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Effect of planting date on morphological characters and biomass production in *Indigofera tinctoria*

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Abstract: An experiment was conducted at the Botanical Garden, Bangladesh Agricultural University, Mymensingh, during March, 2006 to April, 2007 to evaluate the effect of date of planting on some canopy characters and biomass yield in Indigo (*Indigofera tinctoria*). Seeds were sown at seven planting dates (March, May, June, July, August, September and October) and harvested at 180 days after sowing (DAS). Results revealed that stem height, stem base diameter, number of branches, leaf number, leaf area, leaf fresh weight; dry weight of root, leaf, main stem, rachis and branches; total fresh and dry mass yield were significantly increased between March and May planting compared to other planting dates. It may be concluded that March and May planting may be better for higher leaf and total dry mass yield in *Indigofera tinctoria* at 180 DAS.
Keywords: Sowing date, canopy, biomass yield, Indigo

Introduction

lndigo (*Indigofera tinctoria* L.) is one of the ancient blue dyes used by man for textiles. The indigo dye comes from leguminous plant of the genus *Indigofera*, of which over three hundred species have been identified (Mattson, 2004). The pigment (Indigo) is extracted from the leaves and stems of 14 *Indigofera* species of which two are cultivated with the Asian *Indigofera tinctoria* L. (Indian Indigo) and the African *Indigofera arrecta* Hoechst (Natal Indigo) have been the most important source of dye for commercial production. Indigo shrubs can grow 1 to 5 m in height and have semi-wood main stem, with or without branches, dark green leaves that are oval shaped in most species, and clusters of red flowers that look like butterflies and turn into peapods. The morphological variations of three *Indigofera* species are described by Khan (2007). The dye is obtained mainly from the leaves through a process of fermentation. The Indigo dyestuff is not present in the green leaves and stems but is formed post-harvest by hydrolysis of glucoside to indoxyl which is then subjected to oxidation (FAO, 1995). Most of the commercial *Indigofera* species are adaptable to a range of climates in the tropics and sub-tropics. *Indigofera* species may be used as a cover crop and for green manure, especially in tea, coffee and rubber plantations (Rich, 2003). Indigo is primarily used for dyeing and decorating cotton, silk, wool and other natural fibre for clothes, carpets, tents and a host of other products. Indigo has also been used for body ornamentation through the ages. After extraction of dye, the dried plants are also used as firewood. In Bangladesh, *I. tinctoria* is grown in the Nilphamari District for green manuring and fuel (Anon. 2005; 2007). Indigo plants could also be used as excellent hedge plant since it possesses unpleasant smell and is not liked by grazing animals. Indigo plants, therefore, could be an important multipurpose shrub species in Agroforesrty. During colonial regime, before the development of the synthetic blue dye the English forced Bengali farmers for indigo cultivation. In the traditional medicine, Indigo is used in the treatments of epilepsy disease and psychiatric illness (Ahmed, 2002).

Leguminous crops, in general, are characterised by slow juvenile growth (Fakir, 1997). The problem of slow juvenile growth is even more conspicuous in Indigo seedlings (Pervin, 2004). Therefore, some means of enhancing seedling growth and biomass yield is needed. Different means of increasing biomass yield *viz*., optimum planting density and shoot clipping (Rokonuzzaman, 2003), boosting growth by spraying hormones (Kabir, 2003; Pervin, 2004) were tried. Indigo natural dye is extracted mainly from fresh leaves. Positive correlation between fresh leaf weight and dry mass indicate that greater leaf production may increase dry matter production of plant (Kabir, 2003). Researches on nutrient and moisture requirement, different planting and harvesting time have not yet been done for the indigo cultivation in Bangladesh. To obtain higher biomass production of indigo research work is needed to evaluate growth and biomass accumulation as influenced by different time of planting in Indigo cultivation. So, a piece of research work was conducted to investigate the effect of sowing dates on morphological characters and biomass production in *I. tinctoria*.

Materials and Methods

The experiment was conducted at the Botanical garden, Department of Crop Botany, Bangladesh Agricultural University, Mymensingh, during March, 2006 to April, 2007. Geographically the experimental field is located at 24° 75′ N latitude and 90° 50′ E longitude at the elevation on 18 m above the sea level. The experimental field was medium high land belonging to the sonatola soil series of gray flood plain soil type under the agro-ecological zone of Old Brahmaputra Plain (AEZ-9) (UNDP and FAO, 1988). The soil was silty loam, imperfectly poor drain with slow permeability. The climatic condition of the experimental site is sub-tropical and characterized by high temperature (32.45 ^{0}C) and high humidity (88.63 %), heavy rainfall (413.2 mm) during kharif season (April to September) and scanty rainfall (0.2 mm) associated with moderately low temperature (10.80 ^{0}C) during the Robi season (October to March). Seven treatments (sowing dates) were laid out following randomised complete block design (RCBD) with three replications. The seven sowing dates were 1st sowing (mid-March), 2nd sowing (mid-May), 3rd sowing (mid-June), 4th sowing (mid-July), 5th sowing (mid-August), 6th sowing (mid-September) and

7th sowing (mid-October). Seeds were also sown in mid-April but seed did not germinate and hence was excluded from the experiment. The experimental plots were divided into three blocks each representing a replication. Each block was then divided into seven unit plots (120 cm x 180 cm) with plant spacing was 30 cm x 30 cm. Block to block and plot to plot distance was 75 cm and 50 cm, respectively. Five plants were harvested from each plot at 60, 120 and 180 days after sowing (DAS). The following data were recorded: stem height (cm), stem base diameter at 10 cm from base (d_{10}), primary branch number/ plant, total number of branches/plant, leaf number/plant, leaf area/plant (cm^2), leaf fresh and dry weight (g); dry weight (g) of branch, main stem, rachis, root and pod (g); total fresh weight (g), and total dry matter (TDM) (g). Total dry mass of separated plant parts was obtained by summing and weighing of dried weight of samples in an electric balance after at 80 ± 2^0 C for 48 hours. The data were compiled and analysed following RCBD to find out the statistical significance of the experimental results. The means for all recorded data were calculated and the analyses of variance for all the characters were performed. The mean differences were evaluated by Duncans New Multiple Range Test (DMRT) (Gomez and Gomez, 1984). A correlation study of total biomass with different canopy characteristics was also carried out.

Results

Canopy Structure: At 180 DAS, plant height was significantly (P≤0.05) greater between May and August (average of 99.36 cm) than in the others but stem base diameter (d_{10}) was greater at March planting (1.37 cm) than in the others. At 180 DAS, the highest number of primary branches /plant was produced at March, May and August planting (average of 13.69) but lowest in October planting. The number of secondary branches/plant was greater at May (58.73) than at June (35.27), August (24.53) and July, September and October (average of 8.40) (Table 1). March planting produced the highest (75.27) and July and October planting had lowest tertiary branches/plant (average of 0.7). The highest number of leaves was noted at May (1053.13) and lowest at July, September and October planting (average of 145.55). The leaf area (LA) was greater at May and June planting (average of 3580.22 cm^2) than in the others. The leaf fresh weight was greater at May (59.34 g) than June (49.65 g), March (31.03 g), August (19.91 g) and July (9.37 g) (Table 1).

Biomass: The root mass was significantly (P≤0.05) greater at March, May and August (average of 13.06 g/plant) than in June and September (average of 7.78 g/plant) and October (2.93 g/plant) (Table 2). The leaf dry weight was also greater at May (22.18 g/plant) than March and June (average of 13.56 g/plant) July, August, September and October (average of 4.57 g/plant). The main stem dry weight was greater at earlier plantings, March and May (average of 15.89 g/plant) than in the August (12.65 g/plant) June and July (average of 9.44 g/plant) and September and October (average of 2.80 g/plant). The higher rachis dry weight was observed at May and June plantings (average of 4.10 g/plant) than in the others. The branch dry weight was significantly greater at March and June planting (average of 44.48 g/plant) than in the others. The total fresh weight was higher at May planting (209.53 g/plant) than March and June (average of 174.29 g/plant), July and August (average of 100.05 g/plant) and September and October (average of 47.91 g/plant). The total dry mass was higher at March and May (average of 87.84 g/plant) than at June and August (average of 51.09 g/plant), July (39.86 g/plant) and September and October (average of 17.84 g/plant). Flowering and pod development occurred after 120 DAS (data not shown). The pod dry weight was smaller at September (1.55 g/plant) August and October (average of 4.49 g/plant), and June and July planting (average of 6.135 g/plant). The fresh and dry biomass was very small at 60 DAS, increased with aging and became maximum at 120 DAS (Fig. 1). Total of leaf and branches biomass had similar trend.

Table 1. Effect of planting time on some important morphological features in Indigo (*Indigofera tinctoria*) at 180 days after sowing

Planting time	Stem height (cm)	Stem base diameter (cm)	Primary branch/ plant (No.)	Secondary branch/ plant (No.)	Tertiary branch/ plant (No.)	Leaf / plant (No.)	Leaf area/ plant (cm^2)	Leaf fresh wt. / plant (g)
March	60.80 b	1.37 a	14.20 a	32.13 bc	75.27 a	704.27 b	1904.01 b	31.03 c
May	97.27 a	1.07 b	12.60 ab	58.73 a	37.20 b	1053.13 a	3898.64 a	59.34 a
June	96.07 a	0.93 c	9.93 c	35.27 b	14.23 cd	480.87 c	3261.79 a	49.65 b
July	102.43 a	0.48 e	10.93 bc	7.60 d	1.13 e	128.60 d	668.55 c	9.37 e
Aug.	101.67 a	0.89 c	14.27 a	24.53 c	15.67 c	395.33 c	1307.10 bc	19.91 d
Sept.	53.53 b	0.59 d	9.33 c	11.00 d	2.27 de	180.73 d	946.08 c	14.40 de
Oct.	54.27 b	0.49 e	4.80 d	6.60 d	0.27 e	127.33 d	772.49 c	11.76 de

In a column, figures bearing similar letter (s) do not differ significantly at P ≤ 0.05 by DMRT.

Correlation of biomass with canopy character: Total fresh mass was positively and significantly (P≤0.05) correlated with stem height (r = 0.461, data not shown), stem base diameter (r = 0.830), number of primary (1^0) branches (r = 0.573), total number of branches (r = 0.874), leaf/plant (r = 0.905), leaf area/plant (cm^2) (r = 0.884), leaf fresh weight (r = 0.898), root fresh weight (r = 0.623). Total dry mass yield was also correlated with stem height (r = 0.322), stem base diameter (r = 0.609), number of 1^0 branches (r = 0.701), total number of branches (r = 0.947), leaf/plant (r = 0.892), leaf area/plant (cm^2) (r = 0.697), leaf fresh weight (r = 0.720), root fresh weight (r = 0.748).

Table 2. Effect of sowing time on dry mass production, partitioning and total fresh and dry weight at 180 days after sowing in Indigo (*Indigofera tinctoria*)

Planting time	Dry mass/ plant (g)							Total fresh weight (g/plant)
	Root	Leaf	Main stem	Rachis	Branch	Pod	TDM	
March	12.72 a	14.79 b	16.39 a	2.45 b	45.22 a	5.30 ab	90.37 a	175. 97 b
May	12.65 a	22.18 a	15.39 a	3.87 a	33.43 b	5.29 ab	85.30 a	209.53 a
June	5.75 b	12.32 b	8.99 c	4.32 a	43.74 a	6.19 a	54.12 b	172.60 b
July	4.48 bc	3.55 c	9.89 c	0.90 d	17.15 c	6.08 a	39.86 c	91.10 c
Aug.	13.81 a	6.50 c	12.65 b	1.75 bc	15.52 c	4.66 b	48.05 b	108.97 c
Sept.	5.81 b	4.34 c	3.57 d	1.31 cd	6.30 d	1.55 c	20.09 d	50.21 d
Oct.	2.93 c	3.87 c	2.03 d	0.77 d	3.13 d	4.32 b	15.59 d	45.61 d

In a column, figures bearing similar letter (s) do not differ significantly at P ≤ 0.05 by DMRT.

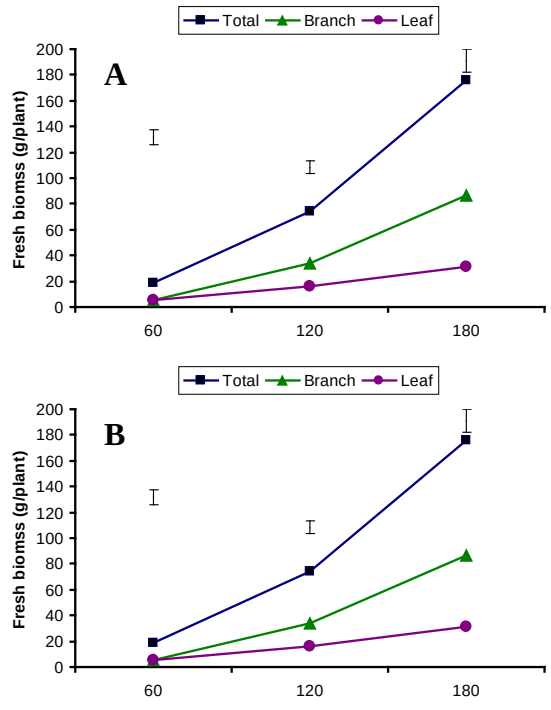

Fig 1. Effect of age (days from sowing) on biomass production, fresh mass (A) and dry mass (B), in *Indigofera tinctoria* at March planting. Vertical bars are standard error of means.

Discussion

Indigofera tinctoria is a natural or vegetable dye yielding shrub and leaf and other plant parts yield blue indigo dye after oxidation (FAO, 1995). Biomass yield, therefore, is an important index of selection criterion. Biomass production in Indigo depends on canopy structures, genotype, cultivation protocol and time of planting. In the present study, effect of planting date on different components of canopy structure was investigated. Canopy structure consists of tallness of the plant, stem thickness, number of branches of different order (1^0, 2^0, 3^0) and their orientation to main stem, and amount of foliage. Plants were harvested at 60, 120 and 180 days after sowing (DAS) after which plants began to senesce leaf. The planting time consisted of series of dates between March and October with monthly interval. In general, components of canopy structure and biomass progressively increased with age. Harvesting, therefore, at 180 DAS produced larger plants with increased biomass. Time of planting (TOP) markedly influenced tallness, stem thickness, branch and leaf production. Larger canopy structure and greater biomass were noted in earlier planting especially between March and June than in the later ones. Significant and positive correlation of biomass yield with plant height, stem base diameter, number of branches and leaf and root mass indicate that increasing growth of the canopy would led improved biomass yield in *Indigofera tinctoria*. Such relationship was also observed by Dehaan (2005). Rokonuzzaman (2003) observed increased biomass after clipping shoot at 60 cm height with densities between 16 and 20 plant/m^2. Hasan (2005) and Pervin (2004) also observed increased biomass production by spraying GA_3 (100 ppm) in *Indigofera tinctoria*. In the current investigation, seven planting dates (March,

May to October) were used as treatments. It is not known about biomass yield potential during other time of plantings. Therefore, further experiment is needed to see the effect of other time of plantings such as before March and after October on biomass yield in Indigo plant. It appears that time of planting is a vital factor for biomass yield of Indigo plant and generally March and May planting may be the better time to get increased biomass at 180 DAS in *Indigofera tinctoria*.

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