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# Assessment of genetic diversity in annatto through progeny evaluation

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**Abstract**
Annatto is a commercially exploited plant species for its seed dye. Bixin is the carotenoid present in the seed arils of the plant. Twenty seed based progenies were raised from plus trees of *Bixa orellana* L. collected from various geographic locations of Tamil Nadu and Telangana. Significant variations were recorded in the growth performance under nursery. Genetic divergence studies revealed that 20 progenies were resolved into seven clusters. Higher cluster mean values were shown in cluster VII (TNBi 0012) and cluster VI (TNBi 008), as they are unique and genetically divergent. The clustering pattern revealed that there is no connection between genetic diversity and geographical nearness. Cluster IV reported maximum intra cluster distance. Maximum inter cluster distance was recorded between cluster I and cluster IV. The progenies of cluster I (TNBi 003 and TNBi 005) and cluster IV (TNBi 002 and TNBi 0013) could be employed for further hybridization for the improvement of the species.

**Key words:** Annatto, Progenies, Growth traits, Clusters, Genetic diversity

## INTRODUCTION

Colours are the smiles of nature as they bring happiness. Natural dyes provide all the colours of the rainbow. Natural dyes are highly demanded products in the current market for their eco-friendly, non-toxic and easy to use abilities. One such important natural dye yielding species is Annatto (*Bixa orellana* L.). It is a small evergreen tree, commonly known as lipstick tree, for its dye is present in the seed arils of the plant. It belongs to the family Bixaceae. Bixa is the only genus under Bixaceae with six species (Umadevi *et al.,*2020). It is well distributed under varied tropical climates. Bixin, orange to red coloured pigment present in this plant, is the most commercially exploited apocarotenoid to extract natural dye (Rivera-Madrid *et al.,* 2006). Bixin is utilized as a natural colorant in food industries, textile and cosmetic industries (Teixeira *et al.,* 2019). Annatto also has high medicinal values and acts as a hedge crop in farms and as an ornamental plant in gardens (Kala and Kumaran, 2015). The high amount of variation is seen in flower colour, pod shape, pod colour, leaf shape, seed characters, and dye content. Genetic diversity is the major component of biodiversity and its assessment plays a vital role in tree improvement (Zobel, 1971). This generates a rich and wider base population for commencing proper selection. The most suitable statistical tool for genetic diversity assessment is $D^2$ statistics. Clustering is done to identify the superior group or an ideal individual for utilization. Plus trees are phenotypically superior trees but have not yet genetically been tested (Zobel and Talbert, 1984). In the present studies, an attempt was made to scale genetic divergence among the twenty progenies of plus trees to find out the potential progenies for further tree improvement in Annatto to meet out the increasing dye demand.

## MATERIALS AND METHODS

Seeds were collected from twenty different plus trees of *Bixa orellana* from Tamil Nadu and Telangana. Seedlings were produced at the nursery in the Department of Forest Biology and Tree Improvement, Forest College and Research Institute, Mettupalayam, India. Plus trees were selected based on the phenotypic characters such as tree height, basal girth, the number of branches, pod colour variation, pod shape variation, yield, pest and disease free.

Twenty progenies were raised in three replications. Each replication had twenty five seedlings. The statistical design used was a Completely Randomized Block Design. The growth traits studied were shoot length, collar diameter, root length, root shoot ratio, the number of leaves, sturdiness quotient and volume index. Data on biometric observations were taken at an interval of 45 days up to six months. Biometrical data recorded at 180 DAS alone was taken for genetic divergence studies. Analysis of data was done statistically as per Panse and Sukhatme (1978). For the assessment of genetic divergence, $D^2$ statistics were used (Mahalanobis, 1928). Clustering of progenies was done using the results of $D^2$ statistics.'TNAUSTAT' statistical package was used to calculate $D^2$ values using Tocher's method as recommended by Rao (1952). Intra and inter cluster relationships were studied after clustering was done. The mutual relationship between the clusters, their intra and inter cluster distances was identified.

## RESULTS AND DISCUSSION

The progeny evaluation studies at the nursery showed significant variation among the progenies due to growth trait performances and the results are given in **Table 1**. The shoot length recorded at 180 DAS showed a range from 27.02 (TNBi 002) to 48.98 cm (TNBi 003). Among twenty progenies, seven progenies showed significantly higher shoot length than the general mean value. The collar diameter taken at 180 DAS showed a range from 0.50 (TNBi 002) to 0.77 cm (TNBi 0014), six progenies showed significantly higher collar diameter than the general mean. The root length taken at 180 DAS showed a range from 28.10 (TNBi 0010) to 46.67 cm (TNBi 0012) whereas seven progenies showed significantly higher

**Table 1. Growth performance of Annatto progenies at 180 DAS in nursery**

| S.No. | Progenies (code) | Shoot length (cm) | Collar diameter (cm) | Root length (cm) | Root to Shoot ratio | Number of leaves | Sturdiness quotient | Volume index |
|---|---|---|---|---|---|---|---|---|
| 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 |
| | $SE_d$ | 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 |

* 5% Significance

root length than the general mean. The root to shoot ratio data taken at 180 DAS showed a range from 0.89 (TNBi 001) to 1.15 (TNBi 0013). Seven progenies showed a significantly higher root to shoot ratio than the general mean.

The data on the number of leaves taken at 180 DAS ranged from 37.00 (TNBi 002) to 45.22 (TNBi 008), six progenies showed a significantly higher number of leaves than the general mean. The sturdiness quotient calculated at 180 DAS ranged from 45.21 (TNBi 008) to 68.32 (TNBi 0012). Among twenty progenies, six progenies showed a significantly higher sturdiness quotient than the general mean. The volume index measured at 180 DAS showed a range from 6.76 (TNBi 002) to 26.13 $cm^3$ (TNBi 003), seven progenies showed significantly higher volume index than the general mean. Thus the variation recorded in the present research helps in finding the superior progenies with higher values for growth attributes. Such variation in growth traits at nursery conditions were recorded earlier in *Bixa orellana* L. (Kala and Kumaran, 2012) and in *Dalbergia sissoo* (Deve and Parthiban, 2014). Considering twenty progenies, only three progenies *viz.,* TNBi 008, TNBi 003 and TNBi 005 showed superior performances for growth traits. Similar studies of such superiority for growth traits were reported in seed sources of *Prosopis julifora* (Sundaram, 2018), progenies of *Acacia nilotica* (Singhdoha *et al*., 2017).

Genetic diversity assessment is the most important part of all breeding programs and possesses greater significance. This plays a vital role in inter-mating of different groups and acts as a pre-requisite for a successful tree improvement, thus producing genetically superior ones (Shree *et al*., 2018). Based on Mahalanobis $D^2$ clustering techniques, the twenty progenies of Annatto were placed into seven clusters (**Fig. 1**). Similar studies were done earlier and reported in *Bixa orellana* (Kiruba, 2012) and also in *Azadirachta indica* (Kumaran, 1997).

Progeny distribution in each cluster is different from one another. Cluster I consisted of two progenies (TNBi 003 and TNBi 004); cluster II constituted five

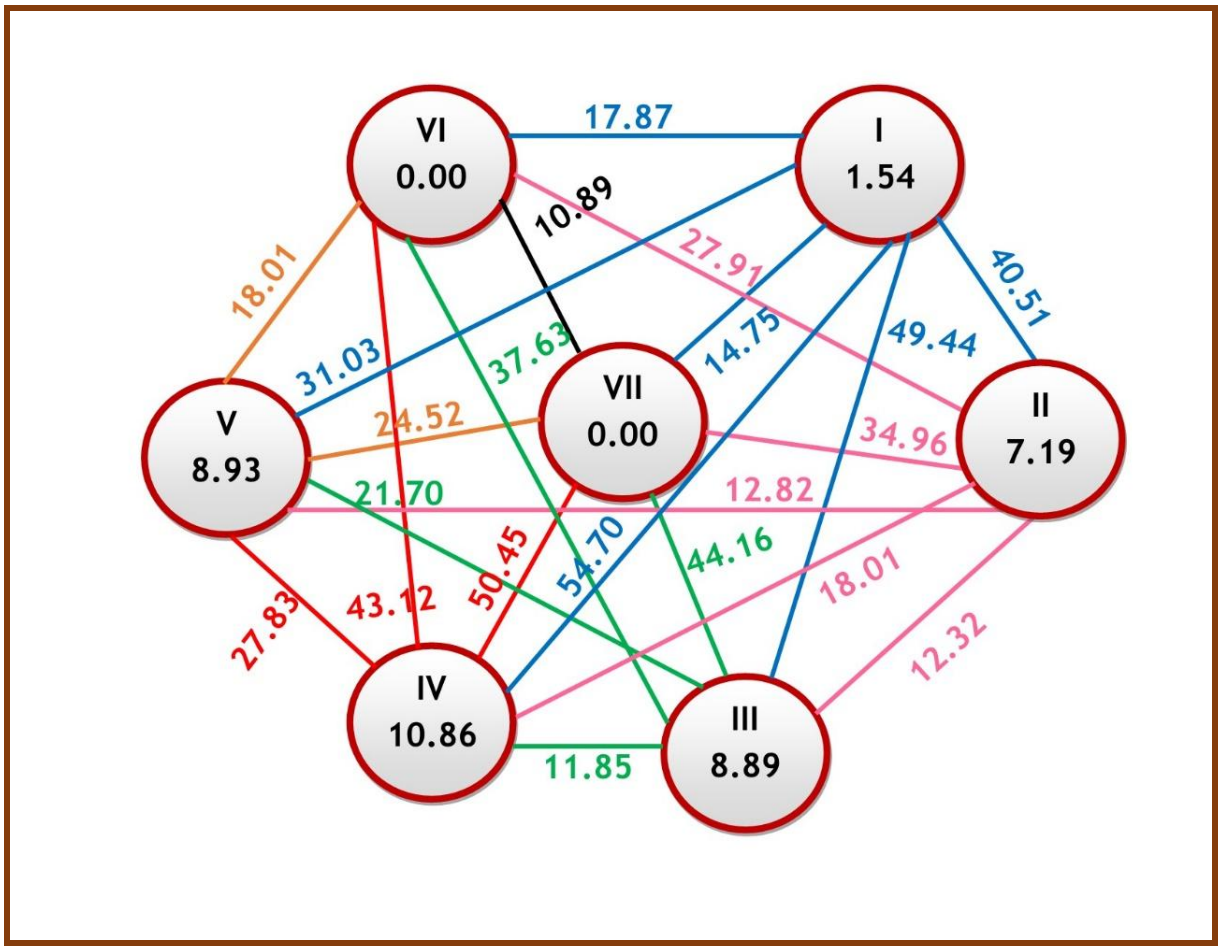


**Fig. 1. Cluster diagram for growth traits of Annatto progenies**

**Table 2.Cluster composition among Annatto progenies**

| Cluster Number | Number of Progenies | Members of clusters |
|---|---|---|
| I | 2 | TNBi 003, TNBi 004 |
| II | 5 | TNBi 0015, TNBi 0020, TNBi 0021, TNBi 007, TNBi 009 |
| III | 6 | TNBi 006, TNBi 0018, TNBi 001, TNBi 0017, TNBi 0016, TNBi 0010 |
| IV | 2 | TNBi 002, TNBi 0013 |
| V | 3 | TNBi 0014, TNBi 0019, TNBi 005 |
| VI | 1 | TNBi 008 |
| VII | 1 | TNBi 0012 |

progenies (TNBi 0015, TNBi 0020, TNBi 0021, TNBi 007 and TNBi 009); cluster III composed of six progenies (TNBi 006, TNBi 0018,TNBi 001, TNBi 0017, TNBi 0016 and TNBi 0010); whereas, cluster IV consisted of two progenies (TNBi 002 and TNBi 0013); Cluster V constituted three progenies (TNBi 004, TNBi 0019 and TNBi 005); cluster VI contained only one progeny (TNBi 008) and similarly cluster VII also had only one progeny (TNBi 0012) and are given in **Table 2.**

From the above results, it was known that cluster III included maximum of six progenies followed by cluster II with five progenies. Cluster VI and VII constituted only one progeny each, which were the most different and distinct among the twenty progenies. The clustering technique in present findings showed that high variability occurs among progenies. For an instance, cluster V and cluster III consisted of progenies from all locations whereas some other clusters included progenies from a similar locality. Progenies in a single cluster need not be from a similar area as it was dependent upon the genetic setup of the progeny (Selvan *et al.*, 2018). Early reports in *Pongamia pinnata*, also indicated that genetic divergence occurs due to other factors apart from geographical nearness (Kumaran, 1991).

The average intra and inter cluster $D^2$ and D values among seven clusters are presented in **Table 3**. The intra cluster distance ranged from 0.00 to 117.96. High intra cluster $D^2$ value was recorded by cluster IV (117.96) and its corresponding D value (10.86) whereas low intra cluster $D^2$ and D values was recorded by cluster VI and VII (0.00). Likewise, the inter cluster distances ranged from 118.17 to 2999.57 and their D value also followed the same pattern from 10.89 to 54.70. The highest inter cluster $D^2$ values were seen between clusters I and IV (2999.57) followed by the clusters IV and VII (2545.43) and clusters I and III (2444.65). These results indicated that there occurs a larger genetic divergence between these clusters. The lowest inter cluster distance recorded was found between clusters VI and VII (118.71). Similar results of inter and intra cluster distances were reported in *Leuceana leucocephala* (Chavan and Keerthika, 2013).

The mean performance of clusters for various growth traits was calculated and presented in **Table 4**. The maximum cluster mean for shoot length was recorded in cluster I (48.89 cm) and the minimum cluster mean was reported by cluster IV (27.46 cm). Clusters I and VI showed a maximum cluster mean (0.73 cm) for collar diameter whereas, cluster II showed a minimum cluster mean

**Table 3. Intra and Inter cluster distances for growth parameters**

| | I | II | III | IV | V | VI | VII |
|---|---|---|---|---|---|---|---|
| I | 2.36 (1.54) | 1641.34 (40.51) | 2444.65 (49.44) | 2992.57 (54.70) | 962.62 (31.03) | 319.42 (17.87) | 217.70 (14.75) |
| II | | 51.73 (7.19) | 151.85 (12.32) | 324.41 (18.01) | 164.36 (12.82) | 778.90 (27.91) | 1222.15 (34.96) |
| III | | | 79.11 (8.89) | 140.45 (11.85) | 470.74 (21.70) | 1416.35 (37.63) | 1950.06 (44.16) |
| IV | | | | 117.96 (10.86) | 774.38 (27.83) | 1859.05 (43.12) | 2545.43 (50.45) |
| V | | | | | 79.81 (8.93) | 324.40 (18.01) | 601.15 (24.52) |
| VI | | | | | | 0.00 (0.00) | 118.71 (10.89) |

Figures in the parenthesis are D values

**Table 4. Cluster Mean values for growth parameters**

| Cluster | Shoot length (cm) | Collar diameter (cm) | Root length (cm) | Root to Shoot ratio | Number of leaves | Sturdiness quotient | Volume index ($cm^3$) |
|---|---|---|---|---|---|---|---|
| I | 48.89 | 0.73 | 45.52 | 0.93 | 39.39 | 60.05 | 26.08 |
| II | 34.65 | 0.69 | 35.13 | 1.01 | 48.58 | 50.46 | 16.99 |
| III | 31.03 | 0.62 | 31.37 | 1.01 | 37.76 | 50.69 | 12.09 |
| IV | 27.46 | 0.55 | 30.51 | 1.11 | 40.00 | 50.22 | 8.47 |
| V | 38.63 | 0.68 | 39.48 | 1.02 | 41.00 | 57.94 | 18.06 |
| VI | 43.92 | 0.73 | 46.67 | 1.06 | 45.22 | 60.24 | 23.43 |
| VII | 46.43 | 0.68 | 49.10 | 1.06 | 38.56 | 68.32 | 21.48 |

(0.62 cm). The members of cluster VII reported higher performance of cluster mean (49.10 cm) for root length and cluster IV members exhibited lower cluster mean values (30.51 cm). Cluster IV showed a maximum cluster mean (1.11) for root to shoot ratio whereas, cluster I showed a minimum value (0.93). The highest cluster mean for the number of leaves was recorded by cluster II (48.58) and the lowest by cluster III (37.76). The maximum cluster mean for sturdiness quotient was recorded by cluster VII (68.32) and the minimum value by cluster IV (50.22). The highest cluster mean for volume index was recorded by cluster I (26.08 $cm^3$) and the lowest by cluster IV (8.47 $cm^3$). Similar results of cluster mean performances were also reported in *Terminalia tomentosa* (Gargi *et al*., 2015) and *Pongamia pinnata* (Jaisankar *et al*., 2014).

The contribution of genetic divergence by each trait and the number of times each trait ranked first was calculated and shown in **Fig. 2**. In the present study, shoot length among the various growth traits contributed maximum genetic divergence (55.78%) continued by root length (31.59%), the number of leaves and sturdiness quotient (4.73%), collar diameter (1.59%), volume index (1.58%) whereas, root to shoot ratio showed no contribution (0.00%) towards genetic divergence. The contributions of shoot length for genetic divergence in the current study indicated that it could be used as an index for *Bixa orellana* improvement programmes. The early record of similar results in *Melia azedarach* (Meena *et al*., 2014) was also supported by the results of the present investigation.

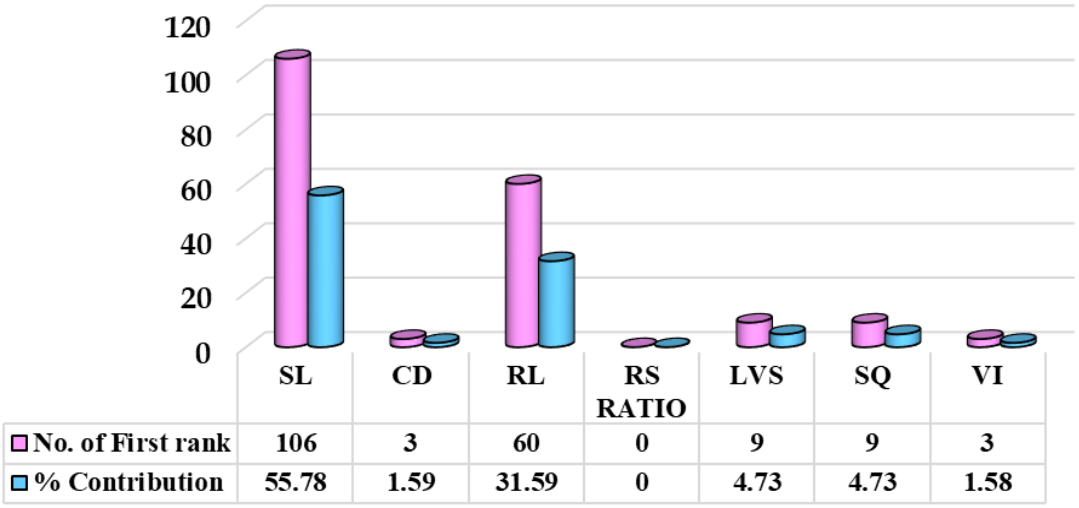


**Fig. 2. Contribution of various growth traits of progenies towards genetic divergence**

SL - Shoot length, CD - Collar diameter, RL - Root length, RS Ratio - Root to shoot ratio, LVS - Number of leaves, SQ - Sturdiness quotient,VI - Volume index

The progeny evaluation test identified three progenies *viz.,* TNBi 008, TNBi 003 and TNBi 005 for their superior performance for growth attributes under nursery conditions. These progenies could be used for further utilization. Higher cluster mean values for seedling growth traits were recorded by cluster VII and VI, as they constituted only one progeny in each cluster *viz.,* TNBi 0012 and TNBi 008, respectively. These two progenies are highly divergent and thus the growth trait contribution towards genetic divergence may help the breeder to use that particular contributing parameter for the further breeding programme. High intra cluster distance was recorded by cluster IV. High inter cluster distance was recorded between cluster I and cluster IV. Therefore, these two clusters were genetically divergent. Thus the progenies of clusters having high inter cluster distance *viz.,* cluster I (TNBi 003 and TNBi 005) and cluster IV (TNBi 002 and TNBi 0013) could be utilized for further hybridization programmes to obtain high yielding genotypes of Annatto.

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