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# Metabolic changes during rooting in pre-girdled stem cuttings and air-layers of *Heritiera*

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**Abstract.** The mangroves of the Mahanadi Delta are unique and include 62 of the 64 mangrove species distributed in India. Vegetative propagation through rooting in stem cuttings and air-layers of *Heritiera fomes* and *H. littoralis,* commonly known as Sundri, using IAA, IBA, and NAA is reported. Significant increase in the root number was recorded in the air-layers and the pre-girdled stem cuttings of *H. fomes* treated together with IBA (5,000 ppm) and NAA (2,500 ppm) as compared to *H. littoralis.* The maximum number of roots was obtained in *H. littoralis* with IBA (5,000 ppm) and NAA (1,000 ppm). The overall rooting response was better in the treatments with IBA and NAA rather than with IAA and NAA combinations. Variation in the rooting response due to the exogenous application of auxins was also reflected in the metabolic changes during the initiation, emergence, and development of roots in the cuttings and the air-layers. An increase in the level of reducing sugars was noted in the pre-girdled tissues at initiation as well as at subsequent stages of root development, which was further enhanced by the use of auxins. A decrease in the total sugar, carbohydrate, and polyphenols and an increase in the total nitrogen and a high C/N ratio were noted at the root initiation stage in both species. There was less accumulation of photosynthates and nitrogen in the above-girdled tissues in air-layers than the pre-girdled cuttings. Interaction of IBA and NAA promoted starch hydrolysis during root development and subsequently reduced the C/N ratio and increased the protein-nitrogen activity during the development of root primordia. The auxin influenced mobilization of nitrogen to the rooting zone promoted the rooting efficiency in *Heritiera*.

**Keywords:** Auxins; C/N ratio; *Heritiera*; Mangroves; Rooting; Sugars.

**Abbreviations: IAA,** indole-3-acetic acid; **IBA,** indole-butyric acid; **NAA,** α-naphthalene acetic acid.

## Introduction

The rich biological diversity of the mangrove ecosystem in the tropical and subtropical inter-tidal regions of the world is an economic resource which has been widely and variously used by coastal people. The mangroves prevent encroachment by the sea by checking soil erosion and thereby stabilizing the shoreline. The organic matter and nutrient flow from the mangrove ecosystem, to a great extent, support the benthic populations of the sea. The Mahanadi Delta is known to have unique communities of plants comprising 62 of the 64 mangrove species distributed in India (Newbold, 1993). Most of these mangroves are threatened, largely by human intervention, resulting in a change in the tidal pattern and salinity gradient. Types of intervention include encroachment of the land for cultivation and shrimp culture, cattle grazing, over exploitation for fuel, timber, and fodder, and others (Das et al., 1994).

*Heritiera fomes* and *Heritiera littoralis,* commonly found along the tidal creeks and channels of the coastal swamps (Banerjee, 1990) regenerate naturally through seeds, but depletion of growing stock, post-dispersal predation of seeds by crabs (Robertson et al., 1990), sporadic flowering, and poor seed set in the remnant mangrove forests necessitate mass vegetative propagation, an alternative to seed propagation, for perpetuation of the species and their re-establishment in the area (Hartmann and Kester, 1989).

The present studies deal with the effect of auxins (IAA, IBA & NAA) on rooting of the pre-girdled stem cuttings and air-layers and the biochemical changes during initiation and development of roots in *H. fomes* and *H. littoralis*.

## Materials and Methods

One-season-old healthy shoots (semi-hard wood) of *Heritiera fomes* and *H. littoralis,* growing in the remnant mangrove forests of the Mahanadi Delta, were girdled 25 cm from the growing tip. After 15 days, the shoots were cut below the girdled point with 2–4 leaves and dipped in tap water. The shoots (2–3 cm from base) were then dipped for 30 sec in IAA, IBA, and NAA solutions alone at 1,000, 2,500, 5,000 ppm concentrations and in combinations of IAA & IBA, NAA & IBA, NAA & IAA at the above concentrations. For air-layering, plant growth regulators in talc were smeared above the girdled portion of the twig,

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covered with moist moss, and wrapped with white polyethylene film. The cuttings and the one month old air-layers were then planted in pots containing coarse sand and kept in a mist chamber at 30±2°C & 85±5% RH. There were 37 treatments in all (including the control in each species), and the treatments were replicated 20 times. Five cuttings were scored for rooting response after 30 days of treatment. The rooted cuttings were established in the soil. ANOVA (Sokal and Rohlf, 1973) following Duncan's multiple range test (Harter, 1960) was performed on the percentage of cuttings rooted, root number, and root length among the treatments of each species.

Subsequently in a separate experiment, the best treatments of each species (IBA 2500 + NAA 5000 for air-layers of *H. littoralis,* IBA 5000 + NAA 1000 for air-layer of *H. fomes* and IBA 5000 + NAA 2500 for the cuttings of both the species) were subjected to biochemical analysis. The basal girdled portions (2–3 cm) of both the treated and the untreated cuttings and air-layers from each species were collected at three different stages, i.e. at the time of girdling (Control), 15 days after girdling ($S_0$), 10 days after treatment ($S_1$), and 30 days after treatment ($S_2$) during rooting. To estimate the reducing sugars, fresh samples were crushed in warm ethanol (80%) on a mortar pestle and centrifuged at 5,000 rpm for 30 min. Supernatants were mixed with copper reagents following the method of Somogyi (1945) and boiled for 20 min in a water bath. After cooling of the mixture, arsenomolybdate color reagent was mixed in the reaction mixture and kept at room temperature for 15 min. Spectrophotometric readings were taken at 500 nm, and sugar content was determined using a standard curve prepared against pure glucose. Non-reducing sugars were estimated following titration with 0.005 N sodium thiosulphate prior to hydrolysis, and the sample materials were subsequently neutralized by adding concentrated sulphuric acid and sodium bicarbonate, respectively. Reserve polysaccharides were estimated by the same method after removing the soluble sugars and hydrolysing the residue in 6N HCl in a 100°C water bath for 3 h. The total carbohydrate content was estimated by Somogyi's (1945) modification of Nelson's (1944) method using anthrone (0.2%) as reagent. Spectrophotometric readings were taken at 620 nm. To estimate buffer soluble protein nitrogen, samples were extracted in 0.0625 M tris-glycine buffer at pH 6.8 and boiled with 10% trichloroacetic acid. The precipitate obtained after centrifugation was estimated for nitrogen with a conventional microkjeldahl apparatus following the method of Pregl (1930). Total nitrogen was obtained from the precipitate fraction of the protein in addition to the residual supernatant protein fraction of the sample. Quantitative estimation of polyphenol was carried out following a modified method of Swain and Hills (1959) using gallic acid as a standard. Spectrophotometric observations of polyphenols were taken at 515 nm in a Jasco UVIDEC-650 double beam spectrophotometer.

## Results

### *Induction and Development of Roots*

Data on adventitious root formation in *H. fomes* and *H. littoralis* revealed that the best rooting response was in the IBA treated cuttings followed by the cuttings treated with NAA and the IAA (data not shown). The cuttings treated together with any two of the three auxins showed better rooting compared to those treated with IAA, IBA, or NAA alone. The maximum number of roots per pre-girdled cutting of *H. fomes* and *H. littoralis* were 22.01 and 13.21, respectively, due to the treatment with 5,000 ppm IBA in combination with 2,500 ppm NAA. The interactions of IBA and NAA induced more roots per pre-girdled cutting than the air-layer in each of the species

**Table 1.** Effect of auxin interactions on mean root number, root length (cm), and rooting percentage in the cuttings and air-layers of *H. fomes* and *H. littoralis.*

| Treatments (ppm) | Species | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *H. fomes* | | | | | | *H. littoralis* | | | | | |
| | Cutting | | | Air-layer | | | Cutting | | | Air-layer | | |
| | RN | RL | R% | RN | RL | R% | RN | RL | R% | RN | RL | R% |
| Control | – | – | – | – | – | – | – | – | – | – | – | – |
| IBA 1000 | 3.5ns | 2.5ns | 46.6* | 2.8ns | 2.4ns | 60.0* | 2.2ns | 2.0ns | 25.0ns | 2.0ns | 2.0ns | 25.0ns |
| IBA 2500 | 4.8ns | 3.2* | 39.9* | 3.5ns | 2.2ns | 50.0* | 3.4ns | 2.5ns | 25.0ns | 2.6ns | 2.3ns | 33.3* |
| IBA 5000 | 12.8* | 4.5* | 85.8* | 6.2* | 3.1* | 75.5* | 7.4* | 3.1* | 50.0* | 3.5ns | 2.8* | 33.3* |
| NAA 1000 | 2.0ns | 2.1ns | 26.6ns | 2.0ns | 2.2ns | 25.0ns | 2.0ns | 2.2ns | 33.3* | 2.8ns | 2.4ns | 25.0ns |
| NAA 2500 | 9.0* | 5.1* | 46.6* | 4.8ns | 3.8* | 50.0* | 5.6ns | 3.0* | 25.0ns | 2.0ns | 2.8* | 26.6ns |
| NAA 5000 | 3.2ns | 2.8* | 33.3* | 2.5ns | 2.5ns | 25.5ns | 2.8ns | 2.1ns | 33.3* | 2.5ns | 2.6ns | 25.0ns |
| IBA 2500+NAA 5000 | 4.2ns | 2.6ns | 39.9* | 8.5* | 2.1ns | 73.3* | 3.0ns | 2.1ns | 40.0* | 8.2* | 3.4* | 60.0* |
| IBA 5000+NAA 1000 | 14.5* | 3.8* | 46.6* | 15.6* | 3.4* | 80.0* | 10.5* | 3.4* | 60.0* | 7.0* | 2.5ns | 40.0* |
| IBA 5000+NAA 2500 | 22.0* | 3.1* | 85.8* | 12.5* | 3.1* | 80.0* | 13.2* | 3.0* | 73.3* | – | – | – |

RN=Root number; RL=Root length; R%=Rooting percentage; ns=Not significant; *=Significant at 5% level.

**Table 2.** ANOVA of percentage of rooting root number and root length per cutting of *H. fomes* and *H. littoralis.*

| Source | DF | SS | MS | F |
|---|---|---|---|---|
| Percentage of rooting | | | | |
| Between treatments | 36 | 48500.00 | 1350.00 | 2.28** |
| Between species | 1 | 71600.00 | 71600.00 | 30.36** |
| Error | 36 | 84900.00 | 2358.33 | – |
| CD = 30.10 at 5% level. | | | | |
| Root number per cutting | | | | |
| Between treatments | 36 | 1970.00 | 54.60 | 2.31** |
| Between species | 1 | 3840.00 | 3840.00 | 40.66** |
| Error | 36 | 3400.00 | 94.44 | – |
| CD = 6.02 at 5% level. | | | | |
| Root length per cutting | | | | |
| Between treatments | 36 | 237.00 | 6.59 | 1.22** |
| Between species | 1 | 312.00 | 312.00 | 14.38** |
| Error | 36 | 781.00 | 21.69 | – |
| CD = 2.80 at 5% level. | | | | |

** = Highly significant at 1% level.

(Table 1). The root number was significantly lower in the air-layer of *H. littoralis* (8.2) than *H. fomes* (15.6). The significant variation in root number and root length was noted among the treatments, and the variation was species specific (Table 2). The number of cuttings that rooted also varied significantly depending upon the treatments, the species, and the method of propagation. The percentage of cuttings responding to root induction and root development varied with the treatments and the species (Tables 1 and 2). The cuttings treated with IAA, or IBA, or NAA alone produced fewer roots than those treated in combination with another auxin.

### *Biochemical Changes During Rooting*

Analysis of the total sugars, polysaccharides, carbohydrates, proteins, and polyphenols during initiation and emergence of roots showed an increase in the total sugar content in the pre-girdled shoots before auxin treatment, and a gradual depletion of sugars was noted during root development following initiation of root primordia (Figures 1–2). A gradual increase in the percentage of reducing sugars was recorded as the initiation and emergence of root took place in all the species. However, no changes were noted in the case of non-reducing sugars. There was significant accumulation of total carbohydrates during the $S_0$ phase in the pre-girdled cuttings compared to the air-layers that declined gradually in subsequent stages of root development (Figures 1–2). A similar trend of accumulation and reduction in the level of polysaccharides was observed in different species. Total nitrogen content increased at the root initiation stage and decreased during the subsequent stages of root development. The highest C/N ratio was observed at the $S_0$ stage, and it declined thereafter during the $S_1$ to $S_2$ stages (i.e. from root initiation to root emergence) in all species. The percentage of polyphenols also varied significantly at different stages, gradually decreasing in the $S_2$ and $S_1$ stages.

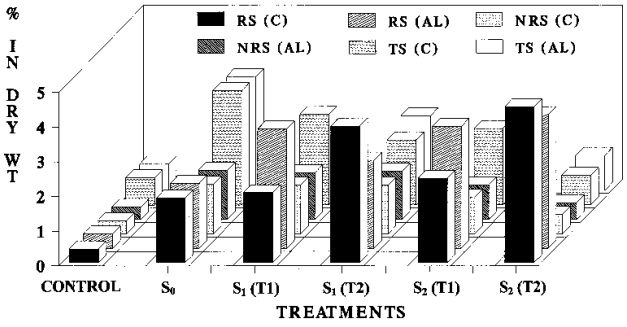


**Figure 1.** Synergistic effects of auxins on sugars during root development of mangrove stem cuttings of *Heritiera fomes.* RS (C) = reducing sugar (cutting); RS (AL) = reducing sugar (air-layer); NRS (C) = non-reducing sugar (cutting); NRS (AL) = non-reducing sugar (air layer); $S_0$ = 15 days after girdling; $S_1$ = 10 days after treatment; $S_2$ = 30 days after treatment. T1 = IBA 2,500 ppm + NAA 5,000 ppm; T2 = IBA 5,000 ppm + NAA 2,500 ppm

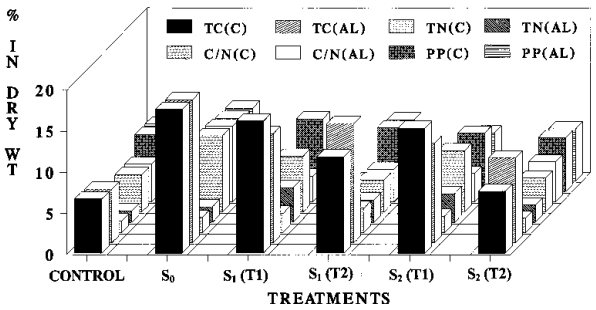


**Figure 2.** Synergistic effects of auxins on total carbohydrate, total nitrogen, C/N ratio, polyphenols and polysaccharide during root development of mangrove stem cuttings of *Heritiera fomes.* TC (C) total carbohydrate (cutting); TC (AL) = total carbohydrate (air layer); TN (C) = total nitrogen (cutting); TN (AL) = total nitrogen (air-layer); C/N (C) = carbon-nitrogen ratio (cutting). C/N (AL) = carbon-nitrogen ratio (air layer); PP (C) = polyphenol (cutting); PP (AL) = polyphenol (air-layer).

## Discussion

The results clearly indicate that, among the three auxins, IBA (5,000 ppm) induced the best rooting. Significantly greater rooting response of the pre-girdled cuttings, however, was obtained when any two of the auxins were applied together. The cuttings treated with IBA and NAA developed the maximum number of roots per cutting in both *H. fomes* and *H. littoralis*. The rooting in the air-layers was better in *H. fomes* than *H. littoralis*. The rooting in cuttings of another mangrove *Avicennia alba* showed a similar response (Reddy et al., 1994). The varied responses of the cuttings suggest that the endogenous promoters and inhibitors greatly determine the nature of interaction with the exogenously-applied auxins (Roy et al., 1972). Girdling before the preparation of cuttings or the air-layers, invariably had better effects on root initiation in both species of mangrove, perhaps due to the increase in rooting cofactors above the girdle (Jauhari and Rahaman, 1959; Basu et al., 1966; Stoitz and Hess, 1966). The differential root-regenerating capacities of different auxins individually or in combination, might depend on their respective capacities to synthesize proteins essential for the regeneration and elongation of roots (Ghosh and Basu, 1974).

The biochemical studies on root initiation clearly indicate that the root inducing effects of the three auxins were not related to the hydrolysis of the polysaccharide reserve during the period of root emergence. Regeneration took place mainly through utilization of soluble sugars. The utilization of reserve polysaccharides was, however, noted under all the auxin combinations. The non-reducing sugars in particular were utilized to a greater extent at the root development phase, and the utilization was greater in cuttings treated with NAA, followed by IBA and IAA treated cuttings. The results therefore confirm the earlier observations that hydrolytic activities assumed some importance only during root development to meet the needs of the developing roots (Ghosh and Basu, 1974; Basu and Pal, 1966). Such activities may be looked upon as aftereffects rather than direct causes of root initiation under influence of the root promoting hormones. Total nitrogen content showed an increase at $S_1$ but subsequently fell at the $S_2$ phase. A synthesis of proteins took place during the period from $S_0$ to $S_1$; at $S_1$, protein-nitrogen was greater in NAA and IBA combinations than in IAA and IBA combinations, but the concentration again fell at the $S_2$ stage.

Furthermore, the decrease of the C/N ratio at the $S_1$ stage promoted root initiation and gradual normalization at the $S_2$ phase and enhanced the root initiation and elongation in the treated cuttings. The biogenesis of rooting cofactors which synergize auxin-induced rooting might have been promoted by the favorable nutritional status of the stock plants; however, the relationship between the root inducing potentiality of auxins with protein synthesis is considered to be of greater significance. The accumulation of endogenous root promoting substances above the girdled portion, the exogenous application of different concentrations and combinations of auxins such as IBA, NAA, and IAA at the same site, and a higher concentration of carbohydrate followed by low nitrogen seemed to act synergistically towards the regeneration of roots (Hartmann and Kester, 1989) in the mangrove trees. The differential root-regenerating capacities of different auxins may, therefore, depend on their respective capacities to regulate the synthesis of proteins essential for the regeneration of roots. The large number of root-primordia induced by the root promoting auxins acted as effective metabolic sinks, drawing on the nutritional reserves of the cutting for their growth and development.

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