

**GENETIC DIVERGENCE STUDIES IN *BIXA ORELLANA* L. ACCESSIONS, AN IMPORTANT NATURAL FOOD DYE PLANT USING MAHALANOBIS  $D^2$  MULTIVARIATE ANALYSIS**Poornima, S., S. R. Ambika<sup>1</sup> and P. Rajalakshmi<sup>2</sup>

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**Abstract**

Twenty accessions of *Bixa orellana* L., were assessed for the genetic divergence using Mahalanobis  $D^2$  statistics. The clustering pattern indicated that there was no association between geographical distribution of the accessions and genetic divergence. The characters namely capsule length and breadth, number of seeds/ capsule and seed bixin content of the accessions were analyzed. All these characters like capsule length and breadth, number of seeds per capsule and seed bixin content were contributing maximum to the divergence. Therefore, selection of divergent parents based on these characters is recommended for producing good hybrids.

**Keywords:** *Bixa orellana* L., Genetic divergence,  $D^2$  analysis.

**Introduction**

*Bixa orellana* L., belonging to Bixaceae is a shrub/bushy tree. It is one of the sources of natural food dye. The dye called annatto is obtained from the oily arils of the seeds and is the world's most important natural colourant (after caramel) yielding yellow-red colours<sup>6</sup>.

*Bixa orellana* L., being highly cross pollinated and known for continued seedling propagation is anticipated for the existence of wide genetic variability in tree and capsule characters. The genetic variation of *Bixa orellana* L., has been based on the phenotypic variation and hence *Bixa* germplasm collection is primarily based on fruit characteristics and seed bixin content. The fruit characteristics include capsule size variation, capsule color, seed number and seed bixin content.

In India, most of the *Bixa orellana* L., cultivation is planted with unselected inferior cultivars. Unavailability or lack of standard varieties has resulted in under exploitation of this very useful tree. As the research work on diversity and multivariate analysis is lacking in this species, the present investigation was carried out to examine the nature and magnitude of genetic divergence (variability) in 20 *Bixa orellana* L., accessions collected from different regions of Bangalore and outskirts of Bangalore, Karnataka, India. So, also it was desired to determine the grouping pattern of genotypes into

different clusters according to their genetic affinity using  $D^2$  statistic.

Genetic diversity is the basic requirement for a successful breeding programme. The more diverse the parents, better are the chances of improving economic characters, which also help to know the relative distance between the accessions. Mahalanobis  $D^2$  multivariate analysis is recognized as a powerful tool in quantifying the degree of divergence among the populations, at the genotypic level.

**Materials and Methods**

20 accessions of *Bixa orellana* L. were evaluated during 2003-06. These were collected from North, South, East & West of Bangalore and also from outskirts of Bangalore, Karnataka, India. The sampling areas were Yelahanka and Hessaraghata (North) comprising of 6 trees, South of Bangalore comprising of Banashankari, Jayanagar and Narasimharaja Colony having 9 trees, East of Bangalore comprising of a Single-1 tree from Krishnarajapuram and west of Bangalore comprising-of 4 trees from West of Chord Road and Sai (Sports Authority of India). The parent accessions were labelled as Y<sub>1</sub>, Y<sub>2</sub>, Y<sub>3</sub>, J<sub>1</sub>, J<sub>2</sub>, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>4</sub>, B<sub>5</sub>, B<sub>6</sub>, N<sub>1</sub>, K<sub>1</sub>, W<sub>1</sub>, W<sub>2</sub>, S<sub>1</sub>, S<sub>2</sub>, H<sub>1</sub>, H<sub>2</sub> & H<sub>3</sub>.

All these 20 accessions were analyzed for 3 parameters, namely capsule length and breadth,

seed number and seed bixin content at 5 replications, per accession.

The mean, data of the above experiments were subjected to multivariate analysis using  $D^2$  statistic<sup>5</sup> and the accessions were grouped into clusters following Tocher's method<sup>8</sup>. Intra and Inter cluster  $D^2$  and cluster wise mean values were estimated.

**Results and Discussion**

The analysis of variance revealed that the genotypes varied significantly for all the characters under study.

According to  $D^2$  analysis, all the 20 accessions of *Bixa orellana* L., could be grouped into different clusters, with respect to 3 different parameters - capsule length and breadth, seed number per capsule and seed bixin content, as given in Table 1-3 & Fig 1-3.

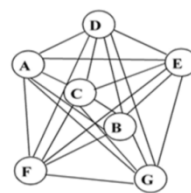
**1. Capsule Length and Breadth:**

All the twenty accessions were grouped into 7 clusters for this trait. The distribution of 20 accessions in 7 clusters is given in Table 1. Cluster G was the largest having 5 accessions, followed by cluster F with 4 accessions, cluster A with 3 accessions followed by clusters B, C, D & E with 2 accessions each respectively.

The highest cluster mean for this trait is shown by cluster G (11.7959), followed by cluster F (1.9047cm), cluster A (1.299cm), cluster E (0.598cm), cluster D (0.578cm), Cluster C (0.555cm) and cluster B (0.392cm). The inter cluster distances were maximum between cluster D and G (15.8535cm), followed by A and G (14.156cm). Therefore, crosses between cluster D and cluster G may be tried (Table-1, Fig. 1)

**Table- 1. Distribution of 20 accessions of *Bixa orellana* L., in 7 clusters with respect to length and breadth of the capsule**

| Sl. No. | Cluster | Total number of Accessions | Accessions   |
|---------|---------|----------------------------|--|
| 1       | A       | 3                          | K <sub>1</sub> , W <sub>2</sub> and B <sub>4</sub>                                   |
| 2       | B       | 2                          | H <sub>1</sub> and H <sub>3</sub>  |
| 3       | C       | 2                          | B <sub>3</sub> and B <sub>5</sub>  |
| 4       | D       | 2                          | B <sub>2</sub> and B <sub>6</sub>  |
| 5       | E       | 2                          | J <sub>2</sub> and S <sub>1</sub>  |
| 6       | F       | 4                          | B <sub>1</sub> , S <sub>2</sub> , Y <sub>2</sub> and Y <sub>3</sub>                  |
| 7       | G       | 5                          | J <sub>1</sub> , W <sub>1</sub> , Y <sub>1</sub> , H <sub>2</sub> and N <sub>1</sub> |



**Fig 1 Mutual relationship among different clusters of *Bixa orellana* L., accessions with respect to Length and Breadth**

- A-B = 7.550
- A-C = 2.690
- A-D = 3.53
- A-E = 4.027
- A-F = 3.443
- A-G = 14.156
- B-C = 5.003
- B-D = 9.002
- B-E = 6.0806
- B-F = 4.938
- B-G = 9.644
- C-D = 4.488
- C-E = 2.276
- C-F = 2.0752
- C-G = 12.469
- D-E = 3.244
- D-F = 3.3640
- D-G = 15.8535
- E-F = 4.5393
- E-G = 11.51026

- F-G = 11.7231
- Intra cluster  $D^2$  Genetic distances among clusters
- A-A=1.299
- B-B=0.392
- C-C=0.555
- D-D=0.578
- E-E=0.598
- F-F=1.9047
- G-G=11.7959

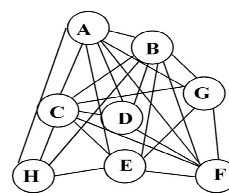
**2. Seed Number:**

All the 20 accessions could be grouped into 8 clusters. The distribution of 20 accessions into 8 clusters is given in the Table 2. Cluster E was the largest having 4 accessions, followed by clusters A & H with 3 accessions & Clusters B, C, D, F & G with 2 accessions each.

The highest cluster mean for this trait is shown by cluster H (2.0371cm), followed by cluster G (0.8043 cm), cluster F (0.7149cm), cluster E (0.40cm), D (0.3574cm), C (0.1341cm), A (0.2896cm) and cluster B (0.089cm). The inter cluster distances between G and H (7.6245cm), B and H (6.7765cm) and A and H (6.2058cm). The crosses between cluster G and Cluster H may be tried (Table 2, Fig 2).

**Table -2. Distribution of 20 accessions of *Bixa orellana* L., in 8 clusters with respect to number of seeds per capsule**

| Sl. No. | Cluster | Total number of Accessions | Accessions  |
|---------|---------|----------------------------|---|
| 1       | A       | 3                          | B <sub>5</sub> , B <sub>6</sub> and B <sub>2</sub>                  |
| 2       | B       | 2                          | W <sub>1</sub> and S <sub>2</sub>                                   |
| 3       | C       | 2                          | Y <sub>1</sub> and B <sub>4</sub>                                   |
| 4       | D       | 2                          | J <sub>1</sub> and H <sub>1</sub>                                   |
| 5       | E       | 4                          | J <sub>2</sub> , B <sub>3</sub> , Y <sub>3</sub> and S <sub>1</sub> |
| 6       | F       | 2                          | Y <sub>2</sub> and B <sub>1</sub>                                   |
| 7       | G       | 2                          | W <sub>2</sub> and K <sub>1</sub>                                   |
| 8       | H       | 3                          | N <sub>1</sub> , H <sub>2</sub> and H <sub>3</sub>                  |



**Fig 2 Mutual relationship among different clusters of *Bixa orellana* L., accessions with respect to Seed Number**

**Inter cluster  $D^2$  Genetic distances among clusters**

- A-B = 0.5905
- A-C = 0.5707
- A-D = 2.6443
- A-E = 1.2459
- A-F = 2.3274
- A-G = 1.4886
- A-H = 6.2058
- B-C = 1.1423
- B-D = 3.2227
- B-E = 1.8159
- B-F = 3.9044
- B-G = 0.9405
- B-H = 6.7765
- C-D = 2.0867
- C-E = 0.7065
- C-F = 2.7722
- C-G = 2.0299
- C-H = 5.6580
- D-E = 1.4509
- D-F = 0.7803
- D-G = 4.0903
- D-H = 3.6551
- E-F = 2.1336
- E-G = 2.6893
- E-H = 5.0207
- F-G = 4.7675
- F-H = 3.0450
- G-H = 7.6245

**Intra cluster  $D^2$  Genetic distances among clusters**

- A-A=0.2896
- B-B=0.089
- C-C=0.1341
- D-D=0.3574
- E-E=0.40
- F-F=0.7149
- G-G=0.8043
- H-H=2.0371

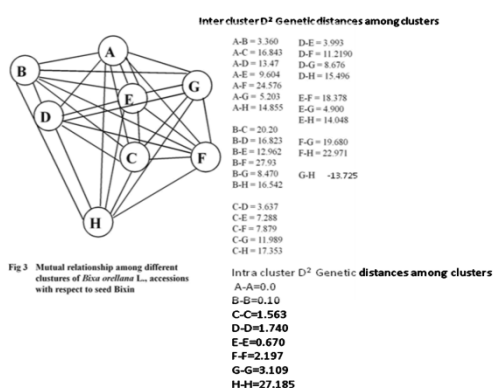
### 3. Seed Bixin :

All the 20 accessions could be grouped into 8 clusters for this trait. The distribution of 20 accessions into 8 clusters is given in the Table 3. Cluster C,D,E & F were largest with 3 accessions each followed by clusters A, B, G & H with 2 accessions each.

The maximum clusters mean for this trait is shown by cluster H (27.185cm), followed by cluster G (3.109 cm), cluster F (2.197cm), Cluster D (1.740cm), cluster C(1.563cm), cluster E (0.670cm), cluster B (0.1 cm). The inter cluster distances were maximum between B and H (27.93cm), F and H (22.971cm), B and C (20.20cm). Therefore crosses between accessions of cluster H and between cluster B and cluster F may be tried (Table 3, Fig 3).

**Table -3. Distribution of 20 accessions of *Bixa orellana* L., in 8 clusters with respect to seed Bixin**

| Sl. No. | Cluster | Total number of Accessions | Accessions   |
|---------|---------|----------------------------|--|
| 1       | A       | 2                          | J <sub>1</sub> , and B <sub>3</sub>                |
| 2       | B       | 2                          | Y <sub>2</sub> and B <sub>1</sub>                  |
| 3       | C       | 3                          | Y <sub>1</sub> , B <sub>6</sub> and K <sub>1</sub> |
| 4       | D       | 3                          | S <sub>1</sub> , W <sub>2</sub> and S <sub>2</sub> |
| 5       | E       | 3                          | B <sub>2</sub> , B <sub>4</sub> and H <sub>2</sub> |
| 6       | F       | 3                          | N <sub>1</sub> , H <sub>1</sub> and H <sub>3</sub> |
| 7       | G       | 2                          | Y <sub>3</sub> and W <sub>1</sub>                  |
| 8       | H       | 2                          | J <sub>2</sub> and B <sub>5</sub>                  |



**Fig 3** Mutual relationship among different clusters of *Bixa orellana* L. accessions with respect to seed Bixin

had large  $D^2$  values showing large distances between them. The grouping of accessions of the same area into different clusters could be due to factors like heterogeneity (different genes), genetic architecture of the populations, developmental traits etc. while, the grouping of accessions of different areas into one cluster may be due to the presence of some common genes controlling the most important characters and these accessions might have some degree of ancestral relationship.

Hence, all these accessions exhibited wide variations in several characters as evidenced by the  $D^2$  analysis. This very clearly indicates the genetic distance between the accessions with respect to different morphological characters. Therefore, accessions growing in South of Bangalore (Jayanagar, Banashankari & N.R. Colony) cluster with that of accessions growing in North (Yelahanka & Hesarghatta,) East (Krishnarajapuram) and West (Sai & West of Chord Road) of Bangalore, indicating no relationship between the area of distribution of these accessions and for closeness or divergence, in their genetic make.

Therefore, the geographic diversity didn't seem to have a direct association with genetic diversity and accessions originating in different geographical areas could form one cluster, while, accessions evolved in the same area could be grouped into different clusters. Hence, genetic divergence in *Bixa orellana* L., accessions / confirmed the earlier studies of genetic diversity in *Solanum melongena* L<sup>3</sup>, *Allium cepa*<sup>7</sup>, *Prunus armeniaca* L<sup>2</sup>, *Capsicum annum*<sup>9</sup>, *Pisum sativum*<sup>10</sup>, Pigeon Pea<sup>4</sup> and *Sesamum indicum* L.<sup>1</sup>.

The accessions within each cluster had smaller  $D^2$  values among themselves when compared to accessions belonging to different clusters. Smaller  $D^2$  values within the cluster indicated that these accessions were very close for a particular character. On the other hand, accessions belonging to two different clusters

**References**

1. Duhoon, S.S., and Raghuwanshi, K.M.S. Genetic diversity and adoptability in Indian germplasm collection of sesame, *Sesamum indicum L.*, J. Oil seeds Res., 2005, 22(1), 29-36.
2. Gul Zaffar, Mir, M.S. and Sofi, A.A. Genetic divergence among apricot (*Prunus armeniaca L.*) genotypes of Kargil Ladak, Indian J. Hort., 2004, 61(1), 6-9.
3. Kale P.N., Rane, D.A. and Tambe, T.B. Diversity Studies in Brinjal (*Solanum melongena L.*), Maharashtra J. hort., 1993, 7(1), 81-87.
4. Katiyar, P.K Singh F. and Singh, I.P. Studies on agro-morphological diversity Vis-a-Vis eco-geographical distribution of germplasm in late Pigeon Pea, Indian J. Pulses Res., 2005, 18(1), 17-20.
5. Mahalanobis, P.C. On the generalized distance in statistics, Proceedings of Natural Science, India, 1936, 2, 49-55.
6. Mercandante A.Z. and II. Pfander. Carotenoids from annatto a review, Recent research developments in Agriculture and Food chem, 1936 ,2, 79-91.
7. Mohanty, B.K. and Prusti, A.M. Multivariate analysis for genetic divergence in onion seed crop, Progressive horticulture ,2001 33 (2), 134-137.
8. Rao, C.R. Advanced statistical methods in Biometric research, 1952, John Wiley & Sons, Inc., New York
9. Rajamony, L. and Sreelathakumary, I. Genetic divergence in Chilli (*Capsicum annum L.*), J. Indian. Hort., 1952 61(2), 137- 139.
10. Rana, M.K, Partap P.S., Tawatia, A.S. and Yadav, P.K. Genetic divergence in Garden Pea, Hariyana J. hort. Sci, 2004, 33 (1&2), 102-105.