

RESEARCH ARTICLE II

Evaluation of Growth Performance of *Bixa orellana* L. Progenies under Nursery Conditions

Saranya Kumari R * and Kumaran K

Department of Forest Biology and Tree Improvement, Forest College and Research Institute, TNAU, Mettupalayam – 641 301

ABSTRACT

Dyeing is a traditional art and the Indians are experts in doing it. Natural dyes find huge scope in the current era of organic interests. *Bixa orellana* L. is commonly known as a lipstick tree. The present study was conducted to identify the superior progenies from plus trees selected from various locations of Tamil Nadu and Andhra Pradesh. The progenies were evaluated for four growth periods in the nursery such as 45 days after sowing (DAS), 90 DAS, 135 DAS, and 180 DAS. The growth performance was recorded for shoot length, collar diameter, root length, root to shoot ratio, number of leaves, sturdiness quotient, and volume index. The results indicated that the two progenies showed significant superiority in growth attributes viz., TNBi 008 and TNBi 003. Higher growth attributes in nursery seedlings may contribute more to better performance in field conditions. Volume index recorded the maximum values for GCV (36.04%), PCV (37.81%), and genetic advance (70.76%). The highest heritability was shown by shoot length (0.99) and root length (0.98). Good tree architecture facilitates them to produce more resources, such energy could be allocated for a further reproductive phase that results in increased seed yield. The superiority of these progenies for growth traits shows that they could be better utilized for developing plantations to meet the dye demand and for further tree improvement programme.

Key words: Natural dyes; *Bixa*; Growth; Variation; Progenies

INTRODUCTION

Natural dyes are God's gift to mankind. Indians are the experts in the field of natural dyeing. Natural dyes are those which are obtained naturally from plants and animals. The extraction of natural dyes does not need any intense mechanism or process (Adeel *et al.*, 2020). There are more than 450 dye-yielding species in India. One such important, commercial species is *Bixa orellana* L. The commercial name of *Bixa* is Annatto. It comes under the Family Bixaceae. *Bixa orellana* is considered to be the domesticated species from *Bixa urucura* Wild. (Moreira *et al.*, 2015). *Bixa* is native to Central and South American countries, but it is highly distributed in the continents of Asia and Africa. The main carotenoid component in annatto seeds is Bixin, an orange-red coloured pigment (Rivera-Madrid *et al.*, 2006). Bixin is highly exploited as a natural colorant in food industries, textiles as well as cosmetic industries (Teixeira *et al.*, 2019). The demand for natural dyes keeps increasing because of awareness of the ill effects of synthetic dyes. It is now necessary to increase the knowledge on dye

yielding species as well as to improve their production by means of genetic improvement of the species. Selection is the first and foremost important step in the tree improvement programme. *Bixa orellana* contains several cultigens that could be delivered from their ideal morphological features viz. variation in flower colour, pod shape, pod colour and so on (Joseph and Siril, 2013). These variations could be analyzed, tested for genetic parameters and the best genotype could be identified. This is possible only by means of selecting the best phenotype i.e. plus tree selection (Zobel and Talbert, 1984). Plus trees are selected based on their superiority in height, basal girth, number of branches, yield, and the variation in pod shape, pod colour and free of pests and diseases. There is an immediate need to identify the best genetic resource of *Bixa* and this paper clearly explains the superiority of growth performance of progenies raised from plus trees under nursery conditions.

MATERIALS AND METHODS

The experimental materials used for the present study include twenty *Bixa* progenies. These progenies

are seed based materials and are raised from twenty plus trees of Bixa orellana from different locations in Tamil Nadu and Andhra Pradesh. These selected plus trees are given with the tree code of "TNBi 00".

Nursery experiments were carried out at the nursery complex of the department of Forest Biology and Tree Improvement, FC&RI, Mettupalayam, Tamil Nadu. Polybags used for the experiment were filled with the proper mixture of soil: sand: FYM in the ratio of 2:1:1. Watering and weeding were done at regular intervals.

Experimental design

The statistical design used was Completely Randomized Block Design (CRBD) with twenty progenies as treatments and 3 replications for each treatment. Each replication carries 25 seedlings.

Biometric observations

The data were taken for every 45 days after sowing (DAS) for 4 growth periods such as 45 DAS, 90 DAS, 135 DAS, and 180 DAS. The growth performance was measured using the traits such as shoot length, collar diameter, root length and number of leaves. From the data taken sturdiness quotient and volume index were derived. Data were analyzed separately using 'TNAUSTAT' software. Estimates of mean, variance, and standard error were done as per procedures described by Panse and Sukhatme, 1978. The significance test was done by referring to the standard 'F' table of Snedecor (1961).

Shoot length: The length of the longest stem was measured from the collar region to the tip of the stem and expressed in cm.

Collar diameter: It was measured at the base of the stem at the root collar region and expressed in cm.

Root length: The length of the longest root was measured from the collar region to the tip of the root and expressed in cm.

Root to shoot ratio: The ratio is obtained by dividing the root length by shoot length and has no unit.

Number of leaves: All the leaves were counted and expressed in the whole number.

Sturdiness quotient: The Sturdiness quotient was calculated using the formula,

$$\text{Sturdiness Quotient} = \frac{\text{Height(cm)}}{(\text{Diameter (cm)})}$$

(Sujitha, 2017)

Volume index: The volume index was calculated using the following formula.

$$\text{Volume index (cm}^3\text{)} = (\text{Diameter})^2 \times \text{Height}$$

(Kiruba, 2012)

Variability studies

i. Phenotypic co-efficient of variability

$$\text{PCV (\%)} = \frac{\sqrt{(\text{Phenotypic variance})}}{(\text{General mean})} \times 100 \quad (\text{Johnson et al., 1955})$$

ii. Genotypic co-efficient of variability

$$\text{GCV (\%)} = \frac{\sqrt{(\text{Genotypic variance})}}{(\text{General mean})} \times 100$$

(Johnson et al., 1955)

iii. Heritability (h^2)

Broad sense heritability (h^2) was calculated according to (Lush, 1940).

$$h^2 = \frac{\sigma^2_g}{\sigma^2_p}$$

iv. Genetic advance

The genetic advance was calculated according to (Johnson et al., 1955).

$$\text{GA} = \frac{\sqrt{(\text{Genotypic variance})}}{(\text{Phenotypic variance})} \times K$$

Where, K = 2.06, a selection differential at 5 per cent selection intensity

v. Genetic advance as percentage of mean

$$\text{GA as percentage of mean} = \frac{\text{GA}}{\text{Grand mean}} \times 100$$

RESULTS AND DISCUSSION

Shoot length

The shoot length recorded at 45 DAS was ranging from 5.40 cm (TNBi 001 and TNBi 002) to 11.18 cm (TNBi 008). At 90 DAS data revealed minimum and maximum shoot lengths as 10.98 cm (TNBi 0017) and 19.33 cm (TNBi 003). Growth data taken at 135 DAS showed that shoot lengths ranged from 21.14 cm (TNBi 002) to 42.33 cm (TNBi 004). The shoot length recorded at 180 DAS showed a range from 27.02 cm (TNBi 002) to 48.98 cm (TNBi 003) (Table 1). In all the 4 growth periods, TNBi 003, TNBi

004, TNBi 008 and TNBi 0012 showed significant superiority consistently in the case of shoot length.

The GCV and PCV recorded by the shoot length were 18.61% and 18.67%, respectively. Shoot length registered the maximum heritability among all other growth parameters (0.99). The value of genetic advance as per cent of the mean was 38.2% (Table 2).

Collar diameter

The collar diameter taken at 45 DAS ranged from 0.11 cm (TNBi 006) to 0.24 cm (TNBi 008 and TNBi 0014). Data recorded at 90 DAS revealed minimum and maximum collar diameter as 0.24 cm (TNBi 006) and 0.43 cm (TNBi 0014). Growth data taken at 135 DAS showed that the values ranged from 0.37 cm (TNBi 006) to 0.59 cm (TNBi 0014). The collar diameter taken at 180 DAS showed a range from 0.50 cm (TNBi 002) to 0.77 cm (TNBi 0014) (Table 1). In all the four growth periods taken, three progenies viz. TNBi 003, TNBi 008 and TNBi 0014 expressed significant high values for collar diameter.

Collar diameter exerted the values of GCV (11.73%) and PCV (12.86%). The values of heritability and genetic advance per cent of the mean were 0.83 and 22.12%, respectively. Collar diameter showed the minimum heritability among other growth parameters (Table 2).

Root length

The root length taken at 45 DAS showed a range from 3.67 cm (TNBi 001) to 13.76 cm (TNBi 0021). Data recorded at 90 DAS revealed that the minimum and maximum root lengths as 9.33 cm (TNBi 001) and 21.87 cm (TNBi 007). Growth data taken at 135 DAS showed that the values ranged from 21.92 cm (TNBi 0010) to 40.03 cm (TNBi 0012). The root length taken at 180 DAS showed a range from 28.10 cm (TNBi 0010) to 48.10 cm (TNBi 0012) (Table 1). Considering all growth periods, five progenies such as TNBi 005, TNBi 008, TNBi 0012, TNBi 0019 and TNBi 0021 showed significant values continuously in the case of root length.

The GCV and PCV recorded by the root length were 16.98% and 17.1%, respectively. Root length registered the heritability value of 0.98 and genetic advance as per cent of the mean was 34.74% (Table 2).

Root-to-shoot ratio

The root to shoot ratio calculated at 45 DAS showed the data range from 1.29 (TNBi 0021) to 0.68 (TNBi 001). Data recorded at 90 DAS revealed that the minimum and maximum root shoot ratios as 0.79 (TNBi 001) and 1.34 (TNBi 0017) respectively. Data calculated at 135 DAS showed that root to shoot ratio ranging from 0.85 (TNBi 001) to 1.17 (TNBi 0017). The root to shoot ratio data taken at 180 DAS showed a range from 0.89 (TNBi 001) to 1.15 (TNBi 0013) (Table 1). In all the four growth periods, six progenies like TNBi 002, TNBi 005, TNBi 006, TNBi 0013, TNBi 0016 and TNBi 0017 expressed significant values continuously in the case of root to shoot ratio.

This trait expressed the GCV and PCV values of 7.84% and 8.29% respectively. Heritability recorded by root to shoot ratio was 0.89 and it also exhibited the genetic advance as per cent of mean as 15.29% (Table 2).

Number of leaves

Data on the number of leaves taken at 45 DAS showed a range from 8.22 (TNBi 0016) to 13.67 (TNBi 008). Data recorded at 90 DAS revealed that the minimum and a maximum number of leaves as 17.22 (TNBi 0016) and 28.00 (TNBi 008). Growth data taken at 135 DAS showed that the values ranged from 28.78 (TNBi 0016) to 39.67 (TNBi 008). The data on the number of leaves taken at 180 DAS showed a range from 37.00 (TNBi 002) to 45.22 (TNBi 008) (Table 1). In all the four growth periods, three progenies like TNBi 008, TNBi 009 and TNBi 0013 showed significant values continuously in the case of the number of leaves.

The values for GCV (6.04%) and PCV (6.54%) registered by the number of leaves was the lowest compared with all other growth parameters. It showed a heritability of 0.85 and recorded the minimum value of genetic advance as per cent of the mean (11.47%) than the other parameters (Table 2).

Sturdiness quotient

The sturdiness quotient calculated at 45 DAS showed the data range from 31.50 (TNBi 002) to 77.01 (TNBi 006). Data recorded at 90 DAS revealed that the minimum and maximum sturdiness quotients as 35.22 (TNBi 002) and 64.12 (TNBi 006). Data calculated at 135 DAS showed that

the sturdiness quotient ranged from 49.26 (TNBi 0017) to 76.60 (TNBi 004). The sturdiness quotient calculated at 180 DAS showed the data range from 45.21 (TNBi 0018) to 68.32 (TNBi 0012) (Table 1). In all the four growth periods, TNBi 006 showed significant superiority consistently in the case of sturdiness quotient.

The sturdiness quotient expressed the GCV and PCV values as 14.46% and 15.40% respectively. It also recorded the values of heritability as 0.88 and genetic advance as per cent of the mean as 27.99% (Table 2).

Volume index

The volume index measured at 45 DAS ranged from 0.09 cm³ (TNBi 0017) to 0.65 cm³ (TNBi 008). Data recorded at 90 DAS revealed that the minimum and maximum volume index values as 0.82 cm³ (TNBi 0017) and 3.35 cm³ (TNBi 0021). Growth data taken at 135 DAS showed that the values ranged from 3.29 cm³ (TNBi 0016) to 14.44 cm³ (TNBi 003). The volume index measured at 180 DAS showed a range from 6.76 cm³ (TNBi 002) to 26.13 cm³ (TNBi 003) (Table 1). In all the four growth periods, TNBi 003, TNBi 008, TNBi 0014 and TNBi 0015 showed significant superior values continuously in the case of volume index.

The volume index showed the maximum values of GCV (36.04%) and PCV (37.81%) among all the growth traits considered. It registered a heritability value of 0.91 and also expressed the highest value for genetic advance as per cent of the mean (70.76%) compared to other traits (Table 2).

Annatto dye is highly used in food industries and is well known for its medicinal and cosmetic values (Kumaran, 2014). Seed extracts of annatto contain several secondary metabolite substances such as Himachol, α -Terpinene, Synaptogenin, and so on (Priyanka *et al.*, 2017). The demand for natural dyes are keep on increasing day by day. This requires systematic tree improvement programmes that could be achieved by selection, evaluation, and conservation of the species. Nursery evaluation of progenies showed significant variations at 4 growth periods viz. 45 DAS, 90 DAS, 135 DAS and 180 DAS for traits such as shoot length, collar diameter, sturdiness quotient, and volume index.

Different researchers revealed that soil and climatic conditions play a vital role in reporting genetic variability among the genotypes (Krishnakumar *et al.*, 2018). Seed characters contribute more to creating variability among the seedlings for growth performances. Nursery growth performance is very important in forecasting the good establishment of seedlings under field conditions. In the present study, two progenies viz., TNBi 008 showed significant superiority in growth traits such as shoot length, collar diameter, root length, number of leaves, volume index, and TNBi 003 exhibited better performance in traits such as shoot length, collar diameter, and volume index as well. Similarly, superiority in few genetic resources was reported in *Ailanthus excelsa* (Kanna *et al.*, 2019) and *Calophyllum* (Palanikumaran *et al.*, 2015). Significant variations could be also obtained from seed sources, provenances, and progenies in tree species like *Bixa orellana* (Kala *et al.*, 2017) and *Leuceanaleucocephala* (Sangram and Keerthika, 2013) and which provided support to the present findings.

Estimating genetic parameters is an ideal way to evaluate the performance of progenies and it is the key to progress of the tree improvement programme (Zobel, 1981). The estimates of GCV and PCV are much needed to understand the environmental effect on different traits. In our study, shoot length and root length recorded high heritability. Similar high heritable estimates were previously reported in *Terminalia bellerica* (Gajendra, 2016), *Madhuca indica* (Wani *et al.*, 2015).

In all the growth traits taken, the values of the genotypic coefficient of variability were lower than those of the phenotypic coefficient of variability. This shows that there is an existence of greater level of opportunity for selection and a further improvement in *Bixa orellana*. Traits with high GCV values are suitable for selection compared to those of moderate GCV values. Similarly, growth parameters with moderate GCV values have more potential for improvement than those with lower values (Kumar *et al.*, 2013). Growth traits with high heritability combined with genetic gain could serve as a better indicator for selection, as reported in *Wrightia tinctoria* (Sujitha, 2017).

Table 1. Variation among the nursery progenies for growth parameters at 180 DAS

S.No	Progeny code	Shoot length (cm)	Collar diameter (cm)	Root length (cm)	Root to shoot ratio	Number of leaves	Sturdiness quotient	Volume index (cm ³)
1	TNBi 001	32.91	0.65	29.27	0.89	38.11	50.71	13.94
2	TNBi 002	27.02	0.50	29.22	1.08**	37.00	54.11	6.76
3	TNBi 003	48.98**	0.73*	45.12**	0.92	39.22	67.17**	26.13**
4	TNBi 004	48.80**	0.73*	45.92**	0.94	39.55	66.94**	26.03**
5	TNBi 005	38.25**	0.57	41.92**	1.10**	42.89**	67.23**	12.45
6	TNBi 006	32.70	0.52	34.95	1.07*	37.78	62.93**	8.85
7	TNBi 007	32.88	0.58	34.78	1.06	39.56	56.40	11.21
8	TNBi 008	43.92**	0.73*	46.67**	1.06	45.22**	60.24*	23.43**
9	TNBi 009	35.13	0.66	31.90	0.91	43.33**	53.73	15.21
10	TNBi 0010	30.69	0.65	28.10	0.92	36.44	47.47	12.83
11	TNBi 0012	46.43**	0.68	48.10**	1.04	38.56	68.32**	21.48**
12	TNBi 0013	27.90	0.60	32.12	1.15**	43.00**	46.33	10.19
13	TNBi 0014	38.74**	0.77**	36.58	0.94	41.67*	50.14	23.17**
14	TNBi 0015	35.86	0.76**	34.71	0.97	38.33	47.24	20.72**
15	TNBi 0016	28.11	0.55	31.41	1.12**	37.45	51.10	8.65
16	TNBi 0017	29.69	0.64	32.67	1.10**	38.78	46.71	12.07
17	TNBi 0018	32.10	0.71	31.81	0.99	38.00	45.21	16.20
18	TNBi 0019	38.90**	0.69	39.96**	1.03	38.45	56.45	18.56
19	TNBi 0020	34.74	0.75**	36.29	1.04	39.00	46.66	19.77*
20	TNBi 0021	34.67	0.72	37.98*	1.10**	42.67**	48.26	18.04
Mean		35.92	0.66	36.47	1.02	39.75	54.67	16.28
SEd		0.46	0.03	0.59	0.02	0.82	2.36	1.52
CD (0.05%)		0.93	0.06	1.19	0.04	1.65	4.77	3.07
CD (0.01%)		1.24	0.08	1.6	0.05	2.22	6.39	4.11

*Significant at 5% level

**Significant at 1% level

Table 2. Genetic estimates of growth traits of the progenies of plus tree

Growth traits	GCV (%)	PCV (%)	Heritability	GA as (%) of mean
Shoot length	18.61%	18.67%	0.99	38.2%
Collar diameter	11.73%	12.86%	0.83	22.12%
Root length	16.98%	17.1%	0.98	34.74%
Root to shoot length	7.84%	8.29%	0.89	15.29%
Number of leaves	6.04%	6.54%	0.85	11.47%
Sturdiness quotient	14.46%	15.40%	0.88	27.99%
Volume index	36.04%	37.81%	0.91	70.76%

CONCLUSION

Among the twenty progenies of Annatto evaluated under nursery conditions, two progenies viz., TNBi 008 performed superiority in growth, especially in shoot length, collar diameter, root length, number of leaves, and volume index at all the three growth periods, and TNBi 003 that showed consistent superiority in growth performance, especially in shoot length, collar diameter and volume index at all the three growth periods and volume index could be taken as a better indicator for breeding work and these two progenies (TNBi 003 and TNBi 008) could be utilized for the further tree improvement programme.

Funding and Acknowledgement

We thank Marudhukumar (B.Sc. Forestry) final year student, who helped in data collection. No other fundings were obtained.

Ethics Statement

No specific permits were required for the described nursery studies because no human or animal subjects were involved in this research.

Originality and plagiarism

Entire research work is a part of my M.Sc. Thesis under Forestry discipline with specialization in Forest Biology and Tree Improvement. Have not copied or used any other work.

Consent for publication

We agree to publish the content.

Competing interests

There were no conflict of interest in the publication of this content.

Data availability

All the data of this manuscript are included in the MS. No separate external data source is required. If anything is required from the MS, certainly, this will be extended by communicating with the corresponding author through corresponding official mail; rsaranyakumari98@gmail.com

REFERENCES

- Adeel, S., Amin, N., Ahmad, T., Batool, F. and Hassan, A. 2020. Sustainable isolation of natural dyes from plant wastes for textiles. Recycling from waste in fashion and textiles: a sustainable and circular economic approach., 363-390.
- Gajendra, C. V. 2016. Genetic Evaluation of Terminalia bellerica Roxb. Progenies. M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore.
- Johnson, H. W., Robinson, H. and Comstock, R. 1955. Estimates of genetic and environmental variability in soybeans. Agronomy journal., 47(7): 314-318.
- Joseph, N., and Siril, E. 2013. Floral color polymorphism and reproductive success in annatto (*Bixa orellana* L.). Tropical plant biology., 6(4): 217-227.
- Kala, S., Kumaran, K., Srimathi, P., Reeja, S. and Singh, R. 2017. Studies on Variability, Correlation and Path Analysis Using Important Seed Traits in *Bixa orellana* (L). Journal of Tree Sciences., 36(1): 93-102.
- Kanna, S. U., Krishnakumar, N., Kather, M. M. A. and Jailani, K. 2019. Growth performance of *Ailanthus excelsa* through progeny test. The Pharma Innovation Journal., 8(7): 204-210.
- Kiruba, M. 2012. Evaluation of Genetic Diversity of *Bixa orellana* L. using biometric, biochemical and molecular tools. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore.
- Krishnakumar, N., Parthiban, K. and Kanna, S. U. 2018. Growth performance of sandal wood (*Santalum album* L.)(An endangered medicinal tree)

- progenies under nursery conditions. The Pharma Innovation Journal., 7(5, Part E): 312.
- Kumar, P., Parthiban, K. and Saravanan, V. 2013. Genetic variations among open pollinated families of selected better trees in *Melia dubia*. Research Journal of Recent Sciences., 2: 189-194.
- Kumaran, K. 2014. Production potential of annatto (*Bixa orellana* L.) as a source of natural edible dye. Paper presented at the the proceedings of the 2014 International workshop on natural dyes, Hyderabad, India.
- Lush, J. L. 1940. Intra-sire correlations or regressions of offspring on dam as a method of estimating heritability of characteristics. Journal of animal science., 293-301.
- Moreira, P. A., Lins, J., Dequigiovanni, G., Veasey, E. A. and Clement, C. R. 2015. The domestication of annatto (*Bixa orellana*) from *Bixa urucurana* in Amazonia. Economic botany., 69(2): 127-135.
- Palanikumar, B., Parthiban, K., Sekar, I., Umarani, R. and Amirtham, D. 2015. Variability studies for seed and seedling traits in *Calophyllum inophyllum* (L.) at south India. Trends in Biosciences., 8(14): 3593-3599.
- Panse, V. and Sukhatme, P. 1978. Statistical methods for agricultural workers New Delhi. ICAR Publication, 41-50.
- Priyanka, V. 2016. Investigation on the performance, chemical characterization and industrial applicability of *Bixa orellana* (L.). Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore.
- Rivera-Madrid, R., Escobedo-Gm, R. M., Balam-Galera, E., Vera-Ku, M. and Harries, H. 2006. Preliminary studies toward genetic improvement of annatto (*Bixa orellana* L.). Scientia Horticulturae., 109(2): 165-172.
- Sangram, C. and Keerthika, A. 2013. Genetic variability and association studies among morphological traits of *Leucaena leucocephala* (Lam.) de Wit. genetic resources. Research Journal of Agriculture and Forestry Sciences., 1(8): 23-29.
- Sujitha, 2017. Studies on the genetic evaluation and propagation in sweet indrajo [*Wrightia tinctoria*] M.Sc Thesis, Tamil Nadu Agricultural University, Coimbatore.
- Teixeira da Silva, J. A., Zeng, S., Godoy-Hernandez, G., Rivera-Madrid, R. and Dobranszki, J. 2019. *Bixa orellana* L.(achiote) tissue culture: a review. In Vitro Cellular and Developmental Biology Plant., 55(3).
- Wani, M. S., Wani, A. and Mughal, A. 2015. Estimation of divergence to genetic variation in half-sib families of *Madhuca indica* Gmel. under greenhouse and open field environmental conditions. Indian Forester., 141(1): 35-40.
- Zobel, B. and Talbert, J. 1984. Applied forest tree improvement, John Wiley and Sons Publications.