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Studies on Variability, Correlation and Path Analysis Using Important Seed Traits in *Bixa orellana* (L).

S. Kala*[1], K. Kumaran, P. Srimathi, S. Reeja and R.K. Singh

[1]*ICAR-Indian Institute of Soil and Water Conservation, Research Centre, Kota, Rajasthan, India.*
E-mail : kalaforestry@gmail.com

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ABSTRACT

Screening of 34 candidate plus trees was carried out to utilize the genetic variation to identify best CPT (Candidate Plus Tree) on seed bixin content. Significant genetic variability, correlation and association were obtained among 34 CPTs using economic seed traits. Variability studies revealed that, CPT-KLBi 3 recorded maximum value for five seed traits *viz.*, 2D surface area, perimeter seed length, seed width, 100 seed weight, seed dye content and seed bixin content. However, CPT- TNBi 1 was recorded maximum seed bixin content (3.13%) followed by KLBi 3,TNBi 4, KLBi 1 and KLBi 5. In general phenotypic coefficient of variation was higher than genotypic coefficient of variation indicating the predominant role of environment. High heritability broadsense and genetic gain observed for 100 seed weight (99.70%, 32.98 %), seed dye content (93.82 %, 29.05%) and seed bixin content (90.58%, 34.32 %) respectively indicate the additive gene actions. Seed dye content, seed width and 100 seed weight showed positive significant correlation with seed bixin content at phenotypic and genotypic level. Path analysis of seed traits indicated, the seed dye content (0.871) is most pronounced character contributing directly to seed bixin content followed by seed width (0.295) and 100 seed weight (0.068). Hence these seed parameters could be considered as selection criteria for early and positive exploitation of higher bixin yielding genotypes. Study confirmed that the existence of substantial genetic variation which can be utilized for genetic resource conservation in gene bank and further tree improvement programmes of this species.

Key words:

Bixa orellana, Bixin, variability, heritability, correlation, Path analysis

INTRODUCTION

Considering the restrictions placed on the use of synthetic dyes by the World Health Organization, interest in natural dyes has been increasing. The recent ban on production and use of benzedine dyes possessing carcinogenic properties has resulted in a search for an alternative by major dyestuff manufacturers. Nowadays, fortunately, there is increasing awareness among people towards natural products. Siva (2003) opined that the revival and use of vegetable dyes is possible by encouraging detailed research on various herbs, shrubs and trees of dye yielding plants. Recent interest in natural dyes has intensified the investigation on biochemical properties of annatto plants and seeds and raised interest in the evaluation of genetic variability in the species. Selection is practiced to enhance seed production and to raise their pigment content to levels above 2.5 per cent, the commercial base line for export quality (Heywood 1993).

Bixa orellana (L), is commonly known as Annatto or Lipstick plant. It belongs to Bixaceae family; it is native to tropical Central and South America but has become naturalized in many countries of Africa and Asia. In India it is well distributed in Karnataka, Andhra Pradesh, Tamil Nadu, Orissa, West Bengal, Gujarat, Maharashtra, Madhya Pradesh and Chhattisgarh and also reported to be cultivated commercially since last five years. It is a tropical multipurpose small evergreen tree, which is widely known for its dye, used for colouring food, dyeing the cloth, painting the skin and other medicinal uses. Being a safe and easy-to-use product, among naturally occurring colorants, Annatto *(Bixa orellana)* ranks second in economic importance, with an average world consumption of 10,650 tons every year (Satyanarayana et al. 2003).

Annatto being tropical plant can survive well under 28^0 to 44^0C temperature and 800-1500 mm annual rainfall. The economic life of plants is about 20-25 years. Annatto commonly propagated through seeds. The plant bears fruit after two years. A plant yields 500 g seeds per annum. It is a highly remunerative crop. Annatto dye is basically a red orange pigment known as *Bixin* (natural carotenoid) extracted from the seed coat of *Bixa orellana*. The colours may reach up to 7 per cent of the seeds dry mass (Katzer 1999). Annatto is grown in garden as ornamental and hedge crop in social forestry/ live fence (Waurren 1997). The crop is now fetching importance in food industry as the seeds are directly powdered and mixed with food products for their attractive colour. The branches with dry pods are used in dry floral arrangements and from the bark, ropes and twines are made (Little et al. 1974). Bark provides fiber. It is also a good hedge and honey plant

Due to the wide geographical distribution, there is considerable scope of genetic variation in annatto. Seeds, which are the principal means of propagation, contain a lot of variation from one origin to another origin with regard to morphological variation and physiological differences (Mathur et al. 1984). Knowledge of variation within species is a prerequisite for developing effective tree improvement/breeding strategies (Vakashaya et al. 1992). Genetic variation is the fundamental component of adaptation and thus, of stability of forest ecosystems. Variation is the basic resource to be explored for genetic improvement in any species and hence play a key role in plant improvement programmes (Hedegart 1975; Zobel and Talbert 1984; Tiwari 1992). A genetic characterization of natural resources is an essential step for a better understanding of genetic resources for the implementation of in situ and ex situ conservation activities (NBPGR 2000). Recent interest in natural dyes has intensified the evaluation of genetic variability in physical and biochemical properties of annatto seeds. The knowledge of genetic variability, association and path analysis on seed traits is considerable to provide considerable help in genetic improvement of the species. Hence, this study was designed to identify the superior CPT's with higher bixin content and their popularization among the commercial growers is highly needed to meet the demand of natural dye.

MATERIALS AND METHODS

An extensive wild germplasm survey was conducted to identify the high yielding CPTs of *Bixa orellana* at fruiting season from predominant bixa grown locations in three southern states *viz.*, Tamil Nadu, Kerala and Karnataka (Table 1). The selection was made on phenotypic assessment of economic interest characters viz., total tree height, basal girth, age of the tree and free from pest and diseases etc . A total of 34 CPTs (morphologically superior trees) were identified from each CPTs 5 kg fruits were collected and the seeds extracted manually from the fruits of each CPTs separately and evaluated for the seed traits with four replication of 50 seeds each. Measurement of morphometric character *viz.*, 2D surface area, seed length, seed width and perimeter was carried out using Image analyser (WinDAS++) by spreading seed s on a glass platform of macro-viewer in replication wise and capturing images. Further captured images were sent to the software called WinDias and calibrated to actual scale. The Qwin identifies the object based on our specification for seed colour and the measurements carried out were 2 dimensional. 100 seed weight was obtained by weighting 100 pure seeds and was expressed ing grams.

Table 1. Geographical description and salient features of candidate plus trees of *Bixa orellana*

Sl. No.	Name of the CPTs	Location	Districts	State	Basal girth (cm)	Height (m)
1	TNBi 1	Coimbatore	Coimbatore	Tamil Nadu	36.50	4.20
2	TNBi 2	Coimbatore	Coimbatore	Tamil Nadu	20.50	3.30
3	TNBi 3	Coimbatore	Coimbatore	Tamil Nadu	19.50	2.15
4	TNBi 4	Kallar	The Nilgiris	Tamil Nadu	36.00	6.15
5	TNBi 5	Kallar	The Nilgiris	Tamil Nadu	34.50	5.60
6	TNBi 6	Savarapoondi	Triuvannamalai	Tamil Nadu	38.00	2.10
7	TNBi 7	Karaikudi	Sivagangai	Tamil Nadu	22.50	2.60
8	TNBi 8	Nadukkuppam	Triuvannamalai	Tamil Nadu	33.00	1.73
9	TNBi 9	Sirumugai	Coimbatore	Tamil Nadu	41.50	4.50
10	TNBi 10	Thachampadi	Triuvannamalai	Tamil Nadu	26.00	1.75
11	TNBi 11	Melkondagalore	Triuvannamalai	Tamil Nadu	36.00	3.00
12	TNBi 12	Nedunkunam	Triuvannamalai	Tamil Nadu	42.00	1.52
13	KLBi 1	Alansuadu	Malapuram	Kerala	24.20	3.40
14	KLBi 2	Alansuvadu	Malapuram	Kerala	32.50	4.00
15	KLBi 3	Kuttichathanpadi	Malapuram	Kerala	21.00	3.20
16	KLBi 4	Kuttichathanpadi	Malapuram	Kerala	45.00	7.50
17	KLBi 5	Peechi	Thrissur	Kerala	36.50	4.20
18	KLBi 6	Peechi	Thrissur	Kerala	32.50	5.50
19	KLBi 7	Peechi	Thrissur	Kerala	28.20	6.00
20	KLBi 8	Kavilakadu	Malapuram	Kerala	30.50	7.50
21	KLBi 9	Kavilakadu	Malapuram	Kerala	22.50	3.50
22	KLBi 10	Vellanikara	Thrissur	Kerala	20.50	4.50
23	KLBi 11	Vellanikara	Thrissur	Kerala	27.00	3.25
24	KLBi 12	Vellanikara	Thrissur	Kerala	28.50	3.50
25	KLBi 13	Vellanikara	Thrissur	Kerala	26.00	4.50
26	KLBi 14	Vellanikara	Thrissur	Kerala	22.50	4.20
27	KABi 1	Mysore	Mysore	Karnataka	22.50	3.20
28	KABi 2	Mysore	Mysore	Karnataka	28.30	2.75
29	KABi 3	Mysore	Mysore	Karnataka	31.50	3.00
30	KABi 4	Mysore	Mysore	Karnataka	25.30	1.75
31	KABi 5	Mysore	Mysore	Karnataka	29.50	4.20
32	KABi 6	Gonikuppal	Kodagu	Karnataka	35.50	5.00
33	KABi 7	Ponnampet	Kodagu	Karnataka	32.50	4.60
34	KABi 8	Ponnampet	Kodagu	Karnataka	29.50	3.95

Seed dye content (%)

Total seed dye content was estimated by following the procedure of Saraswathi et al. (2004). A 100 ml beaker was weighted with 10 gram of annatto seeds and to which 50 ml of water added and the mixture was heated for 10 minutes, which was allowed cooled for 5 minutes. The dye extracted water was filtered through Whatmann No. 4 filter paper. After complete filtration, the filter papers were dried in a shade and then take final weight of filter paper. The following formula used for calculating the seed dye content.

$$\% \text{ dye content} = \frac{\text{Final weight of the filter paper} - \text{Initial weight of the filter paper}}{10\text{ g}} \times 100$$

Assessment of bixin content

The seed bixin content was estimated using scanning spectrophotometer according to Mckeown and Mark (1962). Methodology for bixin estimation is given below.

Approximately 0.1 g of sample was dissolved and diluted in 100 ml of chloroform. The absorbance of the solution is scanned over the wavelength range 400 – 600 nm in a 1 cm quartz cuvette against a reference. The wave length of maximum absorbance (l max) in the region around 500 nm was determined and the absorbance measured at that wavelength and at 404 nm. The total pigment content expressed as 'Bixin', is calculated using following formula:

$$\text{Bixin (\%)} = \frac{[(A_{max}+A_{404})-(0.256_{Amax})] \times 100 \times \text{Dilution volume (ml)}}{-282.6 \times \text{Sample weight (g)} \times 1000}$$

Where A_{max} is the measured absorbance at l max, A_{404} is the measured absorbance at 404 nm, the value 0.256 is the factor relating the absorbance at 404 and 501 nm for bixin in chloroform, and the value 282.6 is the absorptivity of bixin at 501 nm in chloroform.

Analysis of variance was carried out following the procedure given by Panse and Sukhatme (1976). The variability, heritability in broad sense, genetic advance as % of mean, phenotypic and genotypic variance, phenotypic coefficient variation (PCV) and genotypic coefficient variation (GCV) were calculated for seed traits as suggested by Lush (1940), Jhonson et al (1955) and Burton (1952). Correlation coefficients for seed traits were calculated according to the method suggested by Goulden (1952). Path analysis was done after Dewey and Lu (1959) to apportion the genotypic correlation coefficient into direct and indirect effect. F test was assessed the following Panse and Sukhatame (1978).

RESULTS AND DISCUSSION

Variability in Seed traits

In the present investigation, it was observed that a good and significant amount of variability exists among the different CPTs which were expressed in all the seed characters studied such as 2 D surface area, seed perimeter, Seed length, seed width, 100 seed weight, seed dye content and seed bixin content (Table 2). CPTs were ranked based on significant mean seed attributes. Significantly maximum values for 2D surface area (11.63 mm), seed perimeter (14.77 mm), seed length (4.47 mm) and seed width (3.30 mm), 100 seed weight (4.30 g), seed dye content (15.56%) and seed bixin content (3.05%) was recorded by CPT-KLBi 1, hence, it ranked first in the order of prime seed characters (Table 2). The second best CPT was found to be TNBi-1 which exhibited significant values for the traits viz., 2D surface area (10.60 mm), seed perimeter (14.80mm) seed length (4.39 mm) and seed width (3.36mm), 100 seed weight (3.86 g), seed dye content (15.67 %) and seed bixin content (3.13 %). This was followed by CPT-KLBi-5 recorded significant values for 2D surface area (10.20mm), seed perimeter (13.93 mm) seed length (4.45 mm) and seed width (3.16 mm), 100 seed weight (3.76 g), seed dye content (14.57%) and seed bixin content (2.90%). However, CPT- KABi 5 exhibited lowest value in all seed traits viz., 2D surface area (7.88 mm), seed perimeter (10.87 mm), seed length (3.78 mm), seed width (2.91 mm), 100 seed weight (2.25g), seed dye content 9.14(%) and seed bixin content (1.13%). On the basis of overall computation for all seed characters, the following CPTs KLBi 3, TNBi 1, KLBi 5, KLBi 1, TNBi 4 and KABi 3 were found superior for further exploitation of variation among 34 CPTs (Table 2).

Table 2. Influence of Candidate Plus Trees (CPTs) on important seed characters

Sl. No.	Name of the CPTs	2 D surface area (mm)	Perimeter (mm)	Seed length (mm)	Seed width (mm)	100 seed weight (g)	Seed dye content (%)	Seed bixin content (%)
1	TNBi 1	10.60**	14.80**	4.39**	3.36**	3.86**	15.67**	3.13**
2	TNBi 2	6.28	11.10	3.42	2.57	2.44	13.34*	2.77**
3	TNBi 3	6.87	11.81	3.78	2.56	2.71	9.01	1.65
4	TNBi 4	5.86	10.86	3.42	2.43	3.53**	14.62**	2.98**
5	TNBi 5	5.58	11.90	3.50	2.46	2.67	13.95**	2.79**
6	TNBi 6	7.32	11.13	3.69	2.80	3.47**	10.10	1.53
7	TNBi 7	6.57	10.47	3.28	2.75	2.91	10.05	2.28
8	TNBi 8	8.91*	13.33	4.09*	2.93	2.98	12.86	2.65*
9	TNBi 9	8.76*	13.33	4.04*	3.06*	3.43**	14.04**	2.44
10	TNBi 10	7.69	13.80	3.93	2.60	2.74	9.96	1.75
11	TNBi 11	8.73*	12.47	4.04*	3.15*	3.54**	13.92**	2.53
12	TNBi 12	6.68	11.85	3.42	2.84	3.75**	10.53	2.45
13	KLBi 1	8.93*	14.77**	4.05*	3.03	3.35**	15.01**	2.98**
14	KLBi 2	8.04	12.50	4.08*	2.73	3.39**	13.55**	2.67*
15	KLBi 3	11.63**	14.77**	4.47**	3.30**	4.30**	15.56**	3.05**
16	KLBi 4	5.06	11.77	3.28	2.31	2.40	13.09*	2.53
17	KLBi 5	10.20**	13.93*	4.45**	3.16*	3.76**	14.57**	2.90**
18	KLBi 6	6.79	11.97	3.48	2.70	2.92	12.67	2.72*
19	KLBi 7	7.20	12.80	3.53	2.85	3.00	12.03	2.75**
20	KLBi 8	7.52	13.63	3.94	2.87	3.38**	12.62	2.53
21	KLBi 9	8.41	12.10	4.11*	2.65	3.76**	11.54	2.30
22	KLBi 10	7.41	12.93	3.70	2.67	2.63	14.28**	2.42
23	KLBi 11	7.04	11.01	3.46	2.77	2.77	14.02**	2.75**
24	KLBi 12	6.65	12.23	3.49	2.67	2.75	12.99	2.47
25	KLBi 13	5.89	11.52	3.23	2.71	2.72	13.06*	2.42
26	KLBi 14	8.53	12.40	3.85	3.07*	2.86	11.49	2.25
27	KABi 1	9.18*	12.67	4.22*	2.97	2.74	13.07*	2.53
28	KABi 2	9.35**	16.57**	3.78	3.03	3.28**	14.42**	2.86**
29	KABi 3	7.26	11.80	3.82	2.60	3.66**	13.18*	2.28
30	KABi 4	6.24	11.20	3.37	2.72	2.51	11.52	2.23
31	KABi 5	7.88	10.87	3.78	2.91	2.25	9.14	1.13
32	KABi 6	8.85*	12.07	3.88	3.08*	2.63	9.12	2.28
33	KABi 7	7.89	13.17	3.82	3.07*	3.57**	12.10	2.81**
34	KABi 8	6.68	11.85	3.42	2.84	3.54**	13.85**	2.83**
General mean		7.82	12.62	3.80	2.83	3.12	12.68	2.49
SEd		0.74	1.31	0.22	0.20	0.02	0.38	0.12

*** General mean + 1 SE** **** General mean + 2SE**

The occurrence of *Bixa orellana* over a wide range of habitats with diverse geo-climatic conditions was expected to be reflected in the genetic constitution of its populations. In the presnt study, the seeds from various CPTs exhibited significant variability in seed traits could be attributed to isolations that in turn influences gene flow. Significant variability of seed characters like: seed size and weight was observed in selected plus trees (Bagchi and Sharma 1989) and among various provenances of *Santalum album* (Veerendra et al. 1999). This type of variability in seed morphology and germination is attributed to the out-breeding nature of sandalwood. Genetic control of seed size traits has been observed in several tree species like *Faidheria albida* (Ibrahim et al. 1997), *Tectona grandis* (Jayasankar et al. 1999), *Dalbergia sissoo* (Gera et al. 2000) *Strychnos cocculoides* (Mkonda et al. 2003), *Madhuca latifolia* (Umesh kanna 2001) *Simarouba glauca* (Sekar 2003) and *Pongamia pinnata* (Divakara *et al*, 2010)

Assessment of heritability and genetic advance

Among seed characters in 34 CPTs, the estimation of genotypic coefficients of variation were less than that of the phenotypic coefficient of variation all the characters indicating the influence of non additive gene action (Table 3). Estimation of broad sense heritability was highest for 100 seed weight (99.70%) followed by seed dye content (93.82%) and seed bixin content (90.58%) which were governed by additive gene actions. Seed dye content and seed length estimated positive phenotypic and genotypic correlation with seed bixin content (Tables 4 & 5). The present investigation envisaged that high and positive association coupled with intensive direct effect of seed dye content, seed width and 100 seed weight on seed bixin content (Fig 1). Hence, these seed traits could be used as selection criteria for screening superior genotypes of *Bixa orellana* for further tree improvement programmers.

Table 3. Genetic analysis of seed characters

Characters	Phenotypic variance	Genotypi c variance	PCV (%)	GCV (%)	Heritability (%)	GA as % of mean
Seed length	0.186	0.110	11.32	8.72	59.24	13.82
Seed width	0.103	0.043	11.30	7.28	41.53	9.67
100 seed weight	0.251	0.250	16.06	16.03	99.70	32.98
Seed dye content	3.63	3.404	15.03	14.56	93.82	29.05
Seed bixin content	0.210	0.190	18.40	17.51	90.58	34.32

Table 4. Phenotypic correlation co-efficient analysis of seed characters

Characters	Seed length	Seed width	100 seed weight	Seed dye content (%)	Seed bixin content (%)
Seed length	1.000	0.486**	0.383*	-0.005	-0.025
Seed width		1.000	0.294	0.163	-0.161
100 seed weight			1.000	-0.110	-0.034
Seed dye content				1.000	0.751**
Seed bixin content					1.000

*** Significant at five per cent** **** Significant at one per cent**

Table 5. Genotypic correlation co-efficient analysis of seed characters

Characters	Seed length	Seed width	100 seed weight	Seed dye content (%)	Seed bixin content (%)
Seed length	1.000	0.691**	0.488**	- 0.042	0.012
Seed width		1.000	0.452*	- 0.323*	-0.183
100 seed weight			1.000	- 0.115	-0.035
Seed dye content				1.000	0.832**
Seed bixin content					1.000

*** Significant at five per cent** **** Significant at one per cent**

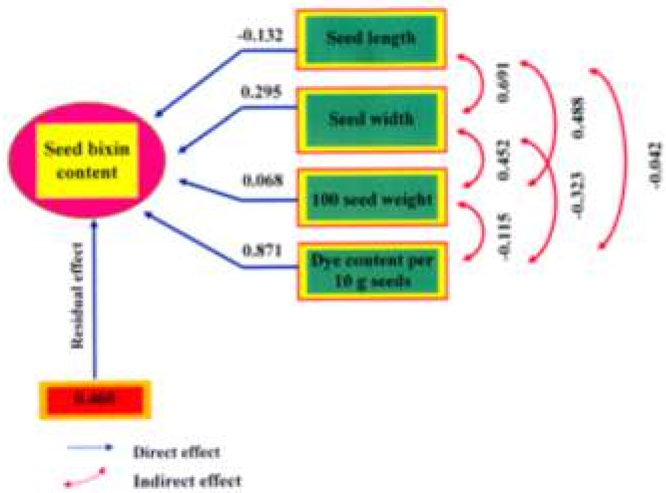

Fig. 1. Path co-efficient analysis showing direct and indirect effects on seed bixin content

The study of seed characters with seed bixin content of natural populations is often considered to be useful step in the study of the genetic variability. Therefore, some of the basic material (seed) from the trees having more seed weight and bixin content may be used for further improvement programme. Similar results have been documented in *Madhuca latifolia* where 100 seed weight and seed width exhibited maximum positive direct effect on oil content (Umesh Kanna 2001). Several authors also reported similar results in many forest tree species *viz.*, *Pongamia pinnata* (Kumaran 1991), *Simarouba gluaca* (Sekar 2003) and *Pongamia pinnata* (Kaushik et al. 2007) and *Madhuca longifolia* (Yadav et al. 2011).

Little difference between phenotypic coefficient of variation and genotypic coefficient of variation and high estimation of heritability (broadsense) for all seed traits under this study clearly revealed the heritable nature of variability present in CPTs. The genotypes coefficient of variation was more than that of the phenotypic coefficient of variation for all the characters indicating the influence of additive gene actions. Higher GCV indicates that worthwhile improvement could be achieved for this through simple selection. This results is concurrence with the finding of genetic parameters in *Azadirachta indica* (Kaushik et al. 2005) and in *Pongamia pinnata* (Kumaran 1991) which lend support to the present study.

High estimates of heritability (99.70%) have also envisaged that environment has comparatively very low influence on the seed traits and bixin content. Heritability has an important place in tree breeding as it provides an index of the relative role of heredity and environment in the expression of various traits. Dorman (1976) reported that heritability estimate is important in tree improvement programme. Kaushik et al (2007) also reported that high heritability in *Pongamia pinnata* for pod-seed ratio (99%), 100 seed weight (100%) and 100 pod weight (98%). Therefore selection based on high heritability will be effective and this offers an opportunity to select genotypes based on these traits.

In general, the genotypic correlation coefficient values were higher than phenotypic correlation coefficient values (Table 3). The genotypic correlation is an estimated value, whereas phenotypic correlation is a derived value from the genotypic and environmental interactions. The genotypic correlation is, therefore, a more reliable estimate for assessing the degree of relationship between characters. Both phenotypic and genotypic correlations between seed dye content, seed width and between seed bixin content were very strong. Correlated quantitative traits are major interest in an improvement program, as the improvement of one character may cause simultaneous changes in the order character. This results agree with the findings in *Pongamia pinnata* (Kaushik et al. 2007) and *Madhuca longifolia* (Yadav et al. 2011), which also exhibited positive correlation between 100 seed weight and seed oil content. Path analysis of seed traits revealed that, seed dye content (0.305), is the most pronounced character contributing directly to seed bixin content followed by seed length (0.345) and the 100 seed weight (0.655) and most other traits associated to seed bixin content are contributing indirectly through seed dye content (Fig.1). This suggests that, seed dye content and 100 seed weight should be given the most attention in the selection for seed with high bixin content in *Bixa orellana*. These results are also line /confirmation with the studies conducted by Yadav et al. (2011) in *Madhuca longifolia and Divakara et al.* (2010) in *Pongamia pinnata.*

CONCLUSION

Potentially huge genetic variability existed in seed traits among various CPTS of Bixa orellana. Among CPTs KLBi 3, TNBi 1, KLBi 5, KLBi 1, TNBi 4 and KABi 3 were found superior in all kind seed traits. Hence, selection, exploitation, mass propagation and popularization of these screened CPTs for commercial cultivation found to be very effective in terms of higher seed bixin yield. Higher genotypic correlation coefficient of seed characters revealed that the traits are genetically controlled and selection can be very effective in tree improvement programmers of these species. Further due to the high positive association coupled with intensive direct effect of seed dye content, seed width and 100 seed weight on bixin content. From studies, overall results revealed the existence of substantial genetic variation resources conservation in gene bank and further tree improvement programmers of the species.

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