (28.06 mm) than in *I. suffruticosa* (14.01 mm) (Table 4). Pod length was the highest in unclipped *I. tinctoria* (28.41 mm) and the lowest in clipped *I. suffruticosa* (13.83 mm) (Table 5).

Pod diameter: There was significant difference in pod diameter between the two *Indigofera* spp. Pod diameter was greater in *I. suffruticosa* (2.80 mm) than in *I. tinctoria* (2.57 mm) (Table 4). There was no significant difference in pod diameter between the shoot clipping treatments; interaction effect of shoot clipping and species was also significant (Tables 4 and 5).

Table 4. Effects of shoot clipping and species on morphological features of pod and seed in two *Indigofera* spp. L: Length, B: Breadth

Treatments	Pod Raceme^{-1} (No.)	Pod Length (mm)	Pod Diameter (mm)	Pod Volume (mm^3)	Seed Pod^{-1} (No.)	Seed size L×B (mm^2)	1000-seed weight (g)	Seed yield (g plant^{-1})
Shoot clipping								
Unclipped	18.16 a	21.30 a	2.68	109.89 b	5.75	2.70 b	4.63	13.17 a
Clipped	16.98 b	20.77 b	2.69	115.85 a	5.66	2.80 a	4.64	8.78 b
Species								
I. tinctoria	10.71 b	28.06 a	2.57 b	139.00 a	7.25 a	3.33 a	5.62 a	14.7 a
I. suffruticosa	24.43 a	14.01 b	2.80 a	86.74 b	4.17 b	2.19 b	3.64 b	7.2 b
$LSD_{0.05}$	0.09	0.09	0.13	5.25	0.10	0.06	0.11	0.35

In a column, figures bearing different letters differ significantly at P ≤ 0.05 by DMRT

Table 5. Interaction effects of shoot clipping and species on morphological features of pod and seed in two *Indigofera* spp. L: Length, B: Breadth

Treatments	Species	Pod Raceme^{-1} (No.)	Pod Length (mm)	Pod Diameter (mm)	Pod Volume (mm^3)	Seed Pod^{-1} (No.)	Seed size L×B (mm^2)	1000-seed weight (g)	Seed yield (g plant^{-1})
Control	*I. tinctoria*	11.43 c	28.41 a	2.56	131.00 b	7.30 a	3.27 a	5.55 a	18.67 a
	I. suffruticosa	24.90 a	14.20 c	2.82	88.81 c	4.22 b	2.14 b	3.71 b	7.67 c
Clipped	*I. tinctoria*	10.00 d	27.72 b	2.60	147.00 a	7.20 a	3.40 a	5.70 a	10.82 b
	I. suffruticosa	23.97 b	13.83 d	2.79	84.68 c	4.13 b	2.25 b	3.58 b	6.75 d
$LSD_{0.05}$		0.13	0.13	0.93	7.42	0.73	0.46	0.16	0.49

In a column, figures bearing different letters differ significantly at P ≤ 0.05 by DMRT

Pod volume: There was significant difference in pod volume between the shoot clipping treatments and also between the species. Pod volume was greater in clipped plant (115.85 mm^3) than in unclipped one (109.89 mm^3) and in *I. tinctoria* (139.00 mm^3) than *I. suffruticosa* (86.74 mm^3) (Table 4). Interaction effect of clipping and species for pod volume was significant. Clipped *I. tinctoria* showed highest pod volume (147.00 mm^3) and both unclipped and clipped *I. suffruticosa* showed the lowest pod volume (av. 86.75 mm^3) (Tables 4 and 5).

Seed per pod: There was no significant difference in seed pod^{-1} between the shoot clipping treatments (Table 4). Significant difference in seed pod^{-1} was also found between the two *Indigofera* species. The interaction effect of shoot clipping and species for seed pod^{-1} was also significant. Seed pod^{-1} was greater in *I. tinctoria* (7.25) than in *I. suffruticosa* (4.17) (Table 4). It was the greatest in both unclipped and clipped *I. tinctoria* (av. 7.25) and the lowest in both unclipped and clipped *I. suffruticosa* (av. 4.18) (Table 5).

Seed size: Shoot clipping and species had significant effects on seed size. Seed size was greater in clipped plant (2.80 mm^2) than in unclipped plant (2.70 mm^2) and in *I. tinctoria* (3.33 mm^2) than in *I. suffruticosa* (2.19 mm^2) (Table 4). The interaction effect of shoot clipping and species for seed size was significant. Seed size was the highest in both unclipped and clipped *I. tinctoria* (av. 3.34 mm^2) and the lowest in both unclipped and clipped *I. suffruticosa* (av. 2.20 mm^2) (Table 5).

1000-seed weight: There was no significant difference in 1000-seed weight between the two shoot clipping treatments (Table 4). Thousand seed weight was also significantly different between the two species. The interaction effect of shoot clipping and species for 1000-seed weight was also significant. *Indigofera tinctoria* (5.62 g) had greater 1000-seed weight than in *I. suffruticosa* (3.64 g) (Table 4). Thousand seed weight was higher in clipped *I. tinctoria* (5.70 g) than clipped *I. suffruticosa* (3.58 g) (Table 5).

Seed yield: Seed yield significantly varied with shoot clipping and with species. The interaction effect of shoot clipping and species for seed yield was also significant. Seed yield was greater in unclipped plant (13.17 g plant^{-1}) than in clipped one (8.78 g plant^{-1}) (Table 4) and in *I. tinctoria* (14.70 g plant^{-1}) than in *I. suffruticosa* (7.20 g plant^{-1}) (Table 4). Seed yield was the highest in unclipped *I. tinctoria* (18.67 g plant^{-1}) and the lowest in clipped *I. suffruticosa* (6.75 g plant^{-1}) (Table 5).

Discussion

Shoot clipping had adverse effect on floral morphology in the both *Indigofera* spp. (Tables 2 and 3), although it increases biomass production in indigo plant (Kabir, 2003; Rokonuzzaman, 2003). Shoot clipping results in loss of leaves, the source of supplying assimilates to sink—flowers, developing pods and seeds in legumes (Hossain *et al*., 2006a,b; Mondal *et al*., 2011). Shoot clipping may, therefore, influence TDM production, and seed and biomass yield through assimilate production and its partitioning into different parts depending on the magnitude of source loss (Hossain *et al*., 2006a,b; Mondal *et al*., 2011). The clipped plants showed fewer flower raceme^{-1} than in unclipped plant (Tables 2 and 3). Shoot clipping also altered the size of different floral parts to a great extent, and had an adverse effect on seed yield in indigo (Tables 4 and 5). Yield is the function of number of flowers raceme^{-1} x number of pods raceme^{-1} x number of seeds pod^{-1} x 1000-seed weight (Mondal *et al*., 2011). The clipped plants exhibited fewer flower raceme^{-1} which resulted in fewer pods raceme^{-1} and finally lower seed yield (g plant^{-1}) in clipped indigo plants. In cowpea, partial source removal induces increase in pod and seed yield (Hossain *et al*., 2006a,b) through the production of higher flowers plant^{-1} with reduced rate of floral abscission (Hossain *et al*., 2006b). In the present study, shoot clipping was done during and/or around first flowering (Jahan *et al*., 2013). Therefore, the flowering was delayed and seed formation was also delayed leading to a reduced seed yield in clipped plants in two *Indigofera* spp. Moreover, the shoot clipping treatments invariably cause a reduction in the amount of biomass partitioned to the reproductive organs, and affects flowering (Gutman *et al.*, 2001). Leaf clipping at flower initiation stage induces the capacity to compensate source loss through the re-growth of leaves in soybean (Board and Harville, 1998; Borras *et al*., 2004). Higher rate of photosynthesis in remaining leaves of partially clipped plants compared to the leaves in intact plants (Rao and Ghildiyal, 1985) may contribute the compensation to source loss by clipping. However, leaf clipping did not affect seed yield in high yielding mungbean genotypes while it reduced yield in low yielding one (Fakir, M.S.A., personal observation).

The variations in floral morphology and seed yield exist between the species (Tables 3 and 5; Jahan *et al*., 2012). Shoot clipping had more adverse effect in *I. tinctoria* especially on seed yield compared to in *I. suffruticosa* (Table 5). The variations between the species in response to clipping might came from the difference in growth rate between the species (Jahan *et al*., 2013). The pods raceme^{-1} was significantly smaller in *I. tinctoria* than in *I. suffruticosa* both in clipping and control conditions, although shoot clipping increases fruit setting percentage in both the species (Tables 3 and 5). At 120 DAS when clipping treatment was done, *I. tinctoria* already exhibited 1st flowering while *I. suffruticosa* showed no flowering (Jahan *et al*., 2013). Therefore, clipping in this experiment produced fewer flowers and pods and finally lowered the seed yield in *I. tinctoria*.

It can be concluded that shoot clipping adversely affected size of flower and pod and seed yield in both of the two *Indigofera* spp.

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