Chapter – I

Section – 2

Progeny

Introduction
Once the embryonic plant comes out of the refuge provided by the parent plant following the seed germination, the young plant has to compete with its neighbouring individuals of the same or other species making similar demands on their environment. The component factors of the environment and their impact on the growth and development of plants have been analyzed and discussed by several authors; (Daubenmire, 1974; Leopold and Kriedemann, 1979; Misra, 1980; Fitter and Hay, 1981). Species having wide ecological amplitude will be successful due to its ability to adapt to the varied environmental conditions. The adaptations might manifest themselves as variations ranging from biochemical to morphologic. The environmental tolerance of a species depend largely on its ability to respond ecotypically (Gregor, 1946) and the wider the ecological range of the species the more numerous are its ecotypes (Daubenmire, 1959). Environmentally induced variations of morphologic nature are acquired only as a result of continued exposure of the individuals to any set up for a long time during their life cycle, but physiologic adjustments may be brought about in a few days. Such effects of environment are simply the inevitable results of the physico-chemical reactions of the protoplasm to different combinations of factors. These are
produced readily and remain in equilibrium with their corresponding environments for long, but disappear when the phenotypic variants are grown under identical conditions. If the variations are genetically based and subsequently picked up during natural selection, these would be irreversible. The variations make the individuals of a species a heterogeneous assemblage of individuals differing to varying degrees depending on the range of their plasticity, governed basically by the hereditary set up.

*Bixa orellana*, the dye yielding plant is an introduced species. It has naturalized in tropical and subtropical regions and is cultivated in warm regions of the world extensively for the food dye. This species, grown as avenue trees and in the road sides is widely cross pollinated. Generally, seedlings obtained from seeds of naturally cross pollinated trees are highly heterozygous. The progeny from a single parent tree will be more genetically uniform than seedlings derived from several parent trees. Most of the characteristics are influenced by a large number of genes. The influence of a single gene on such polygenic traits may be very small. However, the expression of rare recessive genes may result in a trait that is important for the development of a potentially valuable cultivar or subspecies. Hence, it was considered necessary to investigate whether *Bixa orellana* progeny obtained from the 13 parent accessions when grown under
identical controlled conditions retained the variations that were noticed in the parent accessions.

Besides checking the growth rate, and growth equations it was also planned to analyse the quality and the quantity of bixin (marker phytochemical) in different parts of the plant. Such an investigation was undertaken and the findings are discussed briefly.
Materials And Methods
MATERIALS:

Plants of *Bixa orellana* L., were raised from seeds of different accessions collected from 8 areas in Bangalore, Karnataka, in the forest nursery of Bangalore University, Jnanabharathi campus and at Narasimharaja colony in the Backyard of my house.

The saplings were raised in black polythene covers of size (12" ht and 8" dia, 16" ht and 12" dia) bought from Subramanyam stores, Malleswaram, Bangalore.

Sand paper of coarse variety were used for the scarification studies, and was bought from this Subramanyam stores, Malleswaram, Bangalore.

The data on the average temperature and humidity and rainfall were recorded in the area in which the saplings were raised over a period of 3 years as given in the Table I.2.1.

The data on rainfall were collected from India Meteorological Department, Meteorological centre, central observatory Complex, Bangalore.
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<th>Sl. No.</th>
<th>Leaf Shape</th>
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<th>Fruiting Time</th>
<th>Capsule Colour</th>
<th>Capsule Shape</th>
<th>Capsule Texture</th>
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</tr>
<tr>
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<td>White</td>
<td>June &amp; December</td>
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<td>May &amp; August</td>
<td>Pink</td>
<td>Ovoid</td>
<td>Rough</td>
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### Table 1.2.1: Data on Temperature, Rainfall and Relative Humidity of Bangalore City  
*(Courtesy, Meteorological Dept., Blase)*

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<tr>
<td><strong>MONTHLY MEAN RELATIVE HUMIDITY AT 1730 HRS (%)</strong></td>
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<td>MONTHLY MEAN RELATIVE HUMIDITY AT 1730 HRS (%)</td>
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<td>68</td>
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(Courtesy, Meteorological Dept., B'Ive)
METHODS:

Experiment-1: Morphological studies on the progeny of *Bixa orellana* L.

Progeny of thirteen accessions of *Bixa orellana* L., collected from 7 sampling sites namely Y, J, N, K, W, JN and H were considered for this study as in Table I.1.1.

Mature seeds from 13 accessions namely, Y₁, Y₂, Y₃, J₁, N₁, K₁, W₁, W₂, S₁, S₂, H₁, H₂, and H₃ were scarified using sand paper taking care to rupture only the hard seed coat. These were sown in a sand bed maintained in the forest nursery of Bangalore University. The 5 day old-seedlings were transplanted in the garden soil (sand, manure, red soil in 1:2:1 ratio), contained in black polythene bags of size 12” ht and 8” dia. Care was taken to water these plants daily till they reached 2 years old. At one year stage the saplings were transplanted to bigger bags of size 16” ht and 12” dia. For each accession 100 saplings were maintained.

Data were recorded for the height of the plant, number of branches and number of leaves and area of the leaf at the 3rd node during growth stages of 3, 12 and 18 months in 5 replications.
Experiment-1(a):

1) **Growth Pattern**: Growth pattern were studied upto 18 months age of the saplings from all the accessions from the emergence time with respect to linear growth, fresh weight and dry weight.

2) **Growth Studies**: For all the above mentioned accessions of *Bixa orellana* L., the following growth equations were worked out, at a replication of 5 per accession.

(i) **Absolute Growth Rate (AGR)**: Is a simple measure of rate of increase in weight, calculated according to Dhopte (1989).

\[
AGR = \frac{w_2 - w_1}{(t_2 - t_1)}
\]

Where, \(w_2\) and \(w_1\) are the total dry mass of the plant at times \(t_2\) and \(t_1\) respectively. In the present study \(t_2\) and \(t_1\) considered were 12, 3 and 18, 12 months.

(ii) **Relative Growth Rate (RGR)**: Is the increase in dry weight of the plant per unit of time, calculated according to Dhopte (1989).

\[
RGR = \frac{\log w_2 - \log w_1}{(t_2 - t_1)}
\]
Where, \( w_2 \) and \( w_1 \) are the natural logarithms of total dry mass of the plants at times \( t_2 \) and \( t_1 \) respectively. Here \( t_2 \) and \( t_1 \) considered were 12, 3 and 18, 12 months.

(iii) **Net Assimilation Rate (NAR)**: Is the rate of dry weight increase per unit leaf area per unit time (gdm\(^{-2}\) day\(^{-1}\)) and calculated according to Dhopte (1989).

\[
NAR = \frac{w_2 - w_1 \times \log L_2 - \log L_1}{t_2 - t_1} \times \frac{\log L_2 - \log L_1}{(L_2 - L_1)}
\]

Where, \( L_2, L_1, w_1, w_2 \) are the leaf area and the drymass of the plants at times \( t_2 \) and \( t_1 \) respectively. Here, \( t_2 \) and \( t_1 \) considered were 12, 3 and 18, 12 months.

(iv) **Leaf Area Ratio (LAR)**: Is the ratio of total leaf area to the whole plant dryweight

\[
LAR = \frac{LA}{W}
\]

Where, \( LA = \) Leaf area and \( W = \) Plant drymass
Experiment-2: Phytochemical Analysis of Leaves

Total chlorophylls and carotenoids were extracted and estimated from the leaf at the 3rd node of all the 13 accessions of *Bixa orellana* L., following Arnon (1949) and Bansal et al., (1976) as in (Exp-3; 1.1) at 7 months and 12 months stage of all the saplings.
Results
RESULTS:

Morphological studies on the progeny of *Bixa orellana* L., grown for different durations.

Progeny of the 13 accessions of *Bixa orellana* L., collected from 7 sampling sites namely Y, J, N, K, W, JN and H were considered for this study as in (Table 1.1.1).

The progeny of *Bixa orellana* L., were labeled as $y_1, y_2, y_3, j_1, n_1, k_1, w_1, w_2, s_1, s_2, h_1, h_2$ and $h_3$.

The saplings of all the accessions were grown under identical conditions of rainfall, temperature and humidity. The saplings were assessed for their morphological characters at 7-month stage (Plates 1.2.25-1.2.38) and at 14-month stage (Plates 1.2.39-1.2.51).

The saplings had shape, texture and vein colour of the leaves, colour of flowers and Capsule as it their parent plants (Table 1.2.2, Plate 1.1.21, 22 and 23)
Plate 1.2.25: Progeny of *Bixa orellana* raised from seeds of parent accessions in the Forest nursery of Bangalore University under controlled identical conditions. (Refer text for details)
Plate I.2.26: 7 month old saplings of *Bixa orellana* L. \((y_1)\)
Plate I.2.27: 7 month old saplings of *Bixa orellana* L. (y₂)
Plate I.2.28: 7 month old saplings of *Bixa orellana* L. (y₃)
Plate 1.2.29: 7 month old saplings of *Bixa orellana* L. (j1)
Plate 1.2.30: 7 month old saplings of *Bixa orellana* L. (n₁)
Plate I.2.31: 7 month old saplings of *Bixa orellana* L. (k₁)
Plate I.2.32: 7 month old saplings of *Bixa orellana* L. (w1)
Plate I.2.33 : 7 month old saplings of *Bixa orellana* L. (w₂)
Plate I.2.34: 7 month old saplings of *Bixa orellana* L. (s₁)
Plate 1.2.35: 7 month old saplings of *Bixa orellana* L. (s2)
Plate 1.2.36 : 7 month old saplings of *Bixa orellana* L. ($h_1$)
Plate 1.2.37: 7 month old saplings of *Bixa orellana* L. (h₂)
Plate 1.2.38: 7 month old saplings of *Bixa orellana* L. (h₃)
Plate I.2.39: 14 month old saplings of *Bixa orellana* L. ($y_1$ and $y_2$)
Plate I.2.40: 14 month old saplings of *Bixa orellana* L. (y, and j₁)
Plate I.2.41: 14 month old saplings of *Bixa orellana* L. ($n_1$ and $k_1$)
Plate 1.2.42: 14 month old saplings of *Bixa orellana* L. ($w_1$ and $w_2$)
Plate I.2.43: 14 month old saplings of *Bixa orellana* L. (s₁ and s₂)
Plate I.2.44: 14 month old saplings of *Bixa orellana* L. ($h_1$ and $h_2$)
Plate I.2.45: 14 month old saplings of *Bixa orellana* L.
Plate I.2.47: close up of $h_2$ accession with red midrib and green veins
Plate I.2.48: close up of h₃ accession with green midrib and veins
Plate 1.2.49: close up of \( n_1 \) accession with pink midrib and veins
Plate I. 2.50: Progeny of $Y_2$ and $h_3$ (Flowering and Fruiti-
stage)
Plate I. 2.51: 7 and 14 month-old saplings (Nursery view)
Growth Pattern:

The saplings obtained from the seeds of the parent accessions were grown under identical conditions and were analysed for their linear growth at 3, 12 and 18 month stage.

There was increase in linear growth with respect to shoot length and root depth in all the 13 accessions, but the linear growth differed in all of them (Fig. 1.2.1).

Shoot length and root depth increased during the two growth stages studied (3-12 months and 12-18 months). The increase in growth was enormous during the earlier stage (3-12 months), while there was a slight decline in the rate of increase in the 2nd stage. This pattern of linear growth was recorded in the 11 accessions, while there was increase in the root depth alone in the 2nd stage also (12-18 months) as seen in k₁, w₂ and h₁.

In y₁ the increase in shoot length was 74.34cms in 9 months time (from 3-12 months), 24.6cms in another 6 months time (12-18 months) and total increase in shoot length was 98.94cms. from 3 months to 18 months period. Similarly, the root depth increased linearly. It was 23.9cms from 3-12 months, 9.3cms in 12-18 months and total increase was 33.2cms.
Fig. 1.2.1: Plant Height of 3, 12 and 18 month-old saplings of *Bixa orellana* L. accessions raised under identical conditions (given in text)
(Figures in Parentheses represent standard deviation values)

(*Y*, *Y*, *Y*, Yelahanka, J., N. & K., Jayanagar, Narasimharaja Colony & Krishna Rajapuram, W., W., S. & S., West of Chord Road & Sai. H., H. & H., Hesaraghatta)
S2

Root Depth
Shoot Length

Plant Height (cm)

Months

년

_root Depth
Shoot Length

Plant Height (cm)

Months

년

_root Depth
Shoot Length

Plant Height (cm)

Months

년

-root Depth
Shoot Length
In y2 the increase in shoot length was 60.9cms from 3-12 months. 17.4cms in 12-18 months and total increase in shoot length was 78.5cms from 3 months to 18 months period. Similarly, the root depth increased linearly. It was 19.5cms from 3-12 months, 9.6cms in 12-18 months and total increase in root depth was 29.1cms.

In y3 the increase in shoot length was 76.3cms in 3-12 months time. 24.1cms in 12-18 months time and total increase in shoot length was 100.4cms. The increase in root depth was 20.6cms in 3-12 months time. 9.3cms in 12-18 months time and total increase in root depth was 29.9cms.

In J1 the shoot length increased by 70.0cms in 3-12 months time. 22.4cms in 12-18 months time and total increase in Shoot length was 92.4cms. The increase in root depth was 28.60cms in 3-12 months. 4.4cms in 12-18 months and total increase in root depth was 33.0cms.

In n1 the shoot length increased by 76.3cms in 3-12 months time. 6.52cms in 12-18 months and total increase in shoot length was 82.8cms. The increase in root depth was 33.6cms in 3-12 months. 23.48cms in 12-18 months and total increase in root depth was 57.08cms.

In k1 the shoot length increased by 49.4cms in 3-12 months time. 10.68cms in 12-18 months time and total increase in shoot length was
60.08 cms. The root depth increased by 11.2 cms in 3-12 months, 29.92 cms in 12-18 months and total increase in root depth was 41.12 cms.

In w1 the increase in shoot length was more by 67.4 cms in 3-12 months time, 21.2 cms in 12-18 months and total increase in shoot length was 82.8 cms. The increase in root depth was 28.3 cms in 3-12 months time, 10.0 cms in 12-18 months and total increase in root depth was 38.3 cms.

In w2 the shoot length increased by 77.3 cms in 3-12 months time, 1.0 cms in 12-18 months and total increase in shoot length was 78.3 cms. The increase in root depth was 16.7 cms in 3-12 months time, 27.3 cms in 12-18 months and total increase in root depth was 44.0 cms.

s1 showed increase in shoot length by 66.4 cms in 3-12 months time, 23.6 cms in 12-18 months and total increase in shoot length was 90.0 cms. The increase in root depth was 21.1 cms in 3-12 months time, 10.5 cms in 12-18 months and total increase in root depth was 31.6 cms.

s2 showed an increase in shoot length of 55.3 cms in 3-12 months time, 25.7 cms in 12-18 months and total increase of shoot length by 81.0 cms. The increase in root depth was 24.3 cms in 3-12 months time, 2.6 cms in 12-18 months and total increase in root depth was 26.9 cms.
In h₁ the shoot length was 60.4cms in 3-12 months time, 40.07cms in 12-18 months and total increase in shoot length was 101.1cms. The increase in root depth was 10.8cms in 3-12 months time, 13.8cms in 12-18 months and total increase in root depth was 24.6cms.

In h₂ the shoot length was 78.0cms in 3-12 months time, 44.9cms in 12-18 months and total increase in shoot length was 123.0cms. The increase in root depth was 6.9cms in 3-12 months time, 10.1cms in 12-18 months and total increase in root depth was 38.3cms.

In h₃ the increase in shoot length was 65.1cms in 3-12 months time, 5.8cms in 12-18 months and total increase in shoot length was 70.3cms. While root depth increased by 32.7cms in 3-12 months time, 2.2cms in 12-18 months and total increase in root depth was 34.9cms.

The data of this study were subjected to one-way Anova and Multiple Comparison tests. The results of this is represented in Table 1. 2.3
Table I. 2.3

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Linear Growth (3m)</th>
<th>Sum of Squares</th>
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<th>Mean Square</th>
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<td>Linear Growth (12m)</td>
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<td>1320.065</td>
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<td>Linear Growth (18m)</td>
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<td>----------------------</td>
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</tr>
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<td>1.</td>
<td>3m Height</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>12m Height</td>
<td>((h_3, n_1, h_1, k_1), (n_1, h_1, k_1, h_2, y_2), (h_2, y_2, s_2, s_1, w_1, j_1)) and ((y_2, s_2, s_1, w_1, j_1, w_3, y_3, y_1))</td>
<td>--N0--</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>18m Height</td>
<td>((k_1, y_2, s_2, h_1, j_1, w_1, s_1, w_2)) and ((s_2, h_1, j_1, w_1, s_1, w_2, y_3, y_1, h_2))</td>
<td>(n_1) and (h_3)</td>
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</tr>
</tbody>
</table>

Table: 1.2.3
With respect to the linear growth at 3 month stage, none of the accessions were totally different; hence the accessions could be grouped as 

\[(h_3, h_2, h_1, w_1, j_1, n_1), (h_1, w_1, j_1, n_1, y_3, y_1), (n_1, y_2, y_1, y_2, w_2), (y_3, y_1, y_2, w_2, k_1, s_1)\] and \[(y_2, w_2, k_1, s_1, s_2).\]

With respect to the linear growth at 12 month stage, none of the accession is totally different; hence, the accessions could be grouped as 

\[(h_3, n_1, h_1, k_1), (n_1, h_1, k_1, h_2, y_2), (h_2, y_2, s_2, s_1, w_1, j_1)\] and \[(y_2, s_2, s_1, w_1, j_1, w_2, y_3, y_1).\]

With respect to the linear growth at 18 month stage, none of the accession is totally different; hence, the accessions could be grouped as 

\[(k_1, y_2, s_2, h_1, j_1, w_1, s_1, w_2)\] and \[(s_2, h_1, j_1, w_1, s_1, w_2, y_3, y_1, h_2).\]

**Growth Equations:**

The Growth equations worked out at different stages of sapling growth were Absolute Growth Rate (AGR), Net Assimilation Rate (NAR) and Leaf Area Ratio (LAR). These equations were analysed at 3\(^{rd}\) month, 12\(^{th}\) month and 18\(^{th}\) month stages in 5 replications for all the 13 accessions at the above mentioned growth stages.

For these studies, saplings obtained from the seeds of parent accessions namely, 3 accessions from Yelahanka (y_1, y_2, and y_3) single
accession from Jayanagar, N. R. Colony and K. R. Puram (j1, n1, and k1). 2
accessions from West of Chord Road, (w1, and w2). 2 accessions from SAI (s1
and s2) and 3 accessions from Hessaraghata (h1, h2, and h3) were considered.

Absolute Growth Rate (AGR): -

It was determined between 3 and 12 months and 12 and 18
months of Growth stages of the saplings and presented in Fig. 12.2.

It was found that AGR increased at 12–18 months stage, when
compared to 3 months stage, in all the 13 accessions studied.

Between, 3 m – 12m stage, among 3 accessions of Yelahanka,
AGR was maximum in y2, followed by y3 and y1.

Among 2 accessions of West of Chord Road, maximum AGR
was in w1 and among 2 accessions of SAI maximum AGR was found in s1 and
among 3 accessions of Hessaraghata, maximum AGR was found in h1 followed
by h2 and h3.

While, the AGR of single accession of Jayanagar j1 was less than
one of the accessions of West of Chord Road w2 and AGR of single accession
of K. R. Puram k1 is less than that of one of the accession of SAI s2.
Fig. 1.2.2: Absolute Growth Rate in Sapling Bixa orellana L., accessions grown under identical conditions at 3-12 and 12-18 months stage
Therefore, maximum AGR between 3m to 12m stage among all
the 13 accessions was found in single accession of N. R. Colony $n_1$ and
minimum AGR was found in the third accession of Hessaraghata $h_3$.

AGR studied between 12m – 18m stage, is as follows:

Among the 3 accessions of Yelahanka, first accession $y_1$ showed
maximum AGR followed by second and third accessions $y_2$ and $y_3$.

Among 2 accessions of West of Chord Road, maximum AGR
was found in second accession of West of Chord Road $w_2$, among 2 accessions
of SAI. maximum AGR was found in the first accession $s_1$ and among 3
accessions of Hessaraghata, maximum AGR was found in the third accession
$h_3$, followed by first and second accession $h_1$ and $h_2$. While, AGR of single
accession of Jayanagar and one of the accessions of West of Chord Road $w_1$
were similar. AGR of single accession of N.R. Colony $n_1$ was slightly more
than that of second accession of West of Chord Road $w_2$. AGR of single
accession of K. R. Puram $k_1$ was slightly more than that of the second
accession of Hessaraghata $h_3$.

Therefore maximum AGR between 12m – 18m stages was found
in the first accession of Yelahanka $y_1$ and minimum AGR was found in the
second accession of Hessaraghata $h_3$. 
Net Assimilation Rate (NAR): -

It was determined between 3 and 12 and 12 and 18 months of growth stages of the saplings and is presented in Fig. 1. 2. 3.

Out of the 13 accessions studied, it was found that NAR increased at 12-18 months stage, when compared to 3 – 12 months stage, in the nine 9 accessions namely, First accession of Yelahanka y1, single accession of Jayanagar j1, single accession of K. R. Puram k1, second accession of West of Chord Road w2, first accession and second accession of SAI s1 and s2, first, second and third accessions of Hessaraghata h1, h2 and h3, while NAR decreased at 12 – 18 stage when compared to 3 – 12 stage, in 3 accessions namely accession of Yelahanka y3, single accession of N. R. Colony n1 and first accession of West of Chord Road w1 and NAR was found to be equal in the accession of second accession of Yelahanka y2, both at 3 and 12m and 12 and 18 months.

Between 3 – 12m stage, among 3 accessions of Yelahanka, NAR was maximum in third accession y3, followed by second accession y2 and first accession y1.

Among 2 accessions of West of Chord Road, maximum NAR was found in first accession of West of Chord Road w1 and among 2 accessions of SAI, maximum NAR was found in second accession of SAI s2.
Fig. 1.2.3: Net Assimilation Rate in Saplings of *Bixa orellana* L., accessions grown under identical conditions at 3-12 and 12-18 months stage.
Among 3 accessions of Hessaraghat, maximum NAR was found in second accession $h_2$, followed by first accession $h_1$ and third accession $h_3$, respectively.

While, the NAR of single accession of N. R. Colony $n_1$ was less when compared to the second accession of West of Chord Road $w_2$ and first accession of SAI, while AGR of single accession of Jayanagar $j_1$ was more than the single accession of K. R. Puram $k_1$.

Therefore, maximum NAR between 3 – 12m stage, among all the 13 accessions; was found in third accession of Yelahanka $y_3$ and minimum in the third accession of Hessaraghat $h_3$.

NAR studied between 12 – 18m stage, is as follows:-

Among the 3 accessions of Yelahanka, first accession $y_1$ showed maximum NAR, when compared to second and third accessions $y_2$ and $y_3$ showed same NAR.

Among 2 accessions of West of Chord Road, maximum NAR was found in the second accession of West of Chord Road $w_2$ and among 2 accessions of SAI, maximum NAR was found in first accession of SAI $s_1$ and among 3 accessions of Hessaraghat maximum NAR was found in third accession $h_3$, followed by first and second accessions $h_1$ and $h_2$ respectively.
While NAR of second accession of SAI s₂ was more than first accession of West of Chord Road W₁ and NAR of single accession of K. R. Puram k₁ was more than the single accession of Jayanagar j₁ and N. R. Colony n₁.

Therefore, maximum NAR among all the 13 accessions between 12 – 18m stage was found in first accession of Yelahanka y₁ and minimum NAR in the single accession of N. R. Colony n₁.

Leaf Area Ratio (LAR): -

Leaf Area Ratio was determined at 3m, 12m and 18 months growth stages of the saplings and results are represented in the Fig. 1. 2.4.

It was found that in all the 13 accessions, LAR was maximum at 3 months stage followed by 12 months and 18 months stage. At 3 months stage, among the 3 accessions of Yelahanka, maximum LAR was found in the third accession y₃, followed by first and second accession y₁ and y₂.

Among the 2 accessions of West of Chord Road, maximum LAR was found in the first accession of West of Chord Road w₁ and among 2 accessions of SAI, maximum LAR was in the first accession of SAI s₁ and among 3 accessions of Hessaraghata, maximum LAR was found in the second accession h₂ followed by h₁ and h₃.
Fig. 1.2.4: Leaf Area Ratio in saplings of *Bixa orellana* L. accessions grown under identical conditions for 3 months, 12 months and 18 months stage.
Among single accession of Jayanagar J\(_1\), N. R. Colony n\(_1\) and K. R. Puram k\(_1\), maximum LAR was found in the single accession of N. R. Colony n\(_1\), followed by single accession of Jayanagar j\(_1\) and single accession of K. R. Puram k\(_1\). While, LAR of second accession of West of Chord Road w\(_2\) and second accession of SAI s\(_2\) was less than that of the single accession of K. R. Puram k\(_1\).

Thus, maximum LAR among all the 13 accessions at 3m stage was found in the single accession of N. R. Colony n\(_1\) and minimum LAR in the second accession of SAI s\(_2\).

At 12m stage, among the 3 accessions of Yelahanka, maximum LAR was found in the first accession y\(_1\), followed by y\(_2\) and y\(_3\) respectively.

Among the 2 accessions of West of Chord Road, maximum LAR was found in the first accession of West of Chord Road w\(_1\) and among 2 accessions of SAI, maximum LAR was found in the second accession of SAI s\(_2\) and among 3 accessions of Hessaraghatta, maximum LAR was found in the third accession (h\(_3\)). followed by h\(_1\) and h\(_2\).

Among single accessions of Jayanagar j\(_1\), N. R. Colony (n\(_1\)) and K. R. Puram k\(_1\), maximum LAR was found in j\(_1\) followed by n\(_1\) and k\(_1\), while LAR of second accession of SAI s\(_2\) was more than that of second accession of West of Chord Road w\(_2\).
Thus, maximum LAR, among all the 13 accessions at 3 months stage was found in the third accession of Hessaraghatta $h_2$ and minimum LAR in the second accession of West of Chord Road $w_2$.

At 18m stages, among the 3 accessions of Yelahanka, maximum LAR was found in the second accession $y_2$, followed by first and the third accession $y_1$ and $y_3$.

Among the 2 accessions of West of Chord Road, maximum LAR was found in the first accession $w_1$, among the 2 accessions of SAI maximum LAR was in second accession $s_2$ and among the 3 accessions of Hessaraghatta, maximum LAR was found in the second accession $h_2$ followed by first and third accessions $h_1$ and $h_3$.

Among the single accession of Jayanagar $j_1$, N. R. Colony $n_1$ and K. R. Puram $k_1$ maximum LAR was found in $n_1$, followed by $j_1$ and $k_1$, while LAR of second accession of West of Chord Road $w_2$ was more than the first accession of SAI $s_1$.

Thus, maximum LAR, among all the 13 accessions, at 18m stage was found in the second accession of Hessaraghatta-$h_2$ and minimum LAR in the first accession of SAI-$s_1$. 
Leaf Area:

The Leaf Area varied considerably among 3, 12 and 18 month old saplings of all the 13 accessions of *Bixa orellana* L.

Leaf Area studied was maximum in 18 month-old stage, followed by 12 months and 3 months stage, respectively in all the 13 accessions studied.

The data on the leaf area analysed statistically are presented in the Table 1. 2.4.

With respect to Leaf Area at 3 month stage, none of the accession were totally different. Therefore we could group the accessions into \((s_2, y_1, y_2, w_1, w_2, h_3, k_1)\), \((y_1, y_2, w_1, w_2, y_3, k_1, j_1)\), \((y_2, w_1, w_2, y_3, k_1, j_1, s_1)\), \((k_1, j_1, s_1, h_1, h_3)\), \((s_1, h_1, h_2, h_3)\) and \((h_2, n_1)\).

With respect to Leaf Area at 12 month stage, none of the accessions were totally different therefore we could group the accessions into \((w_2, k_1, y_1, h_2, y_2, s_1, w_1, j_1)\), \((y_2, s_1, w_1, j_1, s_2, h_3)\), \((s_2, h_3, h_1, n_1)\) and \((h_1, n_1, y_3)\).

With respect to Leaf Area at 18 month stage, only accession \(j_2\) is totally different, while other accessions could be grouped as \((s_1, s_2, y_3, y_1, y_2, w_2)\), \((s_2, y_3, y_1, y_2, w_2, k_1)\), \((y_3, y_1, y_2, w_2, k_1, h_1)\), \((y_1, y_2, s_1, w_1, h_2, w_2, j_1)\) and \((y_1, h_1, w_1, j_1, h_3, h_2)\).
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Table I. 2.4

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<td>2.</td>
<td>Leaf Area (12 months)</td>
<td>$(w_2, k_1, y_1, h_3, y_3, s_1, w_1, j_1), (y_2, s_1, w_1, j_1, s_2, h_3), (s_2, h_3, h_1, n_1)$ and $(h_1, n_1, y_3)$</td>
<td>--No--</td>
</tr>
<tr>
<td>3.</td>
<td>Leaf Area (18 months)</td>
<td>$(s_1, s_2, y_3, y_1, y_2, w_2), (s_2, y_3, y_1, y_2, w_2, k_1), (y_3, y_1, y_2, w_2, k_1, h_1), (y_1, y_2, s_1, w_1, h_2, w_2, j_1)$ and $(y_1, h_1, w_1, j_1, h_3, h_2)$</td>
<td>only Accession $n_1$</td>
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</table>
Dry matter analysis:

The seeds collected from the parent accessions were used for this study. The saplings obtained from these were analysed for their dry matter at 3, 12 and 18-month stage. The dry matter accumulation in the shoot, root and leaves increased in all the 13 accessions during the growth period of 3 to 18 months stage. The data is represented in Fig. 1.2.5.

There was increase in dry matter with respect to root and leaves in all the 13 accessions (Fig. 1.2.5).

The shoot dry matter increased by 10.64g during 9 months from 3-12 months time and 21.52g during 6 months time (12 - 18 months). Root dry matter increased by 6.40g in 3-12 months time (9 months) and 29.96g in 12-18 months time (6 months time). While leaf dry matter increased by 7.89g in 3-12 months time and 10.56g in 12-18 months time [Fig. 1.2.5(y1)].

Shoot dry matter increased by 14.084g in 3-12 months time and 11.46g in 12-18 months time. Similarly, root dry matter increased by 10.78g and 20.4g in 3-12 month’s time and 12-18 months time, respectively Leaf dry matter increased by 7.59g in 3-12 months time and 7.12g in 12-18 months time [Fig. 1.2.5(y2)].
Shoot dry matter increased by 14.08g in 3-12 months time and 14.94g in 12-18 months time. Root dry matter increased by 8.61g in 3-12 months time and 22.08g in 12-18 months time and Leaf dry matter increased by 9.0g in 3-12 months time and 6.81g in 12-18 months time [Fig. 1. 2.5 (y)].

Shoot dry matter increased by 12.6g in 3-12 months time and 15.8g in 12-18 months time. Root dry matter increased by 9.55g in 3-12 months time and 21.7g in 12-18 months time and Leaf dry matter increased by 6.6g in 3-12 months time and 7.82g in 12-18 months time [Fig. 1. 2.5 (j)].

Shoot dry matter increased by 23.10g in 3-12 months time and 10.0g in 12-18 months time. Root dry matter increased by 17.45g in 3-12 months time and 8.1g in 12-18 months time and Leaf dry matter increased by 7.3g in 3-12 months time and 10.7g in 12-18 months time [Fig. 1. 2.5 (n)].

Shoot dry matter increased by 8.0g in 3-12 months time and 7.7g in 12-18 months time. Root dry matter increased by 7.6g in 3-12 months time and 17.28g in 12-18 months time. Leaf dry matter increased by 6.0g in 3-12 months time and 6.5g in 12-18 months time [Fig. 1. 2.5 (k)].

Shoot dry matter increased by 11.8g in 3-12 months time and 15.4g in 12-18 months time. Root dry matter increased by 14.0g in 3-12 months time and 12.18g in 12-18 months time and Leaf dry matter increased by 9.13g in 3-12 months time and 17.64g in 12-18 months time [Fig. 1. 2.5 (w)].
Shoot dry matter increased by 31.1g in 3-12 months time and 15.2g in 12-18 months time. Root dry matter increased by 8.6g in 3-12 months time and 26.1g in 12-18 months time and Leaf dry matter increased by 8.1g in 3-12 months time and 6.06g in 12-18 months time [Fig. 1. 2.5 (w)].

Shoot dry matter increased by 12.0g in 3-12 months time and 13.6g in 12-18 months time. Similarly, root dry matter and leaf dry matter increased by 9.88g, 15.96g and 6.82g and 6.42g in 3-12 months time and 12-18 months time respectively [Fig. 1. 2.5 (s)].

Shoot dry matter increased by 9.32g in 3-12 months time and 14.4g in 12-18 months time. Similarly, root dry matter and leaf dry matter increased by 8.74g, 11.86g and 5.9g, 7.56g respectively [Fig. 1. 2.5 (s)].

Shoot dry matter increased by 7.70g in 3-12 months time and 17.9g in 12-18 months time. Similarly, root dry matter and leaf dry matter increased by 4.95g, 11.7g and 6.11g, 8.74g respectively [Fig. 1. 2.5 (h)].

Shoot dry matter increased by 9.39g in 3-12 months time and 14.5g in 12-18 months time. Similarly, root dry matter and leaf dry matter increased by 5.1g, 4.8g and 7.0g, 9.0g respectively [Fig. 1. 2.5 (h)].
Shoot dry matter increased by 7.98g in 3-12 months time and 19.4g in 12-18 months time. Similarly, root dry matter and leaf dry matter increased by 4.7g, 24.3g and 3.5g, 11.52 respectively [Fig. 1. 2.5 (h3)].

Shoot dry matter, Leaf dry matter and Root dry matter increased during the two growth stages studied (3-12 months and 12-18 months). The increase in dry matter accumulation was linear during 3-12 months and 12-18 months growth stages in all the 13 accessions, while, there was a slight decline in the rate of increase in dry matter accumulation in shoot during 12-18 months stage in shoot of accessions y2, n1 and k1 (second accession of Yelahanka single accession of N. R. Colony and K.R. Puram).

With respect to the dry matter accumulation in roots there was slight decline in accessions n1, w1 and h2 (accession of N. R. Colony, first accession of West of Chord Road and second accession of Hessaraghata).

With respect to the dry matter accumulation in leaves there was slight decline in accessions of second and third accession of Yelahanka, and second accession of West of Chord Road and first accession of SAI y2, y3, w2 and s1.

The data were subjected to one-way Anova and multiple comparison test (Duncan’s range test) and results are presented in Table 1. 2.5.
With respect to the dry matter accumulation at 3 months stage, only accessions \( s_1 \) and \( s_2 \) were totally different, while other could be grouped as \((n_1, w_1, j_1, k_1, y_3), (w_1, j_1, k_1, y_3, h_2, y_1, h_1), (k_1, y_3, h_2, y_1, h_1, y_2)\) and \((y_3, h_2, y_1, h_1, y_2, h_3, w_2)\).

With respect to the dry matter accumulation at 12 months stage, only accession \( n_1 \) was totally different, while others could be grouped as \((h_3, h_1, h_2, k_1), (h_1, h_2, k_1, y_1, s_2), (h_2, k_1, y_1, s_2, j_1), (y_1, s_2, j_1, s_1, w_2), (s_2, j_1, s_1, w_2, y_3, y_2)\) and \((j_1, s_1, w_2, y_3, y_2, w_1)\).

With respect to the dry matter accumulation at 18 months stage, none of the accessions were totally different and hence the accessions could be grouped as \((h_2, k_1, h_1, s_2), (k_1, h_1, s_2, s_1), (h_1, s_2, s_1, y_3, h_3), (s_2, s_1, y_3, h_3, j_1, y_1)\) and \((s_1, y_3, h_3, j_1, y_1, y_3, n_1, w_2, w_1)\).
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<td>Dry Matter (18months)</td>
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Fig. 1.2.5: Dry weight of 3, 12 and 18 month-old saplings of *Bixa orellana* L. accessions raised under identical conditions (given in text)

Figures in Parentheses represent standard deviation values.
Phytochemical Analysis of Leaves:

Total chlorophylls and carotenoids were estimated in 7 and 12 month-old saplings of all the 13 accessions of Bixa orellana L. and the result are presented in the Fig. 1. 2.6 and Fig. 1. 2.7.

For this study progenies, 3 accessions from Yelahanka y_1, y_2 and y_3, one accession from Jayanagar j_1, N. R. Colony n_1 and K. R. Puram k_1, 2 accessions of West of Chord Road (w_1 and w_2), 2 accessions of SAI s_1 and s_2 and 3 accessions of Hessaraghata h_1, h_2 and h_3 were considered.

Total chlorophylls and carotenoids estimated were more at 12 month-old stage, when compared to the 7 month-old stage, in all the 13 accessions studied.

With respect to the total chlorophylls, at 7 month-old stage among the 3 accessions of Yelahanka total chlorophylls were maximum in the first accession y_1 followed by the same amount found in the second and the third accessions y_2 and y_3.

Among the 2 accessions of West of Chord Road, maximum was in the second accession of West of Chord Road w_2, among 2 accessions of SAI, maximum was in the second accession s_2 and among 3 accessions of Hessaraghata, maximum was in the third accession h_3 followed by h_1 and h_2.
While, in the single accessions of Jyanagar j₁, N. R. Colony n₁ and K. R. Puram k₁, maximum total chlorophylls was found in n₁ followed by k₁ and j₁, respectively and total carotenoids was maximum in the second accession of West of Chord Road w₂, followed by the second accession of SAI s₂.

With respect to the total carotenoids studied at 7 month old stage, among the 3 accessions of the Yelahanka, maximum was found in first accession y₁, followed by the second and the third accession y₂ and y₃ respectively.

Thus, total chlorophylls at 7 months stage was maximum in the single accession of N. R. Colony n₁ and minimum in the single accession of K. R. Puram k₁ and total carotenoids was maximum in the first accession of West of Chord Road w₁ and minimum in the single accession of Jyanagar j₁.

Total chlorophyll and total carotenoids studied at 12 month-old stage is as follows (Fig. 1. 2.7).

With respect to the total chlorophylls at 12 month - old stage, among the 3 accessions of Yelahanka, maximum total chlorophylls were seen in the first accession of Yelahanka y₁, followed by y₃ and y₅ respectively.

Among the 2 accessions of West of Chord Road, maximum total chlorophylls was found in the first accession of West of Chord Road w₁.
among 2 accessions of SAI, maximum chlorophylls was found in the first accession of Sai-s1 and among the 3 accessions of Hessaraghat, maximum chlorophylls was found in the first accession (h1) followed by second and third accession of Hessaraghat h2 and h3 respectively.

Among the single accession of Jayanagar, j1, N. R. Colony n1 and K. R. Puram k1, maximum total chlorophyll was found in k1, followed by n1 and j1 and total chlorophylls was more in the second accession of West of Chord Road w2, when compared to the second accession of Sai-s2.

With respect to the total carotenoids studied at 12 month-old stage, among the 3 accessions of Yelahanka, maximum was found in the first accession y1, followed by second and the third accession y2 and y3 respectively.

Among the 2 accessions of West Chord Road, maximum total carotenoids was found in the second accession w2 and among the 2 accessions of SAI maximum carotenoids was found in the second accession SAI S2 and among the 3 accessions of Hessaraghat maximum total carotenoids was found in the third accession h3 followed by h2 and h1.
While among the single accession of Jayanagar j₁, N. R. Colony (n₁) and K. R. Puram k₁, maximum total carotenoids was found in the n₁, followed by j₁ and k₁ and the total carotenoids of the first accession of West of Chord Road w₁ was more than the first accession of SAI s₁.

Thus, total chlorophylls at 12 month-old stage were maximum in the first accession of Yelahanka y₁ and minimum in the second accession of SAI s₂ and total carotenoids were maximum in the second accession of SAI s₂ and minimum in the first accession of SAI s₁.

The data were analysed statistically and given in the Table I. 2.6.

With respect to the total chlorophyll at 7 month-old stage, only n₁ accession was different, while others could be grouped as (k₁, j₁, s₁), (j₁, s₁, y₁, w₁, y₂), (y₁, w₁, y₂, w₂), (w₂, s₂), (h₁, h₁), (y₁, h₁) and (h₁, h₂).

With respect to the total carotenoids, at 7 month old stage, only accession s₂ was totally different, while other accessions could be grouped into a single group as (j₁, s₁, k₁, y₁, y₂, w₂, w₁, y₁, h₁, h₃, h₂, n₁).

With respect to the total chlorophylls at 12 month-old stage, none of the accessions were totally different; hence the accessions could be grouped into (s₁, y₂, j₁, w₂, s₂), (y₂, j₁, w₂, s₂, h₁), (w₂, s₂, h₁, y₂, h₂), (y₂, h₂, n₁, h₁), (h₂, n₁, h₁, w₁, k₁) and (n₁, h₁, w₁, k₁, y₁).
With respect to the total carotenoids at 12 month - old stage, none of the accession were totally different, hence the accessions could be grouped into \((s_1, j_1, w_1, k_1, s_2 w_2)\), \((w_2, h_1)\), \((h_1, h_2)\), \((h_2, h_3, y_3)\), \((y_1, y_2)\) and \((y_2, n_1)\).
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<td>2.</td>
<td>7 Months (Carotenoids)</td>
<td>(j_1, s_1, k_1, y_2, w_2, y_1, w_1, y_1, h_1, h_2, k_1, n_1)</td>
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<td>12 Months (Chlorophyll)</td>
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Fig. I.2.6: Total Chlorophylls and Carotenoids in 7 month - old saplings of *Bixa orellana* L.

*F* significant at $\alpha = 0.05$

(Figures in parentheses represent Standard Deviation values)
Fig. 1.2.7: Total Chlorophylls and Carotenoids in 12 month-old saplings of *Bixa orellana* L.

* F significant at $\alpha = 0.05$

( Figures in parentheses represent Standard Deviation values )
Discussion
DISCUSSION:

The plants raised from the 13 seed samples of *Bixa orellana* accessions under identical field conditions in Jnana Bharathi campus Bangalore University, Bangalore retained the differences in their vegetative features as did those of their parent generation. The saplings were assessed for their morphological characters at the growth stages of 7, 14 & 18 months. The details of leaf shape, leaf venation colour, flower colour, capsule shape, capsule texture and seed colour of the progenies raised from the seeds of 13 parent accessions are given in Table 1.2.2. These morphological characters were very similar to those of parent accessions. The diversity observed with regard to certain prominent traits like leaf shape, leaf venation colour, flower colour, capsule shape, size, texture and seed colour were expressed even in the progeny that were raised from the seeds of their respective parents. These traits were very prominent and distinct in the parent trees. The fact that these were transmitted to the progeny as such only shows that these characters are inherited from the parent to the progeny. In this connection it has to be stated that the seed storage protein exhibited wide variation between the accessions and the polypeptides that were resolved exhibited mixing up to some extent (Fig. 1.1.24; plate 1.1.24). This mixing up clearly indicates that the different accessions of *Bixa orellana* are not pure and that some of the variations are incorporated into the genome. Though there were wide variations between
accessions in the 6 traits stated above, this genetic diversity may not result in speciation. These may be only resulting in population difference below the level of species.

If these 6 traits were temporarily acquired characters, they would have disappeared when the progeny of these were raised under controlled conditions (edaphic, environment).

The growth rate of *Bixa orellana* progeny were studied during the growth periods of 3 to 18 month stage. In the initial 3 to 12 months time the increase in growth was enormous. In the latter stage of 12-18 months time the rate of growth declined slightly.

The data analysed statistically using one way Ananova and Multiple comparison tests showed that the results are statistically significant and that none of the trees were totally different with respect to the linear growth at 3, 12 and 18 month stage and they were grouped as similar trees (Table 1 2.3). However, slight variations were noticed with regard to the height at 18 month stage. These were in accessions *h*1 and *h*3.

AGR (Absolute Growth Rate), NAR (Net Assimilation Rate) and LAR (Leaf Area Ratio) were the growth equations analyzed at 3, 12 and 18 month stages in all the 13 accessions (Fig. 1. 2.2, 2.3 & 2.4). Though there were minor variations between the accessions for the different growth
equations as stated in the results, drastic differences were noticed in the 18 month – old plants leaf area in accession n₁. Similarly the dry matter accumulation by different accessions varied with growth durations (Table 1.2.5). Based on this the similar and different trees were grouped separately. In 3 months time, accessions s₂ and h₁ were different with respect to dry matter accumulation while accession n₁ was different at 12 months growth stage in dry matter accumulation (Table 1.2.5).

Hence, the progeny of the different accessions varied with respect to the growth equations which reflect the growth rate. Net Assimilation Rate and the Leaf Area Ratio when analyzed at different growth durations. This again speaks about the diversity seen between the accessions; ranging from minute to drastic. Similar growth pattern were reported for 2 Brazilian annatto cultivars, viz. INKA and CPATU113. These two cultivars exhibited different growth and dry matter accumulation in the leaves, stem and roots Soria et al (1994) respectively.

Total Carotenoid and chlorophylls were the two phytochemicals analyzed in the progeny of Bixa orellana at 7 and 12 month stage (Fig. 1.2.6 & 2.7).

The data were found to be statistically significant and is represented in Table 1.2.6.
Only accessions $n_1$ and $s_1$ were different with respect to the phytochemicals at 7 months stage of growth period. All the other trees were very similar to each other.

The discussion stated above very clearly indicate diversity between the various accessions (i.e. intraspecific), specifically because many of the traits are transmitted in the progeny raised from the seeds of the parent accession when grown in the field under identical conditions of temperature, rainfall and humidity. The variations observed for a number of characters further underlines the complexity in describing the different accessions within *Bixa orellana* accessions. The most widely varied quantitative characters were leaf shape, leaf venation color, flower color, capsule color, capsule shape, capsule texture and seed color. The pattern of variations generated by statistical analyses was demonstrated by one way annova and multiple comparison tests.

The foregoing discussion very emphatically state that most of the accessions proved distinct with respect to the morphological characters though the growth pattern and growth equations worked out varied mildly to drastic in different accessions. These variants or races were adopted to the different conditions prevailing in the places of their sampling.

Sampling sites were from Bangalore and neighboring areas where the climatic conditions though have not varied dramatically (Table 1. 2.1), the
soil characters and the environment were quite different. The roadside and the
avenue trees are often subjected to pruning, water stress and hard environment
while those in the Botanical garden and privately owned land are well
protected, nourished and watered regularly but are exposed to the hard
environment most of the time. Hence, the stress for water, nourishment and
hard edaphic conditions caused variations in these accessions.

If these phenotypic variations were acquired and not incorporated
into the genetic make, they would have disappeared when grown under
controlled identical conditions. The very fact that these were retained and were
expressed even in the progeny obtained from the seeds only speak about the
variations which could be genetic that is transmitted into the next generation.

This observation further is supported by the study conducted on
the seed storage proteins. The different accessions had variations in the
polypeptides that were resolved in SDS-PAGE. The Electropherogram very
clearly demonstrated the variation in the genetic make of the accessions
responsible for variations in the phenotype.

This study on *Bixa orellana* accessions from its natural habitat
and the progeny obtained from seeds contributes to our understanding of
genetic diversity and the adaptability of these to their environment.
This is an important research area, in part because of two simultaneous trends:

1) The accelerating pace of environmental change.

2) A consequence of human activities that are affecting the Earths' climate and ecosystem.

The second is the general reduction in size and genetic diversity of plant population as a result of habitat alteration, fragmentation, and destruction.

For a population to survive environmental modification, it must have sufficient genetic diversity to adapt to new conditions. Less genetically diverse populations may be at greater risk.

The combination of mounting environmental change and dwindling genetic diversity means that many species must survive greater stress with reduced genetic resources. A number of species face an increased risk of extinction, and many important conservation decisions must be made.