Journal of Medicinal and Aromatic Plant Sciences 38(1-4) (2016) 29-35

Morphological characteristics and variability among Indian Indigo (*Indigofera tinctoria*) accessions from Kerala

M NEEMA* • BR REGHUNATH[1]

Article History

Received: 21st October, 2014
Accepted: 22nd March, 2016

Key words

Indigofera tinctoria
Morphology
Selection index
Variability
Yield attributes

Abstract

Twenty accessions of Indian Indigo collected from different parts of Kerala state were assessed for their variability. The morphological traits, flowering and seeding behavior, yield attributes and root nodule characteristics were studied. Analysis of variance revealed substantial variations among the accessions. IT 13, accession from Thiruvananthapuram followed by IT 11 (Kollam) and IT 9 (Thiruvananthapuram) were inferred to be superior based on selection indices and morphological characterization. These accessions performed better in termes of Leaf, Shoot and root yields. The study provided an insight into the variations among Indigofera tinctoria accessions and indicated the relative superiority of certain accessions that can further be multiplied and released as high yielding varieties for cultivation.

INTRODUCTION

Indian Indigo (*Indigofera tinctoria* L.) is a leguminous deciduous subshrub of the south-eastern Asia. It is valued in Ayurveda as an important ingredient of hair tonics like Neelibhringadi thailam as well as in the treatment of myelocytic leukemia [2], hepatitis and snake poisoning. The plant is used as a source of blue dye, present in the form of a glycoside namely 'indican' which is used for dying dermatitis free clothes. Lack of authentic varieties of the plant is a major drawback that hinders quality standardization of pharmaceutical preparations made out of them. Screening of existing germplasm for evolving superior genotypes and releasing authentic varieties for cultivation hence assume much importance. Kerala being a state with tremendous biodiversity, this study was undertaken under the aegies of a ongoing project on variability assessment of commercially important medicinal plants of this region for identify superior genotypes.

MATERIALS AND METHODS

Experimental material consisted of 20 accessions of *Indigofera tinctoria* collected from different parts of Kerala state. Details of the accessions and their sources are given in Table 1. The experiment was conducted at College of Agriculture, Vellayani, Thiruvananthapuram. Seeds were sown in earthen seed pan after subjecting to scarification [15]. Seedlings were transplanted after three weeks at five leaf stage to poly bags filled with potting mixture. The experiment was laid out in CRD with three replications. Fifteen plants were maintained in each accession and the crop was raised as per package of practices and recommendations of the Kerala Agricultural University [5].

*Corresponding Author, Email: neema.agri@gmail.com, Division of Crop Improvement, CPCRI, Kasaragod, Kerala - 671124
[1]Department of Plant Biotechnology, College of Agriculture, Vellayani, Thiruvananthapuram Kerala - 695 522

Table 1. Source of germplasm

Sl. No.	Accession No.	Source of germplasm	Selection index scores	Ranks
1	IT 1	TBGRI, Trivandrum	20821.89	4
2	IT 2	Local accession, Kottayam	18867.57	9
3	IT 3	AICRP on Medicinal and Aromatic plants, Thrissur	16452.88	15
4	IT 4	Vrikshabandhu, Kottayam	19502.88	6
5	IT 5	Aromatic and Medicinal plant research station, Ernakulum	19404.40	7
6	IT 6	Local accession, Thrissur	19396.08	8
7	IT7	College of Horticulture, Thrissur	18552.48	11
8	IT 8	Instructional Farm, COA, Thiruvananthapuram	17458.48	13
9	IT 9	Sidha Vaidyasala, Thiruvananthapuram	21000.63	3
10	IT 10	Kuzhipallam botanical garden, Thiruvananthapuram	15789.64	16
11	IT 11	Local accession, Thiruvananthapuram	21986.24	2
12	IT 12	Local accession, Thiruvananthapuram	12597.43	18
13	IT 13	Local accession, Thiruvananthapuram	24227.74	1
14	IT 14	Local accession, Thiruvananthapuram	17737.62	12
15	IT 15	Local accession, Thiruvananthapuram	9504.365	20
16	IT 16	Kottakal Aryavaidyasala, Malapuram	19795.86	5
17	IT 17	Local accession, Thiruvananthapuram	18801.94	10
18	IT 18	Pankajakasthuri, Thiruvananthapuram	15330.32	17
19	IT 19	Local accession, Palakkad	12310.56	19
20	IT 20	Local accession, Thiruvananthapuram	16561.39	14

Morphological observations were recorded at pre-flowering, flowering and pod maturation stage. The observations recorded were plant height, spread, height at first branching, number of branches, stem girth, length, breadth, number and area of leaves, root length and root girth at the collar region. The leaf area was calculated by adopting punch method as defined by Watson [16]. Fifty leaf punches were made. The discs as well as leaves were dried in hot air oven at 70°C and their respective dry weight was recorded. From the data of leaf dry weight, leaf area per plant was computed and recorded.

The yield attributes recorded included fresh and dry weight of leaves, shoots, pods, seeds, roots and also the dry shell weight. The dry weight was obtained by oven drying at 70 ± 5°C to a constant weight. The root nodule characteristics recorded were the number and fresh weight of root nodules. The number of effective root nodules in each accession was also recorded. Root nodules were cut into half and those showing pinkish coloration were considered as effective root nodule.

The flowering and seeding behavior observed were number of days for flowering, time of anthesis, time of anther dehiscence and number of pollen grains produced per anther. The number of days for flowering was obtained by counting the number of days taken for the appearance of first flower from the date of sowing in the observational plants and further the mean value was recorded. To determine the time of anthesis, ten inflorescence of each accession were tagged at 6.00 a.m. and observations were made at two hourly intervals. Later precise observations were made as per Mathew [7]. Time of anther dehiscence was also recorded. For this flower buds were tagged in group of ten and observed with hand lens at two hour interval. Appearance of longitudinal splits in the pollen sac indicated the commencement of anther dehiscence. When more than three anthers in a flower liberated pollen grains it was reckoned as having completed anther dehiscence. The observation was repeated for three days with another group of flowers and the average was worked out [7]. The pollen production per flower

was estimated using haemocytometer as described by Gunasekaran [4].

Analysis of variance as proposed by Panse and Sukhatme [10] was worked out for all the traits to find out the significant difference between the accessions in respect of various traits. The mean value for each of the characters for all the accessions were worked out and compared using critical difference and the significance was tested using F-test. A discriminant function analysis was carried out for isolating superior accessions of *Indigofera tinctoria*. The characters selected for analysis are plant height (X_1), plant spread (X_2), number of branches (X_3), number of leaves (X_4), leaf area (X_5), root length (X_6), fresh weight of leaves (X_7), dry weight of leaves (X_8), fresh weight of shoots (X_9), dry weight of shoots (X_{10}), fresh weight of pods (X_{11}), dry weight of pods (X_{12}), fresh weight of roots (X_{13}), dry weight of roots (X_{14}) and number of effective root nodules (X_{15}). The selection index (I) developed by Smith [13] using the discriminant function of Fisher [3] was worked out as follows:

$$I = 6.741908\ X_1 + 4.057361\ X_2 + 7.054427\ X_3 - 4.464126\ X_4 + 0.002647484\ X_5 - 10.31294\ X_6 + 26.0696\ X_7 + 74.6475\ X_8 + 9.787668\ X_9 - 16.65474\ X_{10} + 2.634035\ X_{11} + 3.339388\ X_{12} + 4.378023\ X_{13} + 7.412608\ X_{14} - 5.666589\ X_{15}$$

RESULTS AND DISCUSSION

The leaf yield, shoot yield and root yield of 20 accessions of *Indigofera tinctoria* at flowering stage are depicted in Fig. 1, 2 and 3.

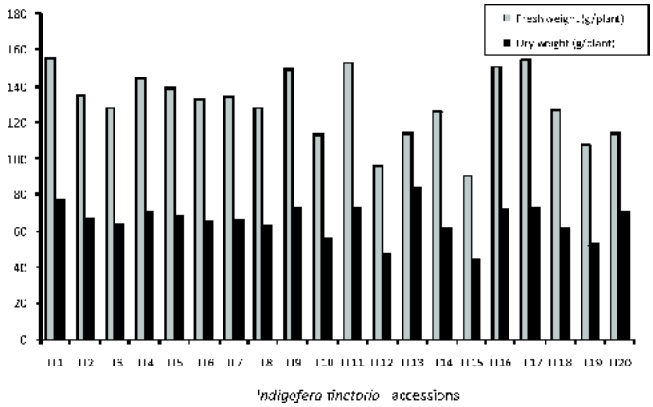

Fig. 1. **Leaf yield of 20 accessions of *Indigofera tinctoria* at flowering stage.**

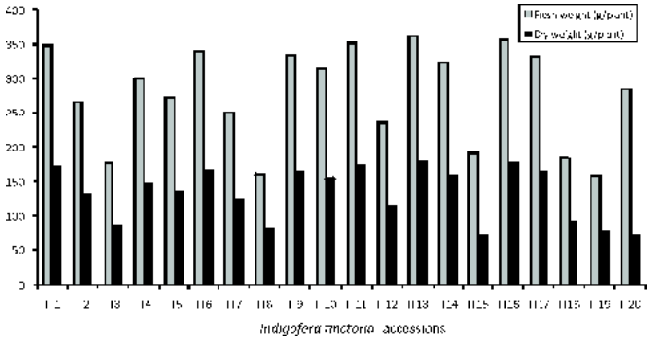

Fig. 2. **Shoot yield of 20 accessions of *Indigofera tinctoria* at flowering stage.**

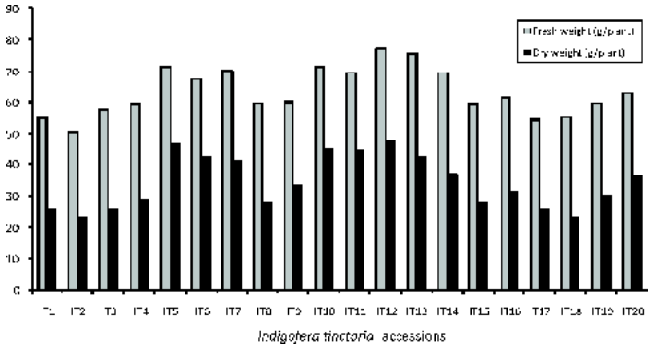

Fig. 3. Root yield of 20 accessions of *Indigofera tinctoria* at pod maturation stage.

The maximum plant length was observed in IT14 at all stages of observation, but minimum plant height was observed in IT12 at pre flowering stage and IT 4 at flowering and pod maturation stage. This indicates that the performance of different accessions differ during various growth stages as reported by Nair [8]. An increase in leaf production was found from pre flowering to the flowering stage, but a decline was found in the seed maturation stage. Nair and Reghunath [9] and Resmi [11] also observed similar trends in *Clitoria ternatea*. This may be attributed to leaf shedding caused by senescence. Variation in the number of leaves among the accessions may be attributed to the fact that it is purely a function of genetic makeup and environmental conditions.

The accessions IT2 and IT 12 recorded the longest and shortest root length at pre flowering stage respectively while in flowering and pod maturation stage, IT 13 produced longest root, IT 1 and IT 2 produced shortest roots respectively. The results revealed that maximum root length was obtained at the pre flowering stage itself and only

Table 2. Growth parameters of *Indigofera tinctoria* at pre flowering, flowering and pod maturation stages of plant growth

Plant characteristics	Stage of growth	Mean	F. ratio	CD
Plant height (cm)	Pre flowering	34.07	64.37**	2.41
	Flowering	100.17	291.11**	1.92
	Pod maturation	178.47	867.19**	2.23
Plant spread (cm)	Pre flowering	53.79	125.21**	2.16
	Flowering	88.57	5157.5**	0.55
	Pod maturation	121.05	3816.12**	0.60
Height at first branching (cm)	Pre flowering	6.15	119.88**	0.32
No. of branches	Pre flowering	11.55	7.88**	2.36
	Flowering	35.48	23.17**	2.00
	Pod maturation	62.17	24.66**	3.58
Girth of stem(cm)	Pre flowering	2.42	27.81**	0.21
	Flowering	3.35	20.58**	0.30
	Pod maturation	6.4	30.2**	0.27
Leaf length (cm)	Pre flowering	10.61	102.42**	0.15
Leaf breadth (cm)	Pre flowering	4.30	8.43**	0.20
Number of leaves	Pre flowering	110.4	132.66**	4.7
	Flowering	448.55	318.57**	11.19
	Pod maturation	378.32	132.43**	16.69
Leaf area (cm^2)	Pre flowering	1665.57	5.11**	371.32
	Flowering	6122.55	77.72**	294.27
	Pod maturation	5109.58	42.87**	413.25
Root length (cm)	Pre flowering	33.79	46.80**	2.41
	Flowering	39.37	49.31**	2.62
	Pod maturation	44.55	105.40**	1.71
Root girth at collar region (cm)	Pre flowering	2.95	0.91	4.23
	Flowering	4.03	26.20**	0.32
	Pod maturation	6.85	24.51**	0.26
Shell weight (g)	Pod maturation	32.82	32.41**	5.67

** significant at 1% level

very small increase in root length occurs in the flowering and pod maturation stage.

Maximum fresh and dry weight of leaves was recorded in the accession IT 13 in all the stages except in pre flowering stage where the maximum fresh weight was recorded in IT 1. The minimum fresh and dry weight was observed in the accession IT 15 for all stages of growth. The fresh and dry weight increased during the first two stages but decreased in the last stage. This could be explained by the production of leaf at all stages and shedding of leaf at the last stage due to senescence as reported by Samuel [12].

Considering the fresh and dry weight of shoots, the maximum shoot yield was produced by the accession IT 13 in the pre flowering stage and flowering stages, but at the pod maturation stage the accession IT 1 produced maximum shoot. Minimum shoot yield was recorded in the accessions IT 19, IT 15 and IT 3 at these stages respectively. The shoot weight was found to increase throughout the growth stage. Among the twenty accessions of *Indigofera* used in the study, the accessions IT 13 and IT 12 produced maximum root yield, while IT 1, 2 and 18 were the lowest root yielder. From the data obtained, an increasing trend

Table 3. Yield parameters of *Indigofera tinctoria* at pre flowering, flowering and pod maturation stages of plant growth

Yield parameter	Stages of growth		Mean	F ratio	CD
Leaf weight (g)	Pre flowering	F*	36.15	13.32**	4.81
		D*	18.42	4.80**	4.14
	Flowering	F*	131.38	43.66**	8.17
		D*	65.47	65.76**	3.42
	Pod maturation	F*	109.54	32.24**	9.98
		D*	54.53	89.26**	3.00
Shoot weight (g)	Pre flowering	F*	104.31	2152.04**	1.48
		D*	55.15	4792.20**	0.52
	Flowering	F*	276.61	19441.90**	1.45
		D*	134.92	7533.55**	1.20
	Pod maturation	F*	620.23	89528.61**	1.22
		D*	311.16	19035.83**	1.34
Root weight (g)	Pre flowering	F*	40.63	117.39**	1.43
		D*	17.07	223.55**	0.77
	Flowering	F*	63.27	98.25**	2.17
		D*	34.09	86.14**	2.52
	Pod maturation	F*	58.06	48.93**	3.27
		D*	30.87	51.32**	3.24
Pod weight (g)	Pod maturation	F*	156.16	47.57**	23.60
		D*	53.69	487.99**	2.57
Seed weight (g)	Pod maturation	F*	52.11	116.03**	5.2
		D*	20.19	209.60**	1.52

F* Fresh weight, D* Dry weight, ** Significant at 1% level

Table 4. Root nodule characteristics of *Indigofera tinctoria* at pre flowering and flowering stage

Root nodule characteristics	Stages of growth		Mean (g)	F ratio	CD
Total Number	Pre flowering		23.22	13.70**	1.70
	Flowering		66	49.08**	7.60
Number of effective root nodules	Pre flowering		8.3	4.95**	3.00
	Flowering		27.92	57.66**	4.08
Weight (g)	Pre flowering	F*	0.35	3.20**	0.004
		D*	0.1585	4.31**	0.004
	Flowering	F*	1.00	51.33**	0.11
		D*	0.45	62.75**	0.004

F* Fresh weight, D* Dry weight, **Significant at 1% level

was observed in the fresh and dry weight of roots up to pre flowering and flowering stage and then exhibited a decline towards pod maturation.

IT 16 recorded the maximum root nodule number at pre flowering and flowering stages. The lowest nodule number was recorded in IT 10 at pre flowering and flowering stages. Nodule number showed an increasing trend up to flowering stage. At pod maturation nodules were absent. This is due to the degeneration of nodules at this stage.

Table 5. The phenotypic (sp^2), genotypic (sg^2) and environmental (se^2) variances for various characters in *Indigofera tinctoria*

Characters	σp^2	σg^2	σe^2
Plant height	527.853	526.031	1.8219
Plant spread	171.020	170.886	0.1344
No. of branches	41.910	37.194	4.7168
No. of leaves	4586.356	4484.006	102.3500
Leaf area (cm^2)	937993.100	875276.3	62716.800
Root length (cm)	38.407	37.334	1.0729
Fresh weight of leaves (g)	417.123	380.580	36.5438
Fresh weight of shoots (g)	16413.950	16413.4	0.5500
Fresh weight of pods (g)	3378.059	3173.609	204.4500
Fresh weight of root (g)	66.750	62.818	3.9320
No. of effective root nodule	121.305	115.205	6.1000

Observations of floral characteristics showed that the number of days for 50 percent flowering ranged from 109.63 (IT 7) to 125.27 (IT 14) days. Anitha [1] reported that *Indigofera tinctoria* needed a mean of 147 days for flowering. The time of anthesis was between 9.10 a.m. (IT 7) to 11.10 a.m. (IT 8). Time of anther dehiscence was between 3.10 a.m. (IT 6) and 5.15 a.m. (IT 7). Number of pollen grains produced per anther ranged from 12,000 in accession IT 14 to 1,96,000 in accession IT 17.

Analysis of variance showed significant differences among the 20 accessions for all the characters studied at all the stages except for the root girth at collar region during the pre flowering stage. These results are in accordance with the observations of Kumar and Choudhary [6] and Solanki *et al*. [14]. The mean values of growth and yield parameters together with the root nodule characteristics studied at different stages of plant growth are presented in Table 2, 3 and 4.

The phenotypic, genotypic and environmental variances for eleven characteristics namely plant height, plant spread, number of branches and leaves, leaf area, root length, fresh weight of leaves and shoots, fresh weight of pods and root and number of effective root nodules are presented in Table 5.

Leaf area exhibited highest phenotypic genotypic and environmental variance followed by shoot fresh weight. A close association between phenotypic and genotypic variance was observed for characters such as plant height, plant spread, root length and fresh weight of shoot. Wide variations were found in number of leaves, leaf area and leaf and pod fresh weight.

The selection index scores were used to identify superior genotypes of *Indigofera tinctoria*. The accession IT 13 showed superior performance followed by IT 11 and IT 9. The accession IT 15 showed the least selection index.

Selection indices worked out as well as morphological characterization as well as yield parameters indicate that the *Indigofera tinctoria* accession IT 13 from Thiruvananthapuram is superior to other accessions. Other promising accessions include IT 11 and IT 9. The study has relevance in the present scenario as the superior varieties of this medicinal plant are scarce and hence the identified accessions could be recommended for cultivation in Kerala state after subjecting to suitable adaptability trials.

REFERENCES

1. Anitha S. 1997. *Mutagenesis in Indigofera tinctoria* (L.) Ph.D. thesis, University of Kerala, Thiruvananthapuram.

2. Aobchey P, Sinchaikul S, Phutrakul S, Chen ST. 2007. Simple purification of indirubin from indigofera tinctoria linn. and inhibitory effect

on MCF-7 human breast cancer cells. *Chiang Mai J Sci* **34**: 329-337

3. Fisher RH. 1936. The use of multiple measurements in taxonomic problems. *Ann Eugen* **7**:179-188.

4. Gunasekaran P. 1992. *Laboratory Manual in Microbiology*. New age International (P) Ltd., 138p.

5. KAU. 2002. *Package of Practices Recommendations – Crops*, Directorate of Extension, Kerala Agricultural University, Thrissur. 155 p.

6. Kumar M. Choudhary BM. 2003. Studies on genetic variability in fenugreek (*Trigonella foenum-graecum* L.). *Orissa J Hort* **31**: 37-39.

7. Mathew SK. 2002. Floral biology, anthesis and fruit development in drum stick (*Moringa oleifera* Lam.). M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur.

8. Nair DS. 2000. Evaluation of genetic stock of sanghupushpam (*Clitoria ternatea* L.) for yield, alkaloid content and nitrogen fixing potential. M.Sc. (Hort) thesis. Kerala Agricultural University, Thrissur.

9. Nair DS, Reghunath BR. 2002. Root growth, yield and nodule formation of *Clitoria ternatea* L. accessions intercropped in coconut garden. *S Indian Hort* **50**: 284-288.

10. Panse VG, Sukhatme PV. 1967. *Statistical Methods for Agricultural workers.* Second edition. Indian Council for Agricultural Research, New Delhi. 381p.

11. Resmi CR. 2001. Evaluation of selected sanghupushpam (*Clitoria ternatea* L.) lines for yield, alkaloid content and nodulation. M.Sc. (Hort.) thesis. Kerala Agricultural University, Thrissur.

12. Samuel A. 2000. Evaluation of genetic stock of *Mucuna pruriens* Baker non DC for yield, L-Dopa content and nitrogen fixing potential in coconut garden. M.Sc. (Hort.) thesis. Kerala Agricultural University, Thrissur.

13. Smith FH. 1936. A discriminant function for plant selection. *Ann Eugen* **7**:240-250.

14. Solanki SS, Sharma RC, Kakani RK, Garg, DK. 2003. Estimation of genetic parameters in moth bean. I**n:** *Proc. National Symp. arid legumes, for food nutrition security and promotion of trade.* 15-16 May, 2002, Hisar, India, pp. 84-87.

15. Sy A, Grouzis M, Danthu P. 2001. Seed germination of seven Sahelian legume species. *J. Arid Environ.* **49:** 875-882.

16. Watson DJ. 1952. The physiological basis of variation for yield. *Ann Bot* **4**: 101-145.