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Material and Methods

Material :

In the present investigation, E. agallocha L. species is selected which grows along West Coast of Maharashtra. The efforts have been done to bring E. agallocha to the laboratory. An attempt has been made to grow the stem cuttings under fresh water conditions in nursery. The well grown plant cuttings were collected from natural population growing at Ratnagiri (73^{O} 21' E, 16^{O} 58' N) and Ganapatipule (73^{O} 2' E, 17^{O} 9' N). Mature plants were selected for regeneration purpose by vegetative propogation. The branches were selected having variation in girth-size. The cuttings were wrapped into moistened jute and brought to the laboratory.

Methods :

In the present work fifteen cuttings of each group with oblique cut were planted in nursery. The cuttings were planted in polybags filled with garden soil and Farm Yard Manure in proportion of 3:1 and were irrigated with fresh water. The portions of the cuttings were planted in the soil upto half of the length. The cuttings were studied for the sprouting potential every week. To find out their sprouting potential, the cuttings were catagorised as follows :

I) Sprouting potential study :

A) Effect of Length Variation - This effect was observed in four groups of variation in length.

a) 10 cm. length of cuttings.

b) 15 cm. length of cuttings.

c) 20 cm. length of cuttings.

d) 25 cm. length of cuttings.

B) Effect of Girth-size Variation - The stem cuttings of various girth-size were seleccted and categorised into following groups :

a) Girth-size ranging from 0.5 to 0.6 mm.

b) Girth-size ranging from 0.9 to 1.1 mm.

c) Girth-size ranging from 1.2 to 1.3 mm

d) Girth-size ranging from 1.5 to 1.6 mm.

C) Effect of Plant Growth Regulators: (PGR): For studying the effects of PGR, two types of plant growth regulators i.e. IAA (Indole acetic acid) and IBA (Indole butyric acid) were prepared in five different concentrations and used for the treatment.

a) IAA - 75, 150, 300, 500 and 1000 ppm.

b) IBA - 75, 150, 300, 500 and 1000 ppm.

For this purpose, one fourth part of the cuttings were dipped into various concentrations for 24 hours. These treated cuttings were allowed to raise in polybags and the effect on sprouting potential was studied.

D) Effect of Keradix treatment : Keradix powder is a rooting medium. Before plantation the moist basal region of cuttings were

just touched to the Keradix powder and the jerk was given to the cuttings to remove excess powder and then the cuttings were planted immediately.

E) Effect of Morphological position : Cuttings from different positions i.e. Apical, Middle and Basal region of the stems were selected and these cuttings were planted in polybags under fresh water conditions by the method described above for further investigations.

II) Response of cuttings to fresh water condition :

When the cuttings were reached at steady state level, their response has been studied by following parameters such as; number of branches, length of branch, number of leaves, leaf thickness, leaf length, leaf breadth from June to December. Above growth parameters were recorded for each week and the statistical data obtained by using the standard deviation formula and has been tabulised.

$$S.D. = \sqrt{\frac{\Sigma f (x - \bar{x})^2}{\Sigma f}}$$

where; f = frequency,

x = individual number of readings,

$\bar{x}$ = mean

III) Response of seedlings to fresh water condition :

Like the cuttings, the seedlings were collected in the month of October to study their response to fresh water condition. The seedlings were planted in polybags filled with garden soil with Farm Yard Manure in proportion of 3:1 and were irrigated with fresh water. Number of seedlings were planted. Initial observations of seedlings i.e. Height of seedlings and diameter of seedlings were recorded and allowed them to stabilize. When they reached at steady state level the data on their growth performance were studied at the approximate age of one year. Growth parameter includes, average height, average girth-size, number of branches, number of leaves per seedling, leaf thickness as well as survival per cent were recorded in each month. The thickness of leaf was measured with the help of micrometer screw. To have the statistical data of the above mentioned parameters the standard deviation has been calculated by using above formula.

Distribution of organic constituents :

In each season of the year i.e. Winter, Summer and Monsoon some physiological aspects i.e. organic constituents have been studied from natural population as well as cultured condition. In the present work Chlorophylls and Polyphenols have been estimated. Fresh material has been used for this purpose.

i) Chlorophylls :

Chlorophylls were estimated according to the method of

Arnon (1949). Chlorophylls were extracted in 80% acetone from 0.5 gm of the plant material. The extract was filtered through Buchner's funnel using Whatman No.1 filter paper. Residue was washed repeatedly with 80% acetone collecting the washings in the same filtrate. The volume of filtrate was made to 100 ml with 80% acetone. The absorbance was read at 663 and 645 nm for chlorophylls 'a' and 'b' respectively.

Chlorophylls (mg/100 gm fresh tissue) were calculated using the following formulae :

Chlorophyll 'a' = 12.7 X A 663 - 2.69 X A 645 = x.

Chlorophyll 'b' = 22.9 X A 645 - 4.68 X A 663 = y.

$$\text{Chlorophyll a/b (mg/100 g fresh tissue)} = \frac{x/y \text{ X vol. of extract X } 100}{1000 \text{ X w't of the material (g)}}$$

ii) Polyphenols :

Polyphenols were estimated by the method of Folin and Denis (1915). Polyphenols were extracted in 80% acetone from the well washed and blotted dry plant material (0.5gm). Extract was filtered through Buchner funnel under suction using Whatman No.1 filter paper. The residue was washed 2-3 times and volume was measured. And this was the source of polyphenols.

1 ml of filtrate was taken in 50 ml marked Nessler's tube.

In others different concentrations (0.5, 0.1, 0.2, 0.3, 0.4 ml) of standard polyphenol solution were taken. 10 ml of 20% Na_2CO_3 were then added to each tube to make medium alkaline. 2 ml of Folin Denis reagent were then added to each test tube and finally the volume was made to 50 ml with water. A blank was prepared similarly, with distilled water. The ingradients were allowed to mix thoroughly. After some time the optical density of each mixture was read at 660 nm on Spectrophotometer (ECIL). Polyphenols were calculated from the calibration curve of standard tannic acid.

1 ml standard = 0.1 mg Polyphenols (tannic acid)

IV) Response of male and female cuttings to fresh water conditions:

During vegetative stage it is very difficult to distinguish between male and female plants. The inflorescence of male and female plants are alike. Both the twigs or plants have been identified morphologically during the flowering periods only. To confirm it, pollen grains of male inflorescence were mounted and the section of ovary was taken. After confirmation the male and female twigs have been marked and labelled. Afterwards these cuttings have been taken for further investigations to study the response of male and female cuttings.

V) Biomass study:

The seedlings and cuttings were uprooted. The measurements

of roots i.e. number of roots, length of roots were taken. And for biomass study the leaves shoots and roots were cut separately (Cintron and Novelli 1984). They were washed and blotted dry. Fresh weight of leaves, shoots and roots were recorded and then the material was oven dried at 60^{o}C. After 20 days when the leaves, shoots and roots show constant dry weight it was recorded. From this moisture content was calculated.

Stem cuttings planted under nursery techniques.

a) Apical region cuttings.

b) Basal region cuttings

c) IAA treated cuttings

d) IBA treated cuttings.

Effect of Length-variation on sprouting potential

a) Sprouting at initial stage

b) Final stage. (after 2 months)

Effect of Girth-size variation on sprouting potential

a) Sprouting at initial stage

Effect of IAA on sprouting potential

a) Initial Stage

b) Final stage (after 12 months)

Effect of IBA on sprouting potential

a) Initial stage

b) Final stage (after 12 months)

Effect of IAA and IBA on sprouting as well as rooting

a) After 9 months growth response of shoot

b) After 9 months growth response of roots.

Effect of Keradix treatment on sprouting and rooting

a) Initial stage sprouting

b) Rooting response at final stage (after 12 months)

Effect of sprouting potential on different morphological positions.

a) Survival cuttings of 'M' region.

b) Survival cuttings of 'B' region.

Planted seedlings under fresh water condition

a) Initial stage

b) Survived seedlings after 5 months

Performance of seedlings under fresh water condition

a) Growth performance at final stage (after 13 months)

b) Rooting response at final stage (after 13 months)

Microphotographs of pollen-grains and T.S. of ovary.

b) Pollen-grains (Magnification - 100 x 3.2 x 1.25)

a) T.S. of ovary (Magnification - 100 x 3.2 x 1.25)

Male and female twigs under natural condition

a) Female twigs with seeds

b) Male twig with inflorescence

Response of male and female cuttings to various concentrations of IAA.

a) 75 ppm IAA treated cuttings

b) 300 ppm IAA treated cuttings

c) 500 ppm IAA treated cuttings

d) 1000 ppm IAA treated cuttings

Rooting in female cuttings

a) Rooting response in 75 ppm IAA treated cuttings

b) Rooting response in 300 ppm IAA treated cutting

c) Rooting response in 500 ppm IAA treated cutting

d) Rooting response in 1000 ppm IAA treated cutting

Response of male and female cuttings to various concentrations of IBA

a) Sprouting in 300 ppm IBA treated cuttings

b) Sprouting in 1000 ppm IBA treated cuttings

c) No response in 1000 ppm IBA treated male cuttings in rooting

E. agallocha

a

b

c

d

a

b

a

a

b


IBA-treatment
Plantation date-20-1-92

a


IBA-treatment
150, 500, 1000 ppm

b


1000 ppm IBA
1000 ppm IAA

a


After 9 months response of 1000 ppm IAA
After 9 months response of 1000 ppm IBA

b


Keradix-treatment
Plantation date - 20-1-92

a


Keradix-treat.
rooting

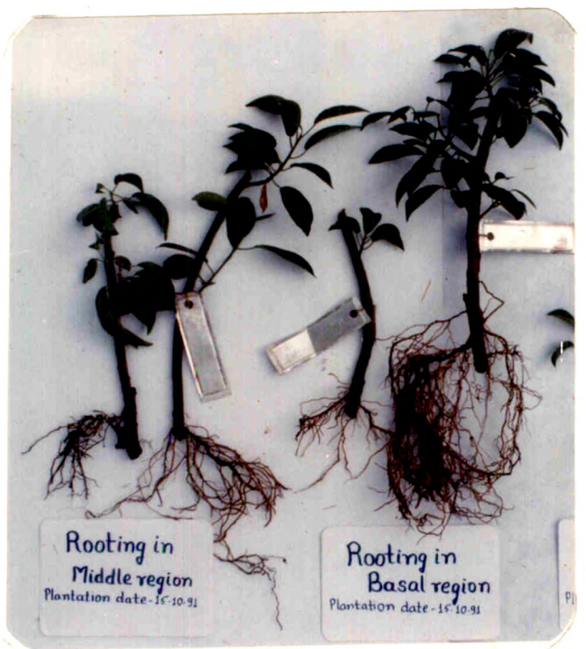
b


Middle region
Plantation date 15.10.91

a


Basal region
Plantation date 15.10.91

b


Rooting in
Middle region
Plantation date-15-10-91
Rooting in
Basal region
Plantation date-15-10-91

a


After 13 months
Middle Position
rooting
After 13 months
Basal Position
rooting

b

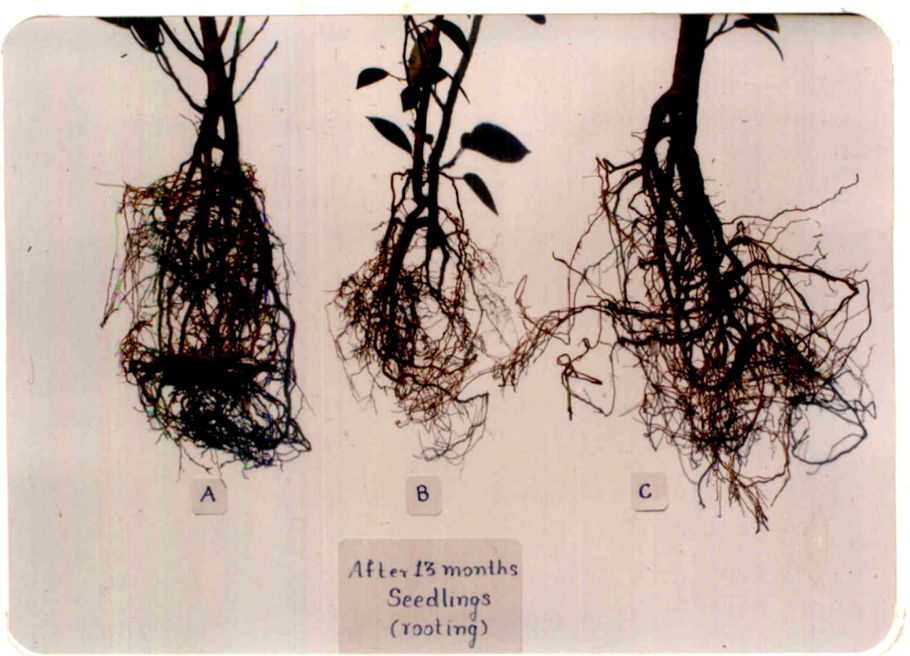

Excoecaria
Seedlings

a

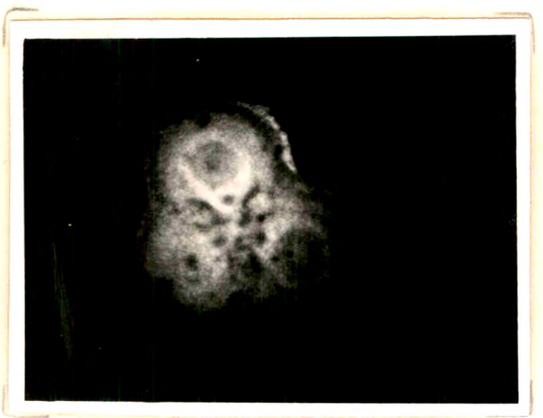

Excoecaria agallocha
Seedlings
Plantation date

b

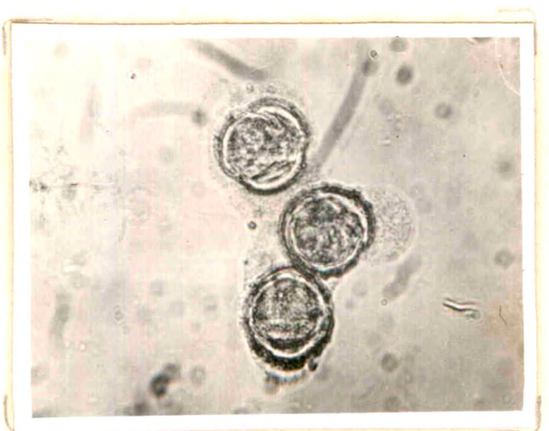
a



b



a



b

a

b


Male
Female
75 ppm IAA

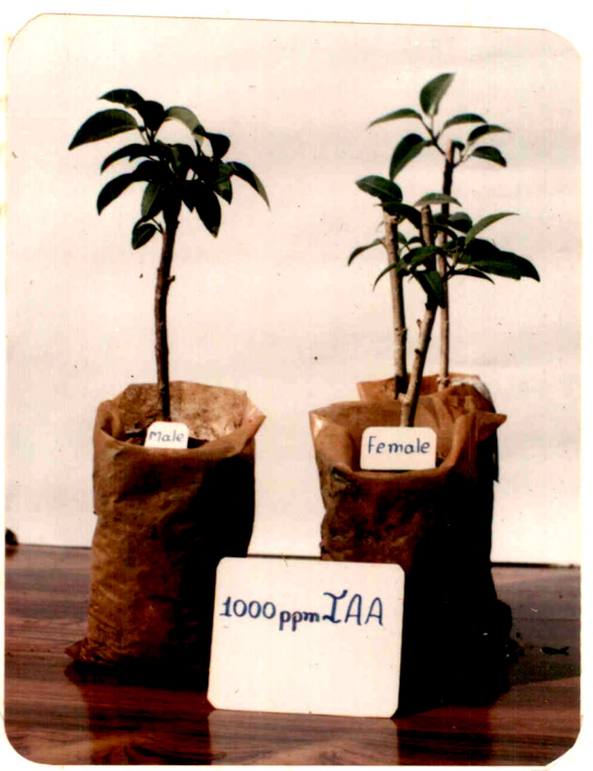
a


Male
Female
300 ppm IAA

b


Male
Female
500 ppm IAA

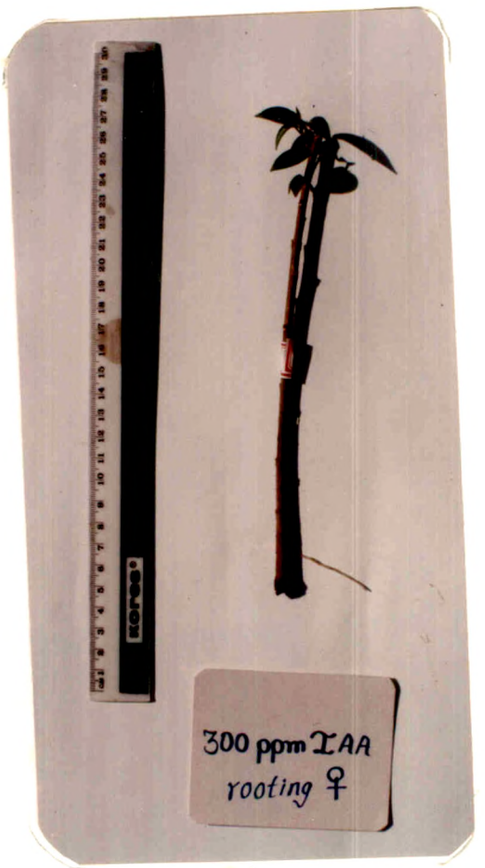
c


Male
Female
1000 ppm IAA

d


75 ppm IAA
rooting ♀

a


300 ppm IAA
rooting ♀

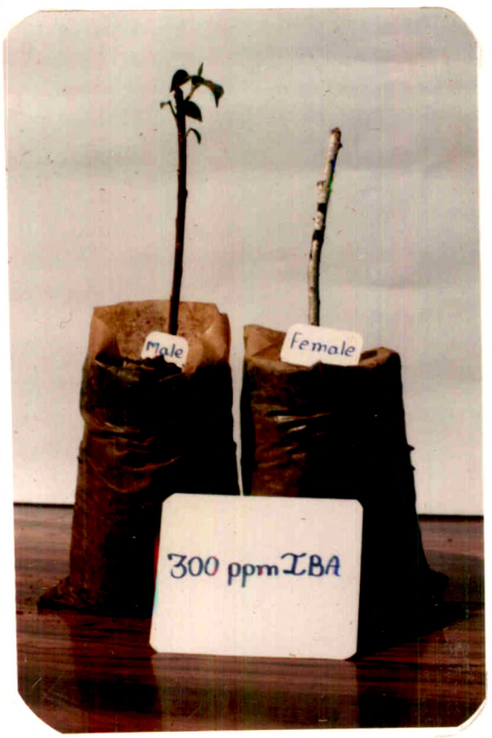
b


500 ppm IAA
rooting ♀

c


1000 ppm IAA
rooting ♀

d


Male
Female
300 ppm IBA

a


Male
Female
1000 ppm IBA

b


1000 ppm IBA
rooting

c