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PHENOLOGY, FLORAL MORPHOLOGY AND SEED YIELD IN *INDIGOFERA TINCTORIA* L. AND *I. SUFFRUTICOSA* MILL.

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Abstract

Phenology, floral and pollen morphological features and seed yield were studied in *Indigofera tinctoria* L. and *I. suffruticosa* Mill. *Indigofera tinctoria* bloomed three times i.e., mid-July, mid-August and early October during the study period. But *I. suffruticosa* bloomed only once i.e., in early September. The number of flowers/raceme and pods/raceme were higher in *I. suffruticosa* than those in *I. tinctoria*. However, the number of seeds/pod, seed size, 1000-seed weight and seed yield were higher in *I. tinctoria*. Pollen with auriculae near the aperture in *Indigofera* is being reported here for the first time. Size and exine thickness of pollen grains were higher in *I. tinctoria* than in *I. suffruticosa*. The highest number of flowers/raceme and seeds/plant were produced at the second flush in *I. tinctoria*. Therefore, *I. tinctoria* plants may be harvested after the maturity of pods from second flush for higher seed yield.

Introduction

Indigo is one of the ancient blue dyes used by man for textiles. The indigo dye comes from plants of the genus *Indigofera* L., the third largest genus in the family Leguminosae (sub-family Papilionoidae), comprises *ca*. 750 species (Schrire 2005). *Indigofera* spp. are grown in many countries of the world as ornamental, for production of indigo dye and also as herbal medicine (Ellison 1999, HealthLink 2001, Puri *et al*. 2007, Luiz-Ferreira *et al*. 2011). Indigo plant may be used as a cover crop and for green manure and erosion control in coffee, tea and rubber plantations (Rich *et al*. 2003). Moreover, recently there has been a resurgence of interest in natural dye to replace synthetic ones. *Indigofera tinctoria* and *I. suffruticosa* are the two most important species frequently used to produce natural blue dye commercially.

In Bangladesh *Indigofera* has been cultivated only in small patches of Nilphamari district as green manure and fuel crop, hedge plant, etc. Although quantitative measurement of some morphological characters *viz*. size of leaf and leaflet, flower and pod in three *Indigofera* species are available (Khan *et al*. 2008), a little is known on phenology, floral morphology and seed yield (Jahan *et al*. 2012, 2013). Knowledge of phenology and floral morphology both are essential for conducting research on breeding programmes. Therefore, the present study was undertaken to study the variation on phenology, floral and pollen morphology, and seed yield between *I. tinctoria* L. and *I. suffruticosa* Mill. Besides, the effect of different flushing time on floral and pollen morphological features, and seed yield of *I. tinctoria* and suitable harvesting time for higher seed yield in *I. tinctoria* were also studied.

Materials and Methods

An experiment was conducted from April to December, 2011. The (two factorial *viz*. species and time of flushing) experiment was laid out following a randomized complete block design (RCBD) with three replications. The seeds of *I. tinctoria* and *I. suffruticosa*, collected from the previous experiment were sown at 2 - 3 cm depth at continuous in the row. Distance between the

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two rows was 30 cm. 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 (Howlader 2013). Final harvesting was done at 240 DAS after the maturation of pods (more than 80% of total number of pods attained characteristics brown colour). Morphological features (colour, size and shape) of different floral parts (corolla, androecium and gynoecium), pods and seeds were studied. Data on both qualitative and quantitative measurements were recorded from randomly selected at least five individual samples of two species. Volume of the pod was determined following Khan *et al*. (2008). 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). Floral, pod and pollen morphological data of *I. tinctoria* have been presented in Tables 1 - 3 as the average of three flushing.

Unopened flowers (buds) from the two *Indigofera* species were collected for pollen morphological study in their respective flowering time. These flower buds were preserved with 50% ethanol solution. Pollen grains were collected from the anther of the buds by teasing with forceps and needle. Pollen grains were mounted in glycerine and examined and measured with a light microscope. The photomicrographs of pollen grains were taken by using an OLYMPUS BX41 microscope fitted with a digital camera. Polar length, equatorial diameter and exine thickness of pollen grains were measured following Perveen and Qaiser (1998) and Masih *et al*. (2005) and the P/E ratio was calculated. The terminology for pollen description was used following Erdtman (1952) and Punt *et al*. (2007).

Results and Discussion

Variation in phenology, floral morphological features and seed yield: *Indigofera tinctoria* started flowering within 2 - 3 months (79 DAS; data not shown) after planting and continued throughout the year. Flowering (flushing) in *I. tinctoria* occurred thrice – at mid-July, mid-August, and early October. On the other hand, *I. suffruticosa* flowered only once at early September during the study period. Both the species reached to a full blooming (80%) within 10 to 15 days after the flowering has been started (data not shown). The number of flowers/raceme was greater in *I. suffruticosa* (41.29) than in *I. tinctoria* (30.45) (Table 1). The size of different members of corolla *viz*. standard, wing and keel, was also distinctly different. The size of standard and wing were greater in *I. tinctoria* (18.65 mm^2 and 7.64 mm^2, respectively) than in *I. suffruticosa* (13.63 mm^2 and 4.40 mm^2, respectively) (Table 1). However, there was no significant difference in keel size in the two *Indigofera* species. Stamens were longer in *I. tinctoria* (5.10 mm) than in *I. suffruticosa* (3.92 mm). The length of carpel and ovary were greater in *I. tinctoria* (5.35 and 4.02 mm, respectively) than in *I. suffruticosa* (4.00 and 2.88 mm, respectively) (Table 1).

Table 1. Variation in morphological features of flower in two *Indigofera* spp.

Species	Flower/ raceme (No.)	Standard (mm^2)	Wing (mm^2)	Keel (mm^2)	Length of stamen (mm)		Length of carpel (mm)	Length of ovary (mm)
		L×B	L×B	L×B	(9)+	1		
*I. tinctoria**	30.45 b	18.65 a	7.64 a	16.38	5.10 a	4.10 a	5.35 a	4.02 a
I. suffruticosa	41.29 a	13.63 b	4.40 b	15.55	3.92 b	3.28 b	4.00 b	2.88 b
$LSD_{0.05}$	10.41	0.84	0.70	1.02	0.18	0.15	0.19	0.90

*Average of three flushing time. In a column, figures having different letter(s) differ significantly ($p \leq 0.05$) according to DMRT. L: Length, B: Breadth.

In *I. tinctoria*, pod was green in colour and it turned to brown when it ripened. The pod is narrow cylindrical, slightly curved at the tip, at maturity indehiscent with smooth surface. In *I. suffruticosa*, pod colour was reddish green and it turned reddish green to brown when it ripened and strongly curved, dehiscent at the middle with densely hairy surface. There was significant variation in pod/raceme between the two *Indigofera* spp. The number of pods/raceme was higher in *I. suffruticosa* (24.90) than in *I. tinctoria* (8.91) (Table 2). On the other word, fruit (pod) setting percentage was significantly higher in *I. suffruticosa* (60.3) than in *I. tinctoria* (29.3). The pod length and volume were also greater in *I. tinctoria* (27.32 mm and 124.10 mm^3, respectively) than in *I. suffruticosa* (14.20 mm and 88.81 mm^3, respectively). However, the pod diameter was greater in *I. suffruticosa* (2.82 mm) than in *I. tinctoria* (2.40 mm) (Table 2).

Table 2. Variation in morphological features of pod and seed in two *Indigofera* spp.

Species	Pod/ raceme (No.)	Pod length (mm)	Pod diameter (mm)	Pod volume (mm^3)	Seed/ pod (No.)	Seed length (mm)	Seed breadth (mm)	Seed size (mm^2)	1000-seed wt. (g)	Seed yield (g/ plant)
*I. tinctoria**	8.91 b	27.32 a	2.40 b	124.10 a	7.09 a	2.23 a	1.37 a	3.08 a	5.43 a	17.39 a
I. suffruticosa	24.90a	14.20 b	2.82 a	88.81 b	4.22 b	1.67 b	1.28 b	2.14 b	3.71 b	7.67 b
$LSD_{0.05}$	2.21	2.22	0.20	23.57	0.50	0.17	0.05	0.31	0.23	1.29

*Average of three flushing time. In a column, figures having different letter(s) differ significantly ($p \leq 0.05$) according to DMRT.

The seeds in *I. tinctoria* are small, solid, cylindrical, and mostly brown in colour. *Indigofera suffruticosa* seeds are solid, cylindrical, dark green in colour, and smaller than those of *I. tinctoria*. Seed/pod had a significant effect on the variation of seed yield in two *Indigofera* spp. Seed/pod was greater in *I. tinctoria* (7.09) than in *I. suffruticosa* (4.22). Seed size was greater in *I. tinctoria* (3.08 mm^2) than in *I. suffruticosa* (2.14 mm^2). Thousand seed weight was higher in *I. tinctoria* (5.43 g) than in *I. suffruticosa* (3.71 g). There was significant difference in seed yield in two *Indigofera* spp. Seed yield was greater in *I. tinctoria* (17.39 g/plant) than in *I. suffruticosa* (7.67 g/plant) (Table 2).

The present study showed significant variation in phenology, floral morphological features – flower/raceme, length of standard, wing, stamen, carpel and ovary, pod size, seed/pod, seed size, and seed yield between *I. tinctoria* and in *I. suffruticosa* (Tables 1, 2). Khan *et al*. (2008) also reported almost similar variation in flower, pod and seed morphology. In all the previous literature, the inflorescence has been described as axillary raceme in both the species, except in Ellison (1999). Ellison (1999) mentioned that inflorescence of *I. tinctoria* was a spike. The flower/raceme, flower colour and size, pod/raceme, pod size, seed/pod and seed size varied between two *Indigofera* spp. The higher fruit setting percentage (lower floral abscission in other words) in *I. suffruticosa* might be due to genetic make-up of the species (Schrire *et al*. 2009) and the environmental factors as well. The *I. suffruticosa* flowered in early September when day-length was relatively shorter with lower atmospheric temperature (data not shown). The higher seed yield in *I. tinctoria* might be due to greater pod size, seed/pod and 1000 seed weight (Table 2).

Variation in pollen morphological features: Pollen grains are monads, medium in size (Table 3); prolate spheroidal in shape (Figs 1A, B), triangular in polar view (Figs 1C, H); 3-colpor(oid)ate, auriculae-like structure present near colpus (aperture) (Figs 1D-H); tected, exine sculpture reticulate in the both species, nexine thicker than sexine (Fig. 1). Both polar length and equatorial diameter were larger in *I. tinctoria* (35.01 and 32.45 µm, respectively) than in *I. suffruticosa* (31.68 and 29.21 µm, respectively). However, the P/E ratio was slightly higher in *I. suffruticosa* (1.09) than in *I. tinctoria* (1.08). The exine was thicker in *I. tinctoria* (1.74 µm) than in *I. suffruticosa* (1.72 µm) (Table 3).

The variation in size and exine thickness of pollen grains of two *Indigofera* spp. might be due to genetic reason that is similar to the description given by Schrire *et al.* (2009). The shape of pollen grains of *I. tinctoria* is prolate-spheroidal (Table 3, Fig. 1A), but reported as almost round by Nwachukwu and Edeoga (2006). Previously, auriculate pollen has not been reported for any *Indigofera* species, although auriculate pollen is observed in *I. tinctoria* from photographs in an online resource (http://www-saps.plantsci.cam.ac.uk/pollen/pollen/pages/Indigofera%20tinctoria.htm). For the first time, we have observed and reported the presence of auriculae-like structures near colpus region in both *Indigofera* species (Figs 1D-H).

Variation in floral, pollen morphological features and seed yield at different time of flush in I. tinctoria: The highest number of flower/raceme (42.07) was recorded in mid-August flushing than other time of flush (30.50 in mid-July and 18.78 in early October) (Table 4). Standard and wing size were highest in mid-July time of flush (20.30 mm^2 and 9.21 mm^2, respectively) than in mid-August (18.16 mm^2 and 7.27 mm^2, respectively) and in early October (17.48 mm^2 and 6.45 mm^2, respectively). Size of keel was highest in mid-August (17.08 mm^2) than other time of flush. Length of stamen was highest in mid-July time of flush (5.37 mm) and lowest in mid-August (4.00 mm). Length of carpel was highest in mid-July (5.58 mm) time of flush. There was no significant difference in ovary length in three time of flush of *I. tinctoria* (Table 4).

Table 3. Variation in palynological features of two *Indigofera* spp. expressed as mean value (in µm) ± standard deviation. Range (in µm) is shown within parenthesis.

Species	Polar length (P)	Equatorial diameter (E)	P/E	Exine thickness
*I. tinctoria**	35.01±2.83	32.45±2.92	1.08	1.74±0.15
	(29.25-47.7)	(24.75-45.9)		(1.13-2.25)
I. suffruticosa	31.68±1.47	29.21±1.98	1.09	1.72±0.10
	(29.25-36.9)	(25.65-36.0)		(1.13-2.03)

* Average of three flushing time.

Table 4. Variation in morphological features of flower in different flowering time in *I. tinctoria*.

Time of flush	Flower/ raceme (No.)	Standard (mm^2)	Wing (mm^2)	Keel (mm^2)	Length of stamen (mm)		Length of carpel (mm)	Length of ovary (mm)
		L×B	L×B	L×B	(9) +	1		
Mid-July	30.50 b	20.30 a	9.21 a	16.94 ab	5.37 a	4.29 a	5.58 a	4.00
Mid-August	42.07 a	18.16 b	7.27 b	17.08 a	5.26 ab	4.00 b	5.47 ab	4.00
Early-October	18.78 c	17.48 b	6.45 b	15.14 c	4.68 c	4.01 b	5.01 c	4.09
$LSD_{0.05}$	4.94	1.39	1.66	1.19	0.21	0.15	0.20	0.21

In a column, figures having different letter(s) differ significantly ($p \leq 0.05$) according to DMRT. L: Length, B: Breadth.

The highest number of pods/raceme was recorded in mid-August (11.43) time of flush than in early October (7.70) and mid-July (7.60) (Table 5). On the other word, fruit (pod) setting percentage was significantly higher in early October flushing (41.0%) followed by mid-August (27.2%) and mid-July flushing (24.9%). Pod length was also differed significantly, but there was no significant difference in pod diameter and volume, seed/pod, seed length, seed size and 1000-seed weight among three times of flush in *I. tinctoria* (Table 5). The highest seed breadth was recorded in mid-August time of flush (1.43 mm). The higher seed yield was also obtained from *I. tinctoria* in mid-August time of flush (18.67 g/plant) than in early October (17.15 g/plant) and in mid-July (16.35 g/plant) (Table 5).

The flowering periodicity in *I. tinctoria* might be due to genetic make-up and growing environment. It seems that *I. tinctoria* appears to be photo-insensitive while *I. suffruticosa* could be a short-day plant (Khan *et al*. 2008). Among three flushing time, in mid-August flower/raceme, pod/raceme, seed breadth was greatest. Although 1000-seed weight, seed size, volume of pod had no significant variation in three times of flush, due to higher number of pod/raceme, the seed yield was highest in mid-August in *I. tinctoria*. It was noteworthy that the floral abscission was highest in mid-July followed by mid-August and early October. The lowest floral abscission (%) in early October flushing might be due to relatively shorter day-length and lower temperature during this period compared with other two time of flushing (data not shown). The pod/raceme and seed yield

Table 5. Variation in morphological features of pod and seed in different flowering time in *I. tinctoria.*

Time of flush	Pod/ raceme (No.)	Pod length (mm)	Pod diameter (mm)	Pod volume (mm^3)	Seed/ pod (No.)	Seed length (mm)	Seed breadth (mm)	Seed size (mm^2)	1000-seed wt. (g)	Seed yield (g/plant)
Mid-July	7.60 b	27.53 ab	2.37	121.24	6.88	2.24	1.28 c	2.87	5.33	16.35 b
Mid-August	11.43 a	28.41a	2.42	130.98	7.30	2.27	1.43 a	3.24	5.55	18.67 a
Early-October	7.70 b	26.00 b	2.42	120.09	7.10	2.20	1.42 ab	3.13	5.40	17.15 ab
$LSD_{0.05}$	1.31	2.06	0.15	17.85	0.59	0.36	0.05	0.57	0.31	1.53

In a column, figures having different letter(s) differ significantly ($p \leq 0.05$) according to DMRT.

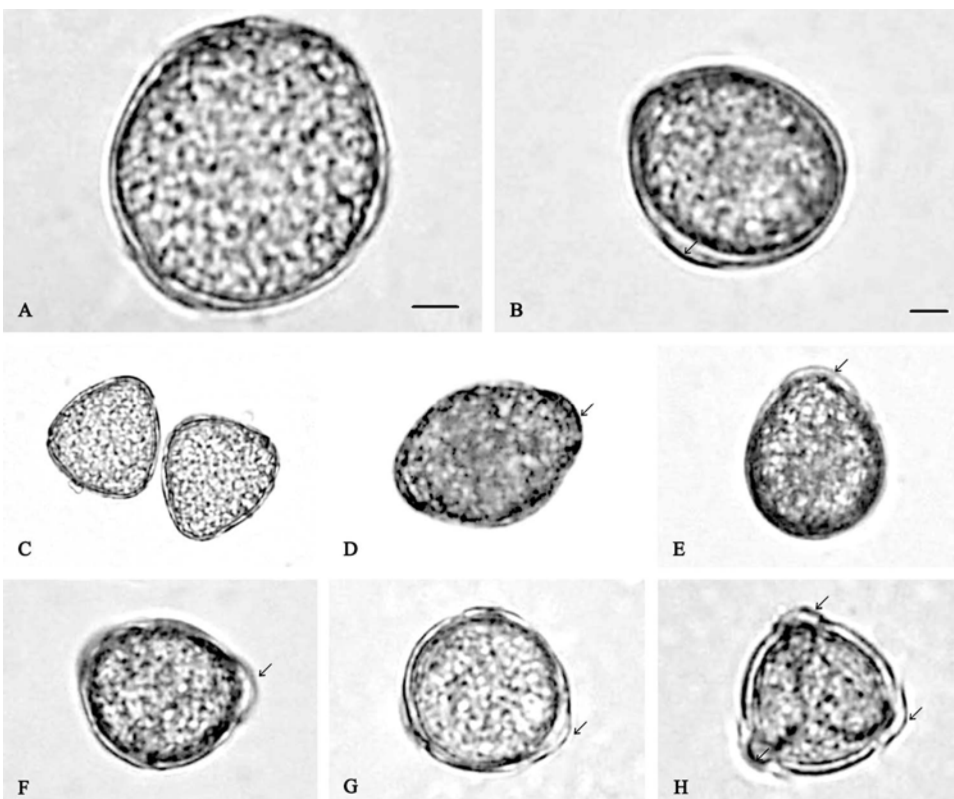

Fig. 1. Pollen morphological features of two *Indigofera* spp. A, C–E: *I. tinctoria.* B, F–H: *I. suffruticosa*. Auriculae near the aperture (arrow). Scale bars – 5 µm.

were lowest in mid-July followed by early October flushing and the highest in mid-August flushing. Therefore, *I. tinctoria* plant may be harvested after the maturity of fruits from 2nd time bloomed flowers for better seed yield and the field will be available for the next winter crop production. Environmental factors such as temperature, rainfall, etc. might control flowering and seed yield which were also stated by Sedi and Humphreys (1992), Borchert *et al*. (2004), Omolaja *et al*. (2009), and Podlesny and Podlesna (2011).

Table 6. Variation in palynological features of *I. tinctoria* expressed as mean value (in µm) ± standard deviation. Range (in µm) is shown within parenthesis.

Time of flush	Polar length (P)	Equatorial diameter (E)	P/E	Exine thickness
Mid-July	35.98 ± 4.34 (31.5 - 47.7)	33.67 ± 4.71 (27.9 - 45.9)	1.07	1.75 ± 0.19 (1.13 - 2.25)
Mid-August	33.36 ± 1.76 (29.25 - 38.25)	30.97 ± 2.21 (27.0 - 36.0)	1.08	1.72 ± 0.16 (1.13 - 2.25)
Early-October	35.69 ± 2.39 (29.25 - 40.5)	32.67 ± 1.82 (27.0 - 39.15)	1.09	1.74 ± 0.11 (1.13 - 2.25)

Among three flushing time of *I. tinctoria*, polar length and equatorial diameter of pollen were higher in mid-July (35.98 and 33.67 µm, respectively) than in early October (35.69 and 32.67 µm, respectively) and mid-August (33.36 and 30.97 µm, respectively) (Table 6). However, the P/E ratio of pollen decreases sequentially from early October (1.09) to mid-August (1.08) and mid-July (1.07). The exine thickness was also higher in mid-July (1.75 µm) than early October (1.74 µm) and mid-August (1.72 µm) (Table 6). These variations might be due to environmental and/or other factors like number of viable anther, size of anther, etc. which was also reported by Omolaja *et al*. (2009).

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References

Borchert R, Meyer SA, Felger RS and Porter-Bolland L 2004. Environmental control of flowering periodicity in Costa Rican and Mexican tropical dry forests. Global Ecol. Biogeogr. **13**: 409-425.

Ellison D 1999. Cultivated Plants of the World: Trees, Shrubs, Climbers, Flora Pub. Inter., Australia. 598 p.

Erdtman G 1952. Pollen Morphology and Plant Taxonomy - Angiosperm (An Introduction to Palynology). Almigvist and Wiksell, Stockholm.

HealthLink 2001. Monograph: indigo naturalis. http://www.healthlink.com.au/nat_lib/htmdata/htm-herb/bhp1016.htm. 3 p.

Howlader MHK 2013. Morphophysiological characters of *Indigofera* spp. in relation to plant growth and biomass yield. Ph D disser., Dept. Crop Botany, Bangladesh Agric. Univ., Mymensingh.

Jahan S, Sarwar AKM Golam and Fakir MSA 2012. Study on floral morphology and seed yield in two *Indigofera* L. species. 3rd International Seed Conference and Fair, Bangladesh Agricultural University, Mymensingh, Bangladesh, February 08-10, p. 44.

Jahan S, Sarwar AKM Golam, Hossain MA and Fakir MSA. 2013. Floral morphology and seed yield in two *Indigofera* spp. as affected by shoot clipping. 2013. J. Bangladesh Agric. Univ. **11**: 61-66.

Khan ABMMM, Prodhan AKMA, Howlader MHK and Fakir MSA 2008. Morphological variation in three *Indigofera* spp. J. Agrofor. Environ. **2**: 7-12.

Luiz-Ferreira A, Cola M, Barbastefano V, Farias-Silva E, Calvo TR, Almeida ABAD, Pellizzon CH, Hiruma-Lima CA, Vilegas W and Souza-Brito ARM 2011. *Indigofera suffruticosa* Mill. as new source of healing agent: involvement of prostaglandin and mucus and heat shock proteins. J. Ethnopharma. **137**: 192-198.

Masih T, Khan MA, Meo AA and Akhtar T 2005. Palynological studies of some genera of Leguminosae. J. Sci. Tech. Univ. Peshawar **29**: 19-27.

Nwachukwu CU and Edeoga HO 2006. Palynomorphology of some species of *Indigofera* L. Pakistan J. Biol. Sci. **9**: 1940-1944.

Omolaja SS, Aikpokpodion P, Adedeji S and Vwioko DE 2009. Rainfall and temperature effects on flowering and pollen productions in cocoa. African Crop Sci. J. **17**: 41-48.

Perveen A and Qaiser M 1998. Pollen flora of Pakistan-VIII Leguminosae (Subfamily: Papilionoideae). Tr. J. Bot. **22**: 73-91.

Podlesny J and Podlesna A 2011. Effect of rainfall amount and distribution on growth, development and yields of determinate and indeterminate cultivars of blue Lupin. Polish J. Agron. **4**: 16-22.

Punt W, Hoen PP, Blackmore S, Nilsson S and Le Thomas A 2007. Glossary of pollen and spore terminology. Rev. Palaeob. Palynol. **143**: 1-81.

Puri A, Khaliq T and Rajendran SM 2007. Antidyslipidemic activity of *Indigofera tinctoria*. J. Herb. Pharma. **7**: 57-64.

Rich J, Wright D, Marois J and Sprenkel D 2003. Selected Legumes Used As Summer Cover Crops. http://edis.ifas.ufl.edu/pdffiles/IN/IN48300.pdf

Schrire BD 2005. Tribe Indigofereae. *In* Lewis G, Schrire B, Mackinder B and Lock M (eds.), Legumes of the World. Royal Bot. Gard., Kew, UK. 361-365 p.

Schrire BD, Lavin M, Barker NP and Forest 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**: 816–852.

Sedi Y and Humphreys LR 1992. Temperature, daylength and the flowering and seed production of *Sesbania sesban* and *S. cannabina*. Trop. Grass. **26**: 100-110.

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