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Research Article

Genetic variability studies in Indigo (*Indigofera tinctoria* L.)

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Abstract
An experiment to estimate the extent of variability, heritability and genetic advance as percent of mean on twenty accessions of *Indigofera tinctoria* collected from diverse geographical locations and raised in the Orchard unit of the Department of Horticulture, Faculty of Agriculture, Annamalai University. The experiment was laid out in a randomized block design with three replications. The biometric observations of plant height, plant spread, the number of branches, the number of leaves, leaf area, biomass, fresh weight of shoots, fresh weight of leaves, glucoside content, indigo content dye yield and dye recovery were recorded at 160 days after sowing. High GCV estimates were recorded for dye yield, leaf area, dye recovery, glucoside content, the number of branches, the number of leaves, indigo content, plant height, fresh weight of leaves and fresh weight of shoot. The high heritability estimate for the glucoside content (82.02 %) revealed the possibility of increasing the glucoside content. The character dye yield was positively and significantly correlated with plant height, plant spread, the number of branches, the number of leaves, leaf area, fresh weight of leaves, fresh weight of shoot, biomass, glucoside content, dye recovery and indigo content both at genotypic as well as phenotypic levels.

Keywords: Indigo, Variability, PCV, GCV, Genetic advance

INTRODUCTION

Until the turn of 19th century all colours came from the natural world, as there were no other means to derive them. With the discovery of synthetic dyes at the end of the 19th century, the cultivation and application of natural dyes disappeared. To reduce the high pollution load characteristic of the modern textile dyeing processes, the partial replacement of synthetic dyes with natural ones in textile production represents a good strategy. The interest in natural dye stuffs has revived recently in India and Europe, Japan, and the United States. Due to environmental awareness, the natural dyes obtained from plants and animals are the dyes of 21st century. It is interesting to note that various parts of plants synthesize over 2000 pigments. Only 150 have been commercially exploited and of these, very few are of industrial importance (Siva, 2007). The current production of natural dyes in India is estimated at 10,000 tonnes per year. Indigo (*Indigofera tinctoria*) is an important and potential plant for commercial exploitation among the various dye plants cultivated. The blue pigment indigo (indigotin) is one of the oldest natural dyes known to man. For centuries, indigo has been obtained from a wide variety of plant sources such as *Indigofera* (Africa, Asia, South America), *Polygonum tinctorium* and *Baphicacanthus cusia* (China, Korea) and Isatis spp (Europe).

In the middle ages, Indigo derived from *Isatis tinctoria* was the basis of a large industry in Europe. This started declining after the 17 th century due to competition from imported indigo, obtained from tropical *Indigofera* spp. (Kokubun *et al*., 1998). *Indigofera tinctoria* produces a higher quantity of quality indigo among the blue dye yielding plants than the temperate plants (Nikkipadden *et al*., 1999). *Indigofera tinctoria* L. (Avuri/ neel in Tamil, neelyamari in Malayalam, Indian indigo in English and neelini/neelika/nenjini in Sanskrit) is a medicinally and commercially useful leguminous plant. It is indigenous to India. Earlier, indigo was cultivated extensively in West Bengal, Odisha, Madhya Pradesh, parts of Maharashtra, Tamil Nadu and Kerala. Considering the importance of this crop and due to limited research, this experiment was designed to estimate the extent of variability and magnitude of genetic divergence among 20 genotypes.

MATERIALS AND METHODS

Twenty accessions of *Indigofera tinctoria* were collected from diverse geographical locations were raised in the Orchard unit of the Department of Horticulture, Faculty of Agriculture, Annamalai University. Ten were collected from Kerala, five from Tamil Nadu, three from West Bengal, and one each from Odisha and Karnataka. The seedlings of each accession were raised in 6 x 4 m plots at a spacing of 90 x 90 cm. The experiment was laid out in a randomized block design with three replications. The following biometric observations *viz*., plant height (cm), plant spread (cm), the number of branches, the number of leaves, leaf area (cm^2), biomass (g $plant^{-1}$), fresh weight of shoots (g $plant^{-1}$), fresh weight of leaves (g $plant^{-1}$), glucoside content (%), indigo content, dye yield and dye recovery were recorded at 160 days after sowing. The data recorded during the investigation were statistically analysed following the standard procedures given by Panse and Sukhatme (1961) and using AGRISTAT software.

The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) was computed by using the formula of Burton (1952). The phenotypic and genotypic co-efficient of variations were calculated and classified into three categories *viz*., Low - Below 10 percent, moderate-10-20 percent and high - above 20 percent according to Sivasubramanian and Menon (1973). The phenotypic, genotypic and environmental correlation co-efficient was worked out following Al-Jibouri *et al*. (1958). The direct and indirect effects of yield attributing traits on dye yield were calculated through path co-efficient analysis as suggested by Wright (1921) and elaborated by Dewey and Lu (1959).

RESULTS AND DISCUSSION

Knowledge of genetic diversity regarding yield and its associated characters is valuable in a planned breeding programme since it helps to choose the best yield determining attributes. The environment highly influences yield and its component characters. Hence, it is difficult to conclude whether the observed variability is heritable or not. It therefore, becomes essential to partition the observed variability into heritable and non heritable components. Agarwal *et al.* (1984) reported that the highest genetic variability was due to the polygenic effect. Such variability in a population can be measured by the phenotypic and genotypic coefficient of variation. The inherent genetic variability that remains unaffected by the environmental fluctuation is very useful for the improvement of any crop. The phenotypic co-efficient of variation ranged from 17.95 to 55.05 percent for plant spread to dye yield per plant respectively (**Table 1**). Moderate PCV was observed for plant spread (17.95 %) and biomass (18.55 %) and high PCV was observed for plant height (21.94 %), the number of branches per plant (27.41 %), the number of leaves (25.65 %), leaf area (37.98 %), fresh weight of shoot (20.88 %), fresh weight of leaves per plant (21.81 %), glucoside content (33.41 %), indigo content (24.50 %), dye yield per plant (55.05 %) and dye recovery (36.96 %). Genotypic co-efficient of variation revealed the same pattern of variability as shown by the PCV. The GCV ranged from 17.88 (plant spread) to 54.38 percent (dye yield per plant). The maximum GCV was recorded for plant height (21.91 %), the number of branches per plant (27.39 percent), the number of leaves (25.63 %), leaf area (37.94 %), fresh weight of shoot (20.71 %), fresh weight of leaves (21.62 %), glucoside content (32.05 %), dye yield per plant (54.38 %), dye recovery (35.92 percent), indigo content (23.74 %) and moderate GCV was observed for plant spread (17.88 %) and biomass (18.43 %).

A comparison of PCV and GCV estimates indicated little difference between them showing that the characters studied are less susceptible to the environmental conditions. The results obtained by Angelini *et al.* (1997), Sarada *et al.* (2005), Campeol *et al.* (2006), Spataro and Negri (2008), and Dalziel (2009), in indigo are in agreement with the results of the present investigation. A quantitative estimate of that portion of variability which is due to genetic effect termed as heritability provides information on the relative practicability of selection. However, for reliable selection, heritability estimates and genetic advances would help to predict under selection than heritability estimates alone (Johnson *et al*., 1955) and (Nivedha et al.,2020).

The heritability values ranged from 59.76 percent for the number of branches per plant to 91.78 percent for leaf area **(Table 1).** Moderate value was observed for the number of branches (59.76 %) and heritability was high for all other characters. Genetic advance as percent of mean was the maximum for leaf area (78.08 %) followed by dye recovery (71.88 %) and dye yield (63.64 %). High heritability and genetic advance as percent of mean was recorded for all the characters and indicated that selection may be effective. Similar results were obtained by Sarada *et al.* (2005) and Sarada and Reghunath (2006). It is predicted that the mean dye yield could be advanced by about 63.64 percent through proper selection and hybridization. The high heritability estimate for the glucoside content (82.02 %) also showed a possibility of increasing the glucoside content. Given the possibility of advancing the dye yield by 63.64 percent, the next step in crop improvement is to select the genotypes for further improvement.

Table 1. Magnitude of variability for various characters

S.No.	Characters	Range	Mean	PCV (%)	GCV (%)	h^2 (%)	Genetic advance as per cent of mean
1	Plant height (cm)	83.7 -173.0	124.5	21.94	21.91	69.77	45.09
2	Plant spread (cm)	62.4 - 124.1	92.3	17.95	17.88	71.26	36.70
3	Number of branches	36.9 - 93.7	63.1	27.41	27.39	59.76	56.36
4	Number of leaves	840.1-1974.2	1363.7	25.65	25.63	88.00	52.84
5	Leaf area (cm^2)	6.20-23.60	14.24	37.98	37.94	91.78	78.08
6	Biomass (g/Plant)	368.0 - 668.2	510.5	18.55	18.43	68.63	37.70
7	Fresh weight of shoot (g/plant)	298.0 - 531.2	410.3	20.88	20.71	78.34	42.31
8	Fresh weight of leaves (g/plant)	144.6 - 282.1	205.4	21.81	21.62	88.23	44.14
9	Glucoside content (%)	0.22 - 0.67	0.43	33.41	32.05	82.02	63.35
10	Indigo content (%)	0.91 - 1.85	1.31	24.50	23.74	83.91	47.41
11	Dye yield (g/100 g)	0.73 - 5.95	3.04	55.05	54.38	87.56	63.64
12	Dye recovery (%)	0.36 - 1.12	0.70	36.96	35.92	84.40	71.88

Estimates of the correlation between yield and yield component characters are presented in **Table 2**. Both at phenotypic and genotypic levels, all the traits established a positive and significant correlation with dye yield. Since yield is a complex trait which is influenced by the number of component characters, it is essential to know the importance as well as the degree of association of various traits. Efforts were made to analyze the correlationship between dye yield with other biometric traits like plant height, plant spread, the number of branches, the number of leaves, leaf area, fresh weight of leaves, fresh weight of shoot, biomass, glucoside content, dye recovery and indigo content.

The genotypic correlations were slightly higher than the phenotypic correlation coefficient in the present investigation. This is in agreement with the earlier reports by Sarada and Reghunath (2006) in indigo and Sreekala and Raghava (2003) in marigold. The character dye yield per plant was positively and significantly correlated with plant height, plant spread, the number of branches, the number of leaves, leaf area, fresh weight of leaves, fresh weight of shoot, biomass, glucoside content, dye recovery and indigo content both at genotypic as well as phenotypic levels. A positive association of these yields attributing characters with dye yield was also reported by Sarada *et al*. (2005), Sarada and Reghunath (2006) and Sreekala and Raghava (2003). Even though correlation studies indicated relationship, some characters contribute directly, while other contributes to yield indirectly. Hence, it is necessary to study the direct and indirect effects. With this caveat in mind, the data were subjected to path analysis.

The estimated residual effect was 0.2816 (**Table 3**). The traits, dye recovery (5.1283), biomass (4.9097) and the number of leaves (1.4611) had a very high positive direct effect and the trait fresh weight of shoot (0.6303) and fresh weight of leaves (0.1147) had a high and low direct positive effect on dye yield, respectively. The characters number of branches (-6.9740), indigo content (-2.0734) and leaf area (-1.5925) had a very high but negative direct effect on dye yield. The traits, plant height (-0.3394) and plant spread (-0.4580) had a high negative direct effect on dye yield.

Plant height had a very high positive indirect effect on dye yield *via* the number of leaves, dye recovery and biomass and also exhibited a high positive direct effect *via* fresh weight of shoot and a negligible indirect effect *via* fresh weight of leaves. While, a very high negative indirect effect was observed on dye yield *via* the number of branches, leaf area, indigo content and a high negative indirect effect through plant height and moderate and negligible influence through plant spread and glucoside content, respectively. Plant spread had a positive and very high indirect effect on dye yield per plant *via* the number of leaves, biomass and dye recovery and had a high positive indirect effect *via* fresh weight of shoot and a negligible indirect effect *via* fresh weight of leaves. While, it exerted a very high negative indirect effect on the number of branches, leaf area, indigo content and moderate negative indirect effect and a negligible negative indirect effect through plant height and glucoside content.

The number of branches had a very high direct positive influence on dye yield per plant through the number of leaves, biomass and dye recovery and high positive indirect effect and a negligible but positive indirect effect througthe h fresh weight of shoot and fresh weight of leaves, respectively. While, the characters leaf area and indigo content had a very high negative indirect effect on dye yield per plant and a high negative indirect effect *via* plant spread and moderate negative indirect effect and a negligible negative indirect effect through plant height and glucoside content.

Table 2. Phenotypic and genotypic correlation between various characters in Indigo genotypes

Characters		Plant height	Plant spread	Number of branches	Number of leaves	Leaf area	Fresh weight of leaves	Fresh weight of shoot	Biomass	Glucoside content	Dye recovery	Indigo content	Dye yield
Plant height	P	1.000	0.598**	0.794**	0.777**	0.789**	0.787**	0.802**	0.800**	0.770**	0.774**	0.729**	0.742**
	G	1.000	0.601**	0.796**	0.777**	0.791**	0.794**	0.727**	0.806**	0.805**	0.796**	0.753**	0.752**
Plant spread	P		1.000	0.693**	0.713**	0.699**	0.710**	0.714**	0.721**	0.668**	0.685**	0.686**	0.667**
	G		1.000	0.696**	0.715**	0.702**	0.723**	0.727**	0.733**	0.698**	0.708**	0.707**	0.679**
Number of branches	P			1.000	0.994**	0.982**	0.988**	0.990**	0.991**	0.929**	0.965**	0.948**	0.983**
	G			1.000	0.995**	0.984**	0.998**	0.998**	0.998**	0.967**	0.995**	0.976**	0.995**
Number of leaves	P				1.000	0.982**	0.986**	0.988**	0.985**	0.928**	0.966**	0.953**	0.980**
	G				1.000	0.983**	0.995**	0.996**	0.992**	0.968**	0.995**	0.983**	0.992**
Leaf area	P					1.000	0.973**	0.977**	0.977**	0.913**	0.957**	0.945**	0.968**
	G					1.000	0.985**	0.989**	0.988**	0.950**	0.987**	0.971**	0.981**
Fresh weight of leaves	P						1.000	0.997**	0.994**	0.919**	0.959**	0.935**	0.978**
	G						1.000	0.998**	0.996**	0.973**	0.995**	0.974**	0.990**
Fresh weight of shoot	P							1.000	0.997**	0.921**	0.966**	0.943**	0.981**
	G							1.000	0.997**	0.975**	0.965**	0.981**	0.992**
Biomass	P								1.000	0.922**	0.963**	0.939**	0.980**
	G								1.000	0.973**	0.995**	0.976**	0.991**
Glucoside content	P									1.000	0.895**	0.897**	0.900**
	G									1.000	0.959**	0.957**	0.949**
Dye recovery	P										1.000	0.930**	0.958**
	G										1.000	0.989**	0.992**
Indigo content	P											1.000	0.947**
	G											1.000	0.976**

** Significant at 1 per cent level ; P - Phenotypic correlation G - Genotypic correlation

Table 3. Genotypic path co-efficient analysis for various characters

Characters	Plant height	Plant spread	Number of branches	Number of leaves	Leaf area	Fresh weight of leaves	Fresh weight of shoot	Biomass	Glucoside content	Dye recovery	Indigo content	Dye yield
Plant height	**-0.3394**	-0.2753	-5.5482	1.1359	-1.2591	0.0911	0.5096	3.9552	-0.0412	4.0846	-1.5611	0.752**
Plant spread	-0.2040	**-0.4580**	-4.8532	1.0453	-1.1175	0.0829	0.4582	3.5979	-0.0357	3.6302	-1.4669	0.679**
Number of branches	-0.2700	-0.3187	**-6.9740**	1.4535	-1.5669	0.1144	0.6293	4.8998	-0.0495	5.1014	-2.0239	0.995**
Number of leaves	-0.2638	-0.3276	-6.9377	**1.4611**	-1.5660	0.1141	0.6278	4.8722	-0.0496	5.1008	-2.0388	0.992**
Leaf area	-0.2683	-0.3214	-6.8619	1.4368	**-1.5925**	0.1130	0.6235	4.8499	-0.0486	5.0631	-2.0125	0.981**
Fresh weight of leaves	-0.2694	-0.3309	-6.9567	1.4539	-1.5692	**0.1147**	0.6290	4.8878	-0.0498	5.1003	-2.0192	0.990**
Fresh weight of shoot	-0.2744	-0.3329	-6.9633	1.4552	-1.5754	0.1145	**0.6303**	4.899	-0.0499	5.1270	-2.0345	0.992**
Biomass	-0.2734	-0.3356	-6.9600	1.4499	-1.5731	0.1142	0.6285	**4.9097**	-0.0498	5.1041	-2.0238	0.991**
Glucoside content	-0.2731	-0.3195	-6.7468	1.4141	-1.5122	0.1116	0.6143	4.7771	**-0.0512**	4.9189	-1.9837	0.949**
Dye recovery	-0.2703	-0.3242	-6.9374	1.4533	-1.5723	0.1141	0.6301	4.8865	-0.0491	**5.1283**	-2.0508	0.992**
Indigo content	-0.2555	-0.3240	-6.8077	1.4367	-1.5458	0.1117	0.6185	4.7924	-0.0490	5.0725	**-2.0734**	0.976**

Residual Effect = 0.2816

The character number of leaves had a negligible but positive indirect effect on dye yield per plant *via* fresh weight of leaves and had a high indirect effect *via* fresh weight of shoot and very high positive indirect effect through biomass and dye recovery. While, it had a very high negative indirect effect on dye yield via the number of branches, leaf area and indigo content, a high negative indirect effect *via* plant spread and a moderate negative indirect effect *via* plant height and glucoside content exerted a negligible negative indirect effect on dye yield. Leaf area haa d positive very high indirect effect on dye yield per plant *via* the number of leaves, biomass and dye recovery and high and negligible indirect influence was noticed throthe ugh fresh weight of shoot and fresh weight of leaves, respectively. While, it exerted a very high but negative indirect effect on dye yield *via* the number of branches, indigo content, and a high negative indirect effect *via* plant spread and moderate and a negligible negative indirect effect through plant height and glucoside content. The character biomass showed a very high positive indirect effect on dye yield per plant the number of leaves and dye recovery, high indirect effect *via* fresh weight of shoot and lower influence through fresh weight of leaves. While, it had a negative but very high indirect effect on dye yield *via* the number of branches, leaf area, indigo content and a high negative indirect effect through plant spread and moderate and a negligible negative influence through plant height and glucoside content, respectively. Fresh weight of shoot had positive very high indirect effect on dye yield per plant *via* the number of leaves, biomass and dye recovery and low indirect influence was noticed through fresh weight of leaves. While, it exerted a very high negative indirect effect on dye yield *via* the number of branches, indigo content, leaf area and a high negative indirect effect *via* plant spread and moderate and negligible indirect negative effect through plant height and glucoside content, respectively. The character fresh weight of leaves had a negligible but positive indirect effect on dye yield per plant *via* fresh weight of leaves and had a high indirect effect *via* fresh weight of shoot and influenced highly through biomass and dye recovery. While, it had a very high negative indirect effect on dye yield *via* the number of branches, indigo content and leaf area and high indirect effect on plant spread and a moderate negative indirect effect *via* plant height and negligible influence through glucoside content.

Glucoside content had a positive and very high indirect effect on dye yield per plant *via* the number of leaves, biomass and dye recovery and had a high indirect effect via fresh weight of shoot and low indirect effect *via* fresh weight of leaves. While, it exerted a very high but negative indirect effect on the number of branches, leaf area, indigo content and a high negative indirect effect via plant spread and moderately influenced through plant spread. Dye recovery percent had a very high positive indirect effect *via* the number of leaves and biomass, and a high indirect effect *via* fresh weight of shoot and lower influence through the fresh weight of leaves. While, it had a negative very high indirect effect on dye yield *via* the number of branches, indigo content and leaf area and high negative indirect effect on plant spread, moderate indirect effect *via* plant height and negligible influence through glucoside content.

Indigo content had a positive and very high indirect effect on dye yield per plant *via* the number of leaves, biomass and dye recovery and had a high indirect effect *via* fresh weight of shoot and lower influence through the fresh weight of leaves. While, it exerted a very high but negative indirect effect on dye yield *via* the number of branches, leaf area and a high negative indirect effect *via* plant spread and moderate and negligible indirect influence through plant height and glucoside content, respectively.

Path analysis furnishes a method of partitioning the correlation coefficients into direct and indirect effects and measures the relative importance of the causal factors involved. Among the eleven characters, which showed a significant positive association with yield, dye recovery, biomass, fresh weight of shoot, fresh weight of leaves and the number of leaves indicated a positive direct effect on dye yield. The highest positive direct effect was found in dye recovery (5.1283) and it was followed by biomass (4.9097). Thus, it is concluded that by improving the dye recovery and biomass, the potential dye yield could be increased. As biomass is a complex trait, it is revealed that the number of leaves had influenced through positive indirect effect. However, the number of leaves was indirectly correlated with plant spread (1.0453) and the number of branches (1.4535). From this analysis, it may be concluded that the ideal genotype should have a bushy habit with more number of branches and more number of larger leaves. In this context, the genotypes IT-5, IT-3 and IT-13 were identified as the top three genotypes for maximizing the yield of dye. It was observed that IT-5 recorded superior performance for the traits *viz.,* the number of branches, the number of leaves, biomass, fresh weight of shoot, fresh weight of leaves and indigo content. Based on the mean performance for dye yield and quality, IT-5 was found to be the best genotype.

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