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Research Article

INTRASPECIFIC DIVERSITY AND CONSERVATION OF AN IMPORTANT NATURAL FOOD DYE YIELDING PLANT *BIXA ORELLANA* L

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ABSTRACT
Diversity is an important attribute of living organisms that has for centuries fascinated the human mind and captured the imagination in an effort to unveil the secrets of its origin and continuity. Plants show variations in the physical structure caused by external environmental conditions like accidents, temperature, food abundance etc,. Such somatic variations die within the organism and not inherited. Hence, they have no effect on evolution. While, heritable variations which are genetic variations arise from changes in DNA and are passed on within families and to the offspring from the parents. An important step in a tree improvement programme in the case of wild species is defining the selection criteria. The type and intensity of selection will depend on the pattern of variability within and between populations which is both Intraspecific and Interspecific variations and such variations are the building blocks of trait/character improvement through Breeding or Genetic selection. Intraspecific studies presently has become very important in Botanical, Breeding, Plant science research and Gene bank activities related to preservation of integrity in viable seed materials. *Bixa orellana* L., is a medicinally important and is also a Natural Dye yielding plant. The dye-Bixin and Nor Bixin are used in Dairy industry and in preparation of cosmetics. The study reports the Morphological and Genetic diversity among the 5 accessions of *Bixa orellana* L., and the Field Gene Bank was established to conserve these accessions.

Key Words: *Bixa orellana* L., Intrasecific diversity, Natural dye yielding plant, D^2 analysis, Bixin, RAPD

INTRODUCTION
Diversity is of 2 types – Interspecific diversity and Intraspecific diversity. Interspecific diversity is the variations which are found between the populations, i.e. between the different species of a single genus or among different genera themselves. While, Intraspecific diversity is the variations within populations which are found within a single species of a particular genera i.e. A monospecific species or the variations exhibited by a single species among themselves. Ex: *Bixa orellana* L., belonging to bixaceae is a monospecific genera, i.e. we have only species 'orellana' reported under the genus '*Bixa*' and can be called as Monogeneric and monospecific species. But this single species grown in different areas of Bangalore still exhibited lot of variations and hence, we call such a type of variations as "Intraspecific Variations or diversity". Intraspecific and Interspecific variations are the building blocks for trait / character improvement through breeding or genetic selection hence has become very important in botanical, breeding, plant science research and gene bank activities related to preservation of integrity in viable seed materials.
Bixa orellana L., is an important Natural Food dye yieliding plant which yields Non toxic Food dye called Bixin and Nor –Bixin also called as Annatto. Annatto is one of the 13 basic food pigments approved by US FDA and ranks 2nd in the world. Annatto is used in dairy industry for colouring butter,

cheese, ice-creams and cosmetics for colouring hair oils, lipsticks and in textile industry for dyeing cotton, silk clothes etc. (Mercandante *et al.*, 1998).

All the parts of *Bixa orellana* L., is used as a traditional medicine and by Ayurvedists for various medicine preparations. The acid extract of leaf is used as an anticancer drug. The seed pulp is antidysentenic, useful in epilepsy, skin and kidney discases and prevents blister formation in burns (Guha *etal* 1999).

Bixa orellana L., is a highly cross pollinated plant producing variability in the saplings obtained from the seeds. There is also variation in the composition of the seed bixin content among the trees and this limits marketability. A wide variation for most of the characters is observed in *Bixa orellana* L., and there is much scope for identification of divergent types. As research work on this species is very limited in India, hence a detailed study was undertaken to understand intraspecific diversity in this species.

MATERIALS AND METHODS

5 accessions of *Bixa orellana* L. were identified from North (Yelahanka & Hesarghatta) and South (N.R. Colony) of Bangalore, Karnataka. The above mentioned 5 accessions were studied for the following parameters mentioned below:

Bixa orellana* L. *Accessions Collected from Different Areas of Bangalore, Karnataka

Identification of *Bixa orellana* L., accessions from different areas of Bangalore and outskirts of Bangalore, Karnataka labelled as parent accessions- Yelahanka and Hessaraghata - North of Bangalore- Y_2 and H_1, H_2 and H_3, NR Colony- South of Bangalore-N_1.

Genetic Divergence Studies on morphological variations among the parent accessions, using RAPD analysis.

All the 5 accessions were highly diverse and exhibited maximum variations with respect to habit, leaf shape, colour of flower, stamen and capsule, capsule size and texture of hence these 5 accessions were subjected to RAPD (Randomly Amplified Polymorphic DNA) assay.

Genomic DNA was extracted from fresh leaf samples by CTAB method.

The data on the morphological parameters of all the above accessions were analyzed statistically using one way Annova and Duncan's multiple range tests.

Progenies of Bixa orellana L., Grown From Selected Parent Accessions Under Identical Conditions

The saplings obtained from the seeds of different parent accessions were grown in the Gandhi Bhavan Nursery of Bangalore University, Bangalore and were monitored for 3 years (2003 – 2006) for various growth parameters – Habit, Height of the plants, Branching pattern, Fresh weight, Dry weight, leaf area ratio (LAR) and Net assimilation rate (NAR) and so also of its flowering and fruiting periods.

Establishment of Field Gene Bank of Bixa orellana L., at Department of Botany, Bangalore University, Bangalore

The above mentioned progenies of *Bixa orellana L.* were **e**stablished in the field gene bank for the conservation of the Intraspecific diversity of all the accessions of *Bixa orellana* L., collected from North, South, East & West of Bangalore in the field allotted behind the Department of Botany, BUB.

RESULTS AND DISCUSSION

All the 5 accessions of *Bixa orellana* L., collected from different areas of Bangalore and outskirts Bangalore, Karnataka showed wide variations with respect to habit, branching pattern, flowering time flower colour, fruiting time, fruit (capsule) colour, capsule shape, texture, number of capsules per branch and number of seeds per capsule (Table 1; Figures 1-4)

1a (Y_2)

1b (N_1)

1c (H_1)

1d (H_2)

1e (H_3)

Figure 1: Habit of *Bixa orellana L.*, accessions collected from different areas of Bangalore (1a: $1Y_2$- Yelahanka; 1b: N_1 – N.R. Colony, 1c; 1d and 1e- H_1, H_2 & H_3 – Hessarghatta).

Table 1: Morphological Characters of *Bixa orellana L.* Accessions

S. No.	Area of Collection	Habit (Size)	Ht of the tree	Branching pattern	No. of Branches	Leaf shape	Flowering Time	Flower and Stamen colour	Fruiting period	Capsule colour	Capsule shape	Capsule size (Length & Breadth)		Capsule texture	Seed Col. Time	Seed colour (unripe)	100 seed wt
								Bangalore North									
								Yelahanka									
1	Y2	Medium	3.9	3/4'''	24	Cordate (Narrow)	July	White	August	Green	Cordate	3.86	4.82	Rough	December	Orange	3.25 gm
								Hessaraghatta									
2	HI	Shrub	2.1	From base	15	Cordate (broad)	July	White	August	Green	Ellipsoid	5.25	4.4	Soft	November	Orange	3.0 gm
3	H2	Shrub	2	From base	16	Cordate (broad)	May	Rose with rose	July	Red	Ellipsoid	5.38	4.57	Soft	November	Reddish Brown	2.99 gm
4	H3	Small	2.6	From base	8	Cordate (broad)	March & May	Rose with Pink	May& August	Maroon	Ovate	5.08	4.42	Rough	July& November	Reddish Brown	3.39gm
								Bangalore South									
								N.R. Colony									
5	Nl	Big	4.5	3/4'''	30	Cordate (broad)	March September	Rose with Pink	June & September	Pinkish Green	Ellipsoid	9.01	7.41	Soft	October & December	Pink	2.5gm

2a (Y_2)

2b (N_1)

2c (H_1)

2d (H_2)

2e (H_3)

Figure 2: Variation in Leaf Shape, Size and Colour in *Bixa orellana* L., accessions

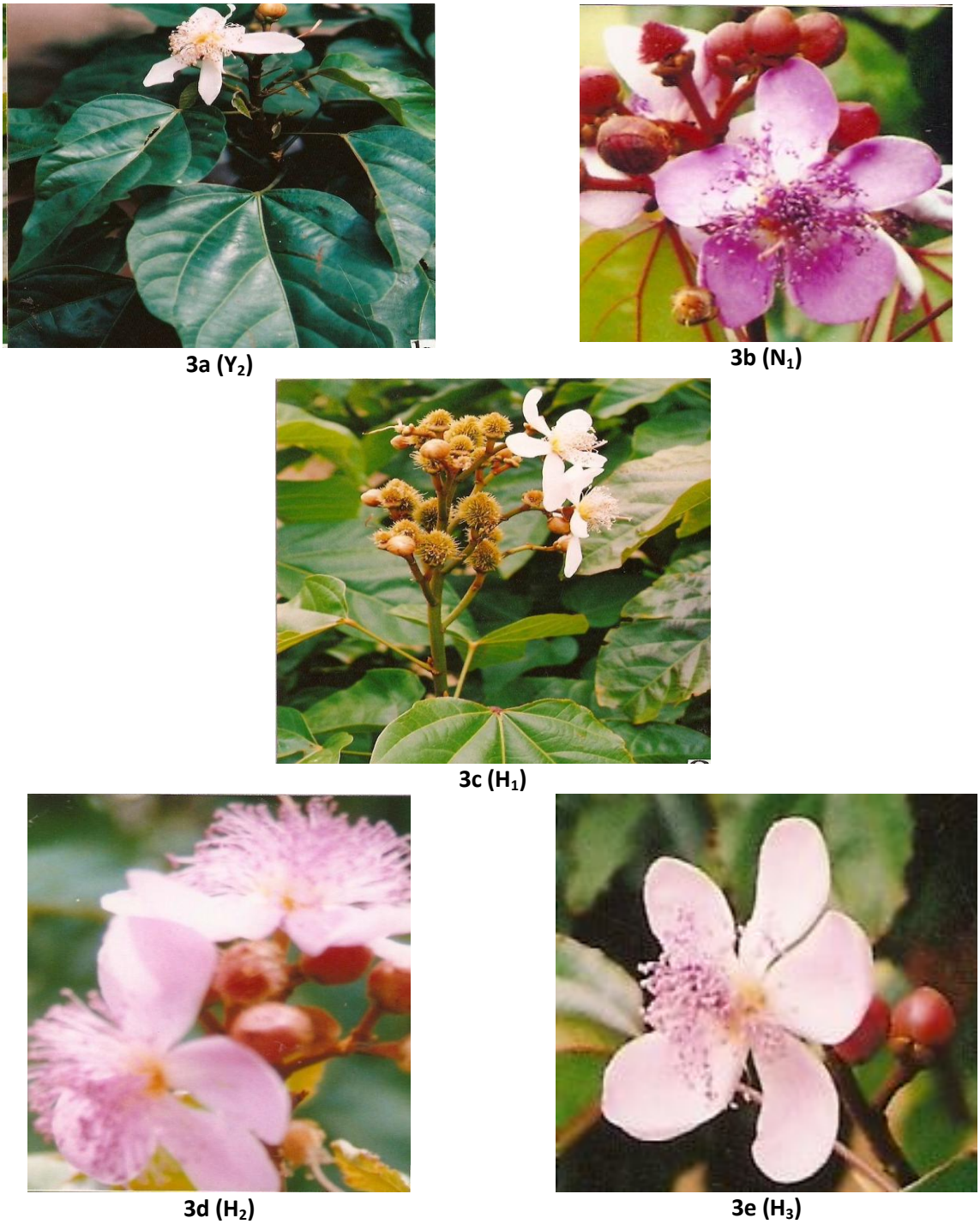
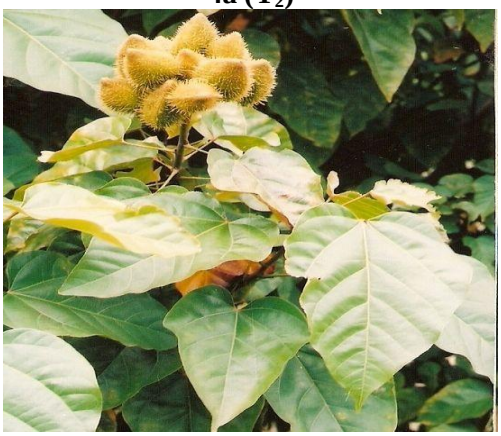


Figure 3: Variation in Flower Colour in *Bixa orellana* L., accessions.

4a (Y_2)

4b (N_1



4c (H_1)

4d (H_2)

4e (H_3)

Figure 4: Variation in Colour, Shape & size of the capsules in *Bixa orellana* L., accessions

These 5 highly diverse accessions were subjected to RAPD analysis and analysis of RAPD data of all the 5 accessions showed higher levels of genetic variation (Poornima *et al.*, 2009).
The genetic relationship among 5 accessions of *Bixa orellana* L., is given in Table 2.

Table 2: Genetic relationship among 5 accessions of *Bixa orellana* L.

S.No.	Name of the Accessions	H_1	N_1	H_2	Y_2	H_3
1	H_1	-	0.8923	0.8217	0.6902	0.7377
2	N_1	0.1139	-	0.8659	0.6779	0.6724
3	H_2	0.1264	0.1440	-	0.7944	0.4999
4	Y_2	0.3708	0.3888	0.2301	--	0.3658
5	H_3	0.3043	0.3968	0.6934	1.0057	--

The Cluster analysis based on UPGMA reveals 1 major cluster comprising of accessions H_1, N_1 and H_2. Accessions Y_1 and H_3 aligns separately indicating its maximum diversity from the other accessions. Among the 5 accessions, H_1 and N_1 shows more similarity while H_3 is the least similar with other (Table 2 ; Figure. 5)

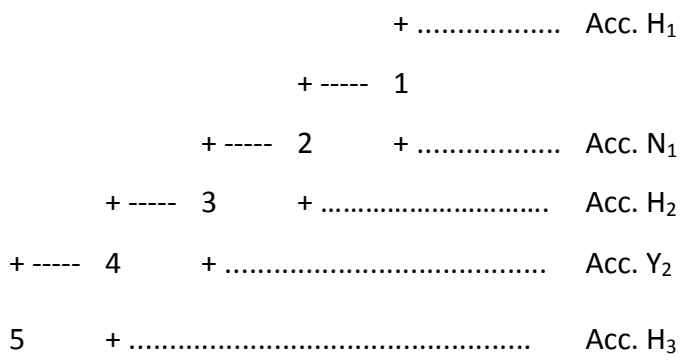


Figure 5: Dendrogram showing genetics of RAPD data following the UPGMA method

The accessions H_1, N_1 and H_2 were found to be Morphologically, Physiologically and Genetically very close (Figures 1-4).The accessions H_1, N_1 and H_2 shared similar Capsular characters like Ellipsoid large Capsules and found to be closer to each other but differed slightly in Capsular colour like green, creamish pink and red respectively. The H_3 accession was showing the widest distance from the others and has ovate maroon Capsules. While the accession Y_2 was found intermediate to H_2 and H_3 genetically and had cordate green Capsules (Fig.5). The H_1, N_1 and H_2 had large Ellipsoid Capsules compared to Y_2 and H_3 which fell apart having small and cordate Capsules.

The 5 progenies of the above mentioned 5 parent accessions grown under identical conditions of climate, water and soil in the Gandhi Bhavan Nursery of Bangalore University, Bangalore and monitored for 3 years also showed variations in the morphological characters as per their parent accessions, which were growing in different areas of Bangalore (Figure 6).

Figure 6: Progenies from North of Bangalore, Yelahanka and Hessarghatta – Y_2 and H_1, H_2 and H_3 and South of Bangalore – NR Colony – N_1 grown in the Gandhi Bhavan Nursery of Bangalore University, Bangalore (under identical conditions of climate, water and soil)

Hence, the genetic diversity recorded in the parents and progenies of *Bixa orellana* L., testifies that this species has high plasticity to accommodate variations within its genome.

So, *Bixa orellana* L., being monospecific genera, we could establish the 5 diverse accessions under the level of species and because it is an important natural food dye yielding plant, it was found necessary to establish a field gene bank to conserve the Intraspecific diversity in *Bixa orellana* L., accessions.

In this regard the field gene bank was established to maintain the different accessions of *Bixa orellana* L., in the field allotted behind the Department of Botany, Bangalore University, Bangalore, being established in the year 2005, the plants are now 7 years old and are flowering and fruiting (Poornima, 2006) (Figures 7-10).

Figure 7: Progeny of parent accession Y_2 from Yelahanka, North of Bangalore growing in the field gene bank of Bangalore University, Bangalore

Figure 8: Progeny of parent accession N_1 from N.R. Colony, South of Bangalore growing in th field gene bank of Bangalore University, Bangalore

Figure 9: Progeny of parent accession H_1 from Hessarghatta, North of Bangalore growing in the field gene bank of Bangalore University, Bangalore

Figure 10: Progeny of parent accession H_2 from Hessarghatta, North of Bangalore growing in the field gene bank of Bangalore University, Bangalore

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