Metabolic changes during adventitious root primordium development in Tectona grandis Linn. f. cuttings as affected by age of donor plants and auxin (IBA and NAA) treatments

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Metabolic changes during adventitious root primordium development in Tectona grandis Linn. f. (teak) cuttings as affected by age of donor plants and auxin (IBA and NAA) treatment

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Abstract   Aging of the donor tree decreased adventitious root formation in shoot cuttings of Tectona grandis Linn. f. (teak). Exogenous application of auxins, i.e., α-naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA) has a significant positive effect on the percentage of rooting. The maximum percent rooting was obtained with 4,000 ppm IBA as compared to other treatment. Significant increase in root number was recorded in shoot cuttings treated with 4,000 ppm NAA. The overall rooting response was better in the treatment with IBA rather than with NAA. Further periodic samples (0, 10, 20, and 30 days) were taken to assess the total soluble sugar, starch, protein, and peroxidase (PER) activity in the rooting zone of shoot cuttings of teak during adventitious root formation. Application NAA and IBA to shoot cuttings resulted in an increase in the level of total soluble sugar, starch, protein, and PER-activity in the rooting zone. The stored carbohydrates were utilized during adventitious root formation. Hence, total soluble sugar and starch contents of cuttings, irrespective of age of donor plants, decreased with the passage of time in cuttings planted for rooting. Significant fluctuations were observed in the protein content of cuttings during the time of root induction. There was an increase in the protein content with the passage of time from the day of planting up to its 20th day, followed by a sharp decline in the protein content of cuttings at the 30th day of planting, irrespective of the age of donor plants or the treatment of cuttings with auxins. Irrespective of donor plant age, PER-activity in the cuttings increased from the day of their planting for rooting up to the 20th day, and then declined at its 30th day of planting. It was interesting to note that PER-activity remained higher at all stages in the cuttings of 2-month-old seedlings which rooted profusely as compared to the cuttings of 15- and 30-year-old donor plants those rooted poorly. This study...
suggested that the exogenously applied NAA and IBA at different concentration seems to activate sugar metabolism for release of energy, protein and PER-activity which are necessary for cellular division and differentiation during adventitious root primordium initiation or development in the rooting zone of shoot cuttings.

**Keywords**  
Cuttings · Rooting response · Rooting zone · Total soluble sugar · Starch · Protein · Peroxidase activity · Auxins · Aging · *Tectona grandis*

**Introduction**

The response to exogenous application of auxins plays an important role in metabolic changes (viz., specific enzyme, carbohydrates, RNA, DNA, protein metabolism, etc.) during the initiation, emergence and development of root primordia in the cuttings rooting zone. Attempts have been made by a number of researchers to envisage the macromolecular changes during initiation and development of adventitious roots in different plant species in order to better understand the underlying physiology and biochemistry (Nanda et al. 1972; Nanda 1975; Bhattacharya and Nanda 1978a, b; Haissig 1986; Mato et al. 1988; Haissig and Davis 1994; Das et al. 1997; Hartmann et al. 1997; Druege et al. 2000; Husen and Pal 2001; Metaxas et al. 2004; Qaddoury and Amsaa 2004). Metabolic changes in the rooting zones of cuttings due to phytohormones/auxins are capable of either inhibiting or promoting adventitious root regeneration.

Adventitious root formation has been reported to be involved in the process of redifferentiation, in which predetermined cells switch from their morphogenetic path to act as mother cells for the root primordia initiation (Friedman et al. 1979; Aeschbacher et al. 1994). Once root primordia have been developed/initiated in cuttings, then considerable metabolic activity occurs as new root tissues appeared and the roots grow through and out of the surrounding stem tissues. Among these activities, the process of lignification in the cell wall, catalyzed by a particular peroxidase (PER), occurs during the rooting (Fukuda and Komamine 1982; Church and Galston, 1988; Bruce and West 1989; McDougall 1992; Sato et al. 1993). Auxins play an important role in mobilization of carbohydrates in leaves and upper stem, also increase transport to the rooting zone (Nanda et al. 1972; Altman and Wareing 1975; Andersen et al. 1975; Middleton et al. 1980; Haissig 1982, 1986; Veierskov et al. 1982). Altman and Wareing (1975) reported that indole acetic acid (IAA) promoted the rooting of cuttings because it increases sugar availability at the site of primordium development. An increase in the activity of hydrolyzing enzyme following auxin treatment has been reported by many workers (Nanda et al. 1967; Nanda and Anand 1970; Haissig 1986; Liu et al. 1998). Loss of carbohydrates from the rooting zone of cuttings indicates that sugars are utilized during the root growth (Husen and Pal 2001). The current rate of photosynthesis may also contribute and translocate sugar to the base of cuttings and thus play an important role in adventitious root formation of certain species (Davis and Potter 1981; Bakshi and Husen 2002). Changes in protein synthesis and RNA production were reported to be involved in adventitious root development. Moreover, an auxin treatment has also been shown to influence nucleic acid and protein metabolism during rooting (Haissig 1974).

The activities of enzymes in the rooting zone of cuttings may provide an easy, fast and reliable means of assessing cellular differentiation into roots. Molnar and
LaCroix (1972) reported that PER was the first enzyme whose activity increased during the initiation and development of roots in *Hydrangea* spp. cuttings. Gasper (1980) correlated an induction phase in rooting with a rise in total PER activity for the whole cuttings. Haissig (1986) has reviewed the involvement of PER in lignification, apart from its mediation of auxin levels in cuttings during rooting. Furthermore several researchers have reported that an auxin-induced change in PER and IAA oxidase occurs during the rooting processes (Gasper et al. 1985; Mato et al. 1988; Fett-Neto et al. 1992).

Loss of adventitious root regeneration potential in cuttings of forest trees with age is a common observation (Hartmann et al. 1997; Hamann 1998; Greenwood et al. 2001; Husen and Pal 2006). In teak (*Tectona grandis* Linn.f.) aging of donor trees suppressed rooting and sprouting of cuttings, but increased callus formation at the base of cuttings. It appears that the auxin requirement for causing and promoting rooting in teak cuttings increased with increasing age of donor plants. The higher auxin requirement for causing and promoting rooting in cuttings of older trees may be due to a decrease in the content on endogenous auxins or decreased sensitivity of aging tissues to rooting promoters and/or accumulation of inhibitory substances which inhibit rooting (Husen and Pal 2006).

Studies on the role of auxins in teak adventitious root formation showed maximum rooting response occurring within 35–45 days (Husen and Pal 2001, 2003a–d, 2006). Moreover, observations from 10th day onwards in the mist chamber revealed that cuttings (control/auxins treated) exhibited root primordium initiation/or emergence at the base. Variation in metabolic changes viz., total soluble sugar, starch, protein and PER activity during root initiation has not previously been studied in teak. Therefore, the present study was undertaken with the objective of investigating the rooting response and metabolic changes in the rooting zone of shoot cuttings of teak during adventitious root primordia development in the different age group of donors.

**Materials and methods**

**Plant materials**

Branches were collected from 2-month, 15- and 30-year-old plants of teak (clone FG11) growing in New Forest campus, Forest Research Institute, Dehra Dun, India. Also 2-month-old donor plants (seedlings) were raised in nursery beds using seeds collected from a teak (clone FG11) seed orchard. About 250 seedlings and 25 mature teak plants were used as donors for collection of cuttings. These were carefully selected for the uniformity of age, size and free from disease, insect pest, physiological disorder. Seedlings were maintained by regular watering and weeding. Complete protection was provided against diseases and insects by foliar spray with insecticides and fungicides, as and when required.

**Coppicing**

Branches of 15- and 30-year-old donor plants were pruned in the month of March 1999 to encourage bud sprouting and new shoot formation while in case of 2-month-old seedlings the main shoots were used for making cuttings.
Collection and preparation of cuttings

The new shoots which grew on the pruned branches were harvested (at that time shoots were about 2 months old) during the first week of May 1999 from 15- and 30-year-old donor plants. Simultaneously, the main shoots of 2-month-old seedlings were also harvested from the nursery stock. For the rooting trial each shoot was made into a mono-nodal leafy soft-wood cuttings. Each nodal shoot cutting retained about 25.0 cm² leaf area per cutting and the total length of the cutting was about 4.0 cm. The cuttings collected from donor plants of different ages were prepared and kept separately.

Treatments

The main treatments were (a) age of donor plants and (b) auxin treatment. As described above the shoots were collected from three different age groups of donor plants. Two different auxins were used at two different concentration. The auxins and their concentration used were α-naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA) at 2,000 and 4,000 ppm. The auxins were applied in their powder formulation (auxins × talcum powder), which also contained 0.05% fungicide, i.e., Bavastin. Other sets of cuttings from each age group were taken and treated with talcum powder containing Bavastin as controls.

Planting

After treatment the cuttings were planted into plastic trays, which were filled with sterilized vermiculite (pH 7.0). The vermiculite was presoaked in tap water for 24 h before filling the trays. The cuttings were planted immediately after auxin treatment and kept inside a mist chamber where the relative humidity was maintained at 85 ± 2% with maximum and minimum day-night temperature at 32 ± 1 and 26 ± 1°C, respectively.

Observation on rooting

After 30 days cuttings from each treatment were carefully removed from the rooting medium and observations were recorded on percent rooting and number of roots grown per cutting.

Collection of samples

The cuttings rooting zone (1–2 cm) from every age group of donor plants (2 months, 15 and 30 years old) treated with 2,000 and 4,000 ppm of NAA and IBA along with control cuttings were sampled at 0, 10, 20, and 30 days for the estimation of total soluble sugar, starch, protein and PER-activity.

Analysis metabolic changes

In order to estimate the metabolic changes viz., total soluble sugar, starch, protein and PER-activity in stem segments composite samples were taken. There were five replications with ten shoot cuttings per replicate. Each replicate contained five
composite samples such that two stem segments clubbed together. Extracts for total soluble sugar of stem tissues were prepared as per the method described by Sawhney et al. (1968). However, total soluble sugar content was estimated by a phenol-sulfuric-acid method (Dubois et al. 1956). The pellet left behind after the extraction of total soluble sugar was hydrolyzed with 52% perchloric acid (w/v) and centrifuged at 5,000 rpm. Then the supernatant was used for starch estimation following a phenol-sulfuric-acid method and the conversion factor 0.9 was used to derive the volume of starch (in mg g\(^{-1}\) DW). The protein was measured in phosphate buffer extract using the Perkin Elmer UV/VIS spectrophotometer Lamda 25 at 595 nm after reaction with trichloroacetic acid (TCA) and the Bradford reagent (Bradford 1976). For PER enzyme activity, fresh tissue (100 mg) from the rooting zone of cutting was homogenized in a chilled mortar pestle in 4.0 ml ice-cold 0.1 M phosphate buffer (pH 6.1) containing 30 mg of insoluble polyvinyle pyrivate (PVP) and 15.0 mg sodium ascorbate. The homogenate was filtered through four layers of muslin cloth and centrifuged at 12,000 rpm for 10 min at 4°C. The supernatant was used for the peroxide assay. The assay mixture contained 0.1 M phosphate buffer (pH 6.1), 4.0 mM guaiacol as donor, 3.0 mM \(\text{H}_2\text{O}_2\) as substrate, and 0.4 ml crude enzyme extract. The total reaction volume was 1.2 ml. The optical density was measured at 420 nm using a Perkin Elmer UV/VIS spectrophotometer Lamda 2S. The enzyme activity was expressed as the rate of optical density change min\(^{-1}\) mg\(^{-1}\) protein (Barnett 1974).

Statistical analysis

A completely randomized factorial design (Panse and Sukhatme 1967) was used with five replications (ten shoot cuttings per replicate) and three factors, i.e., age of donor plants (2-month, 15- and 30-year-old donors), auxin treatment (0, 2,000, and 4,000 ppm of NAA and IBA) and day of sampling (0, 10, 20, and 30 days). Statistical analysis was conducted using Statistical Package for Social Sciences (SPSS). In the analysis of variance (ANOVA) for studied parameters, the mean values of each replication were estimated. For the comparison of different means of different treatment; the critical differences (CD) were calculated based on the Student \(t\)-test at the \(P \leq 0.05\) level.

Results

Rooting response

Formation of adventitious root at the base of stem cuttings of teak declined with the age of donor plants. Exogenous application of 4,000 ppm IBA induced higher rooting percentage, while the maximum number of roots per cutting was observed by 4,000 ppm NAA (Figs. 1, 2).

Total soluble sugar

Significant variation was observed in total soluble sugar content in the rooting zone of cuttings at the \(P \leq 0.01\) level caused by the differences in age of donor plants, auxin treatment and the day of sampling (Tables 1, 2). A decrease in the content of
total soluble sugar was observed with the increase in age of donor plants; higher total soluble sugar content (111.80 mg g\(^{-1}\) DW) was recorded in cuttings of 2-month-old donor plants followed by those of 15-year-old donors (104.13 mg g\(^{-1}\) DW) and the lowest (94.66 mg g\(^{-1}\) DW) in cuttings of 30-year-old donor plants (Table 1). NAA and IBA treated cuttings exhibited higher total soluble sugar content than the control cuttings (Table 1). Initial total soluble sugar content was high and decreased in cuttings after planting; the maximum (126.30 mg g\(^{-1}\) DW) total soluble sugar content was observed at the time when cuttings were planted for rooting, i.e., at the 0 day while the minimum values (52.89 mg g\(^{-1}\) DW) occurred at the 30 days after planting (Table 1). The variation in total soluble sugar content due to the two way interaction effects of age of donor plants with day of sampling were significant at the \( P \leq 0.05 \) level (Table 2). Further the interactive effects between auxin treatment and day of sampling were also significant at the \( P \leq 0.05 \) level (Table 2). The two factor interaction (auxin treatment and age of donor plants) and the three factor interaction (age of donor plants, day of sampling and auxin treatment) were significant at the \( P \leq 0.05 \) level (Table 2).
interaction (auxin treatment, age of donor plants and the day of sampling) were not significant even at the $P \leq 0.05$ level. The combined effect of age of donor plants and day of sampling showed a maximum (139.12 mg g$^{-1}$ DW) total soluble sugar content in cuttings from 2-month-old donor plants on day 0 and a minimum (51.86 mg g$^{-1}$ DW) in the cuttings from the same age of donor plants at the 30th day from planting (Fig. 3a). However, the interactive effects of auxin treatment and day of sampling

<table>
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<th>df</th>
<th>Mean sum of square</th>
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<td></td>
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Critical differences at $P \leq 0.05$ level

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<tr>
<td>$T \times A \times D$</td>
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NS non-significant

* and ** reflects significant at $P \leq 0.05$ and $P \leq 0.01$ level, respectively
showed a maximum (126.65 mg g⁻¹ DW) total soluble sugar content in cuttings treated with 2,000 ppm NAA at the day 10 and a minimum (50.81 mg g⁻¹ DW) in cuttings treated with 2,000 ppm NAA at the day 30 (Fig. 4a).

Fig. 3 Changes in content of a total soluble sugar, b starch, c protein, and d peroxidase activity in rooting zone of teak stem cuttings as affected by interaction of age of donor plants and day of sampling
Starch

Significant variations in starch content of cuttings were observed at the \( P \leq 0.01 \) level for the auxin treatment, age of donor plants and day of sampling (Table 1, 2). A decrease in starch content was found as age of donor plants increased; the highest starch content (100.62 mg g\(^{-1}\) DW) was observed in the rooting zone of cuttings taken from 2-month-old donor plants, followed by those taken from 15-year-old donors (97.99 mg g\(^{-1}\) DW) while the lowest (88.70 mg g\(^{-1}\) DW) was observed in cuttings from 30-year-old donor plants (Table 1). Starch content of auxin-treated cuttings was higher than that of control cuttings (Table 1). Starch content decreased in all cuttings with the passage of time; the maximum starch content was observed at the time of planting, i.e., at the day 0 (118.03 mg g\(^{-1}\) DW) while the minimum (49.60 mg g\(^{-1}\) DW) occurred at the day 30 (Table 1). The interaction between age of donor plants and day of sampling caused a significant variation in starch content of cuttings at the \( P \leq 0.01 \) level (Table 2); the maximum (125.21 mg g\(^{-1}\) DW) starch content was recorded at the day 0 in cuttings of 2-month-old donor plants while the minimum (46.67 mg g\(^{-1}\) DW) occurred at the day 30 in cuttings from 2-month-old donor plants (Fig. 3b). The data on interactive effects of auxin treatment and day of sampling were also significant at the \( P \leq 0.05 \) level (Table 2); the maximum (118.03 mg g\(^{-1}\) DW) starch content occurred at the 0 day while the minimum (47.73 mg g\(^{-1}\) DW) was observed at the day 30 in cuttings treated with 2,000 ppm NAA (Fig. 4b). The two factor interaction (auxin treatment with age of donor plants) and three (auxin treatment, age of donor plants and day of sampling) factor interactions effects were not significant even at the \( P \leq 0.05 \) level (Table 2).

Protein

Individually auxin treatment, age of donor plants and day of sampling significantly influenced the protein content at the \( P \leq 0.01 \) level (Table 1, 2). The maximum protein content was recorded in cuttings taken from 30-year-old donor plants (64.64 mg g\(^{-1}\) FW) followed by those from 15-year-old ones (51.70 mg g\(^{-1}\) FW) and the minimum (45.09 mg g\(^{-1}\) FW) for cuttings taken from 2-month-old donor plants (Table 1). The control cuttings exhibited minimum (51.62 mg g\(^{-1}\) FW) protein content in the rooting zone. Treatment with NAA or IBA caused an increase in the content of protein (Table 1). The effectiveness of auxins did not vary significantly with the concentration or type of auxin. The protein content increased from the days 0 to 20 but decreased at the day 30 (Table 1). The two factor interaction effect between age of donor plants and day of sampling showed significant variation at the \( P \leq 0.05 \) level (Table 2). The minimum value (37.91 mg g\(^{-1}\) FW) of protein content was recorded in cuttings of 2-month-old donor plants at the day 0 while the maximum (81.58 mg g\(^{-1}\) FW) occurred in cuttings of 30-year-old donor plants on the day 20 (Fig. 3c). Further the two factor interaction between auxin treatment and day of sampling also showed significant effect at the \( P \leq 0.05 \) level (Table 2). The highest level of protein content was recorded in auxin treated cuttings on the day 20, but there was a decline as compared to the protein content of control cuttings, i.e., higher than that of the auxin treated cuttings on the day 30 (Fig. 4c). However, the different auxin concentration treatment did not exhibit any significant effect on protein content in the rooting zone. The two (auxin treatment and age of donor plants) and three (auxin treatment, age of donor plants and day of sampling) factor interactions effects were not significant even at the \( P \leq 0.05 \) level (Table 2).
plants) and three (auxin treatment, age of donor plants and day of sampling) factor interaction effects were insignificant at the $P \leq 0.05$ level (Table 2).

Peroxidase activity

PER-activity was significantly influenced by auxin treatment, age of donor plants and day of sampling at the $P \leq 0.01$ level (Table 1, 2). PER-activity decreased as age
of donor plants increased; the maximum (0.16 min⁻¹ mg⁻¹ protein) PER-activity was recorded in cuttings from 2-month-old donor plants followed by those for 15-year (0.10 min⁻¹ mg⁻¹ protein) and the minimum (0.08 min⁻¹ mg⁻¹ protein) in cuttings of 30-year-old donor plants (Table 1). Further, the minimum (0.10 min⁻¹ mg⁻¹ protein) PER-activity was observed in control cuttings and the maximum activity (0.12 min⁻¹ mg⁻¹ protein) was observed in cuttings treated with 2,000 ppm IBA (Table 1). Maximum (0.21 min⁻¹ mg⁻¹ protein) PER-activity was recorded on the day 20. This was followed in decreasing order by day 10 (0.10 min⁻¹ mg⁻¹ protein) and the day 30 (0.07 min⁻¹ mg⁻¹ protein) and finally at the zero sampling day with a minimum value (0.06 min⁻¹ mg⁻¹ protein) (Table 1). The two factor interaction effect (age of donor plants and day of sampling) exhibited a significant variation at the $P \leq 0.01$ level (Table 2). The minimum (0.04 min⁻¹ mg⁻¹ protein) PER-activity was observed in 30-year-old donor plants at the sampling days 0 and 30 while the maximum activity (0.29 min⁻¹ mg⁻¹ protein) was recorded in cuttings of 2-month-old donor plants on the day 20 of sampling (Fig. 3d). The interaction between auxin treatment and day of sampling significantly affected PER-activity at the $P \leq 0.05$ level (Table 2). PER-activity was observed minimum (0.06 min⁻¹ mg⁻¹ protein) at the day 0, thereafter, it increased and reached a maximum (0.22 min⁻¹ mg⁻¹ protein) on the day 20 with auxin treated cuttings showing higher PER-activity than the control cuttings in the rooting zone (Fig. 4d). The two (auxin treatment and age of donor plants) and three (auxin treatment, age of donor plants and day of sampling) factor interaction effects exhibited insignificant variation at $P \leq 0.05$ level (Table 2).

**Discussion**

Adventitious root formation in teak stem cuttings declined with the age of donor plants; per cent rooting was higher in cuttings taken from 2-month-old seedlings than cuttings taken from 15- or 30-year-old trees. Decrease in the rooting potential of stem cuttings due to aging and maturity of donor plants has already been reported (Husen and Pal 2006). Further auxin treatment has a strong effect on the rooting response of stem cuttings. In the present study, treatment with 4,000 ppm IBA produced the highest rooting percentage while the maximum mean number of roots per cutting was produced by treatment with 4,000 ppm NAA. In the case of teak stem cuttings IBA was more effective than NAA (Husen and Pal 2006). The stimulatory effects of auxins on rooting of stem cuttings of several other plant species have been reported by many workers (Nanda 1970; Davis and Haissig 1994; Hartmann et al. 1997). However, the mechanism of this physiological response yet remains disputed.

The biochemical studies on root initiation clearly indicate that the root inducing effects of the treatment, i.e., auxins and age of donor plants, were related to the variation of total soluble sugar, starch, protein, and PER-activity. Our findings suggested that stored carbohydrates are utilized during adventitious root formation in shoot cuttings. Total soluble sugar and starch contents in rooting zone, irrespective of age of donor plants, decreased with the passage of time. For this decrease it has been suggested (Nanda 1970; Haissig and Davis 1994; Hartmann et al. 1997; Husen and Pal 2001) that stored carbohydrates, mainly sugar and starch, are mobilized by the activity of hydrolytic enzymes and translocated to the rooting zone of cuttings where they are utilized to provide the necessary energy for cellular
division and differentiation. Along with this a significant fluctuation in the protein content of shoot cuttings during the rooting was observed. There was an increase in protein content with the passage of time from the day of planting up to the day 20, with a sharp decline in the protein content of cuttings at the day 30 irrespective of the age of donor plants or the treatment of cuttings with auxins. A similar trend in protein during adventitious root regeneration in cuttings of several other species has also been reported (Nanda 1970; Rout et al. 1996). The peak value of protein content has generally been observed at the time of induction of root primordia and it has been related to increased synthesis of enzymatic proteins during the root regeneration process (Nanda 1970; Haissig 1986). These findings further show that increased PER-activity is associated with induction of root primordia and/or the growth of roots in shoot cuttings. Thus, irrespective of the age of donor plants, PER-activity in the cuttings increased from the day of their planting for rooting up to the day 20 of planting, and declined thereafter. It is interesting to note that PER-activity remained higher at all stages in the cuttings of 2-month-old seedlings which also rooted more profusely than the cuttings of 15- and 30-year-old donor plants (Husen and Pal 2006). An increased PER-activity concomitant with root induction in cuttings has also been reported in a number of plants by others (Haissig 1986; Bhattacharya 1988; Pythoud and Buchala 1989) and in *Cynara scolymus*, PER activity could be used as a rooting marker in efforts to improve rooting (Moncousin and Gaspar 1983).

Treatment of shoot cuttings with NAA or IBA increased levels of total soluble sugar and starch in the rooting zone. Numerous investigators have reported the same increase in sugar content in the rooting zone of cuttings caused by auxins which may be attributed to an increase in starch hydrolysis (Nanda 1970; Haissig 1974) and/or increased sugar transport towards rooting (Altman and Wareing 1975; Middleton et al. 1980; Haissig 1982). Moreover, auxin–carbohydrate interactions are also observed to be vital for rooting (Nanda et al. 1972; Anderson et al. 1975; Veierskov and Anderson 1982; Das et al. 1997). The levels of protein and PER-activity were also found to be increased due to NAA or IBA treatment. A marked increase in the activity of hydrolyzing enzymes concomitant with rooting following auxin treatment has been reported by many workers (Nanda et al. 1967; Nanda and Anand, 1970; Haissig 1986). Studies (Haissig 1986) have also shown that nucleic acid and protein synthesis are necessary for adventitious root formation. Nitrogen required during fresh growth of shoots mainly comes from protein and soluble nitrogen compounds in bark and wood (Suzuki and Kohno 1983). Soluble nitrogen in the form of inorganic and organic compounds may be redistributed to the rooting zone following auxin treatment (Haissig 1974). Further, Haissig (1986) reported that the naturally occurring auxin IAA and synthetic auxins affect adventitious rooting of woody stem cuttings. Thus, enhancing initiation of root primordia by stimulating de novo synthesis of specific enzymes, sugar availability through hydrolysis and translocation, amounts and redistribution of amide and amino nitrogen compounds, and promotion of cell wall extensibility.

In conclusion, aging of donor tree decreases rooting response. Auxin treatment (IBA or NAA) increased rooting performance in stem cuttings of all age groups of donor plants. The overall rooting response was best with 4,000 ppm IBA. Furthermore, variation in the rooting response due to exogenous application of auxins was also reflected in the metabolic changes and enzymatic activity during adventitious root formation in the rooting zone of teak shoot cuttings. Both total soluble sugar
and starch contents declined as rooting progressed. Treatment with IBA and NAA increased the levels of total soluble sugar, starch, protein and PER-activity. Aging of the donor tree decreased all parameters except protein content. Protein content and PER-activity increased after the day 10 (planting cuttings) and continued up to the day 20; after that both were found to have decreased at the day 30.

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