https://doi.org/10.26525/jtfs2022.34.2.149

RELATIONSHIP BETWEEN ENDOGENOUS AUXIN (INDOLE-3-ACETIC ACID) AND ADVENTITIOUS ROOTING IN *DALBERGIA* SPECIES OF DIFFERENT ROOTING ABILITY

Kumar P[1,] *, Chaturvedi A[2] & Vaishnav V[3]

[1]*Genetics and Tree Improvement Division, Tropical Forest Research Institute, PO-RFRC, Mandla Road, Jabalpur-M.P., 482021 India*
[2]*Department of Zoology, Indira Gandhi National Tribal University, Amarkantak – M.P., 484887 India*
[3]*Department of Forestry and Environmental Science, Manipur University, Canchipur, Imphal, Manipur, 795003 India*

**pramod_kt@rediffmail.com*

Submitted November 2020; accepted September 2021

A vegetative multiplication trial was conducted in two commercially important timber species with different rooting abilities from the genus *Dalbergia. Dalbergia latifolia,* globally known as 'Indian rosewood', is a premium-quality high priced Indian timber species. It is vulnerable in the forest and bears the constraint of poor adventitious rooting through branch cuttings, hence classified as a difficult-to-root (DR) species. On the other hand, *D. sissoo* is a multipurpose timber species of North India known for its guaranteed rooting, hence classified as an easy-to-root (ER) species. Among the commercially synthesised auxins viz. indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and 1-naphthalene acetic acid (NAA), and rooting synergists, thiamine and piperazine, 5.0 mM IAA resulted in the highest rooting percentage (12%) in the branch cuttings of the DR species. High genetic variation was observed among the accessions of the DR species on rooting ability. No significant correlation was obtained between the endogenous auxin (IAA) level and rooting ability in both DR and ER species. The results indicated that the adventitious rooting potential varies from tree to tree within a species and is significantly not dependent on their endogenous IAA level. The findings did not support the concept that endogenous auxins directly affect root formation. However, a more refined extraction procedure and assay, with advanced instrumentation, at different stages, can validate the possible role of IAA in adventitious rhizogenesis.

Keywords: Clonal forestry, difficult-to-root tree, rhizogenesis, mass multiplication

INTRODUCTION

The genetic improvement program for forest trees includes clonal forestry, through which the desirable traits of the elite trees (progeny-tested trees) are maintained in filial generations. Clonal forestry aided with macro-propagation technique plays a significant role in tree improvement and breeding (Nehra et al. 2005). The method is applied to maintain the genetic homogeneity among the ramets (clonal siblings from the same parent tree) to its ortet (parent tree of the ramets), maintaining desirable characters for commercial utilisation and conservation of forest resources. Clonal plantations of temperate poplar, and tropical and sub-tropical eucalypts and conifers were widely established in the 1970s and 1980s for increased productivity (Libby and Ahuja 1993, Rezende et al. 2013, Wu 2019). In traditional clonal forestry, the vegetative propagation is carried out through rooted cuttings for adventitious rooting. But the desirable success could be achieved only in horticultural fruit trees (Bisognin 2011). In forest trees, clonal multiplication is being practiced at various stages of development. Nevertheless, the adventitious root formation mechanism has not been fully understood in forest trees.

Plant roots generated from non-rooting tissues are termed adventitious roots. The adventitious rooting ability and rooting behavior of forest trees differ significantly. Some tree species with preformed or latent root initials on their branch cutting, e.g., willows and poplars, emerge root immediately on placing the branch cuttings in the proper soil and environment conditions favorable to its rooting and growth (Pijut et al. 2011). On the other hand, there are some species where root formation takes place as a wound response. In wound-induced adventitious root formation, the role of the plant hormone, auxin, is considered more crucial.

It regulates the division of parenchyma cells to synthesise callus in the place of the wound, and later, the adventitious root differentiate from the callus. Specifically, in branch or stem cuttings of trees, wound-induced rooting represents a significant kind of adventitious root formation mechanism. This mechanism has been discussed in detail by Hartmann et al. (2002). However, studies explaining the physiology and molecular genetics of adventitious root formation in forest trees are few. Most of the findings are confined to two factors significantly associated with successful rooting, i.e., first, the auxin regulation at various growth-phase, and second, the genetics of the parent plant material (Blakesley et al. 1991, Mullin et al. 1992).

Auxin is one of the plant growth regulators (PGR), also known as 'rooting hormone' because of its involvement in adventitious root differentiation and formation (Davis et al. 1988, Kevers et al. 1997). Auxin is known for its significant role in the interdependent physiological stages of the rooting process associated with changes in endogenous auxin concentrations (Heloir et al. 1996, Gasper et al. 1997, Nag et al. 2001). Woody cuttings represent a highly complex system in which endogenous levels of PGR transport, dormancy and storage, as well as inhibitory compounds influence adventitious root growth, by preconditioning treatments (Howard 1994, Wilson 1994). The role of endogenous PGR, other than auxin, as a signal to promote adventitious root formation has been extensively examined (Blakesley 1994). The adventitious root formation at the base of a stem cutting consists of different stages, influenced by extrinsic factors, e.g., bioclimatic variables, nutrient supply, site factors, etc., and intrinsic characteristics, e.g., genetic and phenology, as well as the physiology of the tree species. It has been characterised that the rooting ability of an ortet declines with maturity due to the regulation of PGR (Thorpe et al. 1991). It has also been considered that branch cuttings with a low rate of basipetal transport of endogenous auxin would be slow in root formation (Wilson 1994). Hence, the branch cuttings have been treated with commercially synthesised exogenous auxin to increase the basal free endogenous auxin, which stimulates the adventitious root formation in the cuttings (Ford et al. 2002, Quaddoury & Amssa 2004). Therefore, it is now a generalised practice for mass multiplication to collect the branch cuttings from juvenile trees, and to exogenously treat them with commercially synthesised auxins that promote adventitious rooting. For ease of multiplication, tree species have been categorised as 'easy-to-root' (ER) and 'difficult-to-root' (DR) species based on the rooting ability. Although the adventitious root formation is possible only through the activity of endogenous auxin and without the application of exogenous auxin in a model plant (Liu et al. 2014), it has not been studied in long-lived forest tree species.

Therefore, the current study investigated the relationship between endogenous auxin level and adventitious rooting percentage in two species of the *Dalbergia* (Fabaceae) with different rooting abilities, i.e., *D. latifolia* (DR) and *D. sissoo* (ER). *Dalbergia latifolia,* globally known as 'Indian rosewood' is a premium-quality high priced Indian timber species. It is vulnerable in the forest and bears poor adventitious root formation through branch cuttings, hence classified as a DR species. On the other hand, *D. sissoo* is a multipurpose timber species of North India, known for its guaranteed adventitious root formation, hence classified as an ER species. Due to the non-availability of a standardised treatment for *D. latifolia,* an experiment was conducted to test the efficacy among commercially synthesised auxins, IAA, IBA and NAA, and further used in the present study for a better exogenous treatment of shoot cuttings in *D. latifolia.*

MATERIALS AND METHODS

Plant sampling and preparation of cuttings

Shoot cuttings were sampled from 10 phenotypically superior trees from each species, *D. latifolia* and *D. sissoo,* established and grown in the Tropical Forest Research Institute, Jabalpur, India (N 23.099253, E 79.989649). Trees were selected from a natural population of mixed species, based on growth traits like straightness, clear bole height, branches at a climbing/ approachable height for collection of shoot cuttings, and no disease incidence. The samples were collected following the phenology of the species, during the period of new leaf flushing. It was assumed that the physiologically active growth period supports adventitious rooting. Detailed characteristics of the species are given in Table 1. Shoots were cut to prepare cuttings of

15 to 20 cm in length and were surface sterilised with 1% Bavistin (fungicide) before exogenous auxin treatment. The experiment was performed on 15 branch cuttings per tree in three replications, along with a control set. The branch cuttings of *D. latifolia* were planted in polythene bags containing a potting mixture of soil, sand and farm-yard manure (FYM) in a ratio of 1:2:1, and those of *D. sissoo* were planted in sand bed (Figure 1a,c,e). The polythene bags were kept in a partially shaded open environment during the experiment.

Selection of auxin suitable for adventitious rooting

Literature does not have a standardised exogenous treatment for adventitious rooting in *D. latifolia*. Therefore, an experiment was conducted in a simple randomised design with different plant growth regulators, IAA, IBA and NAA, and rooting synergists *viz.* thiamine and piperazine, to select the most suitable auxin for adventitious rooting in *D. latifolia*. The branch cuttings of *D. latifolia* were basal-dipped for four hours in 2.5 and 5.0 mM concentrations of the auxins mentioned above. As a model of ER species, the branch cuttings of *D. sissoo* were compared with DR species, *D. latifolia*. Rooting ability was determined by recording the number of cuttings (in percentage) with successful adventitious rooting on the total number of cuttings planted.

Estimation of endogenous auxin level

The endogenous auxin level in the branch cuttings was estimated after collection from the trees. The method by Stoessel & Venis (1970), with some modifications, was used to standardise a spectrophotometric method to estimate the quantity of endogenous IAA. One gram of tissues (from bark to pericycle) scratched from the basal region of the branch cuttings were grounded in liquid nitrogen (N_2), assuming equal loss in all samples due to photooxidation, and homogenised in 80% methanol (HPLC grade). The homogenate was filtered and the filtrate was treated with a saturated ammonium sulfate solution. Precipitated protein was filtered off and the pH of the supernatant was adjusted to 2.5. A clear solution was taken into a 60 ml separating funnel, and IAA was extracted in diethyl ether. The extraction process was repeated thrice. Ether layer containing IAA was collected, and the ether was evaporated at low temperature. Extracted IAA was dissolved in 96% ethanol. After evaporation of ethanol, 0.1 ml ice-cold solution of acetic anhydride and trifluoroacetic acid (TFA) in 1:1 v/v and 4.5 ml ice-cold Na_2CO_3 (5% w/v) was added. The absorbance of the solution was recorded at 449 nm wavelength using ultraviolet-visible (UV-Vis) double beam spectrophotometer. A standard curve for IAA was prepared using the IAA standard of known concentration, to quantify extracted endogenous IAA.

Assessment of adventitious rooting ability between species

The rooting ability of selected trees of the species was determined by a factorial experiment with randomisation of their branch cuttings. The experiment was conducted twice in May and June for *D. latifolia*. Branch cuttings of *D. latifolia* were surface sterilised with 1% Bavistin and treated with the most effective treatment of 5.0 mM IAA for four hours through basal-dip in the solution. Fifteen cuttings per tree along with the control set was maintained in three replications. Cuttings were planted in poly bags containing soil, sand and FYM in a ratio of 1:2:1, and maintained for 75 days in a partially shaded open environment at 35 ± 2 °C temperature and 60–70% humidity, by watering with a fine nozzle sprayer, four times during daytime. After 75 days, cuttings were sampled and rooting ability was recorded. The

Table 1 Characteristics of trees from *Dalbergia* species sampled for the investigation

Characteristics	*Dalbergia latifolia*	*Dalbergia sissoo*
Age	15–30 years	12–20 years
GBH (girth at breast height, 1.37 m)	56–106 cm	64–102 cm
Phenology (month of new leaf flushing)	May–June	February–March
Adventitious rooting ability	Difficult-to-root	Easy-to-root

experiment was conducted with ER species, *D. sissoo*, in March, at the time of new sprouts, following a successfully applied standard protocol given by Ansari et al. (1995) with modification in IAA concentration. The sterilised branch cuttings of three replications and control were treated with 0.5 mM IAA for 18 hours through basal dip in the solution. Due to the easy-rooting ability of the species, the adventitious roots were adequately developed and became strong enough to be recorded in 45 days.

Data analysis

Estimation of basic statistics, analysis of variance (ANOVA) and the correlation between endogenous IAA level and adventitious rooting ability were performed through SYSTAT programme (version 10).

RESULTS

The IAA resulted in the highest rooting percentage (12%) of exogenously applied auxins at 5.0 mM concentration in *D. latifolia* (Table 2). Therefore, in further experiments, to determine the relationship of endogenous auxin with rooting ability in the species, endogenous IAA was estimated in the branch cutting tissues.

In March, the experiment on *D. sissoo* resulted in adventitious rooting in all tree accessions of the species with rooting ability of 60.67 ± 23.61%, after 45 days of the establishment of branch cuttings treated with IAA (Figure 2a, 1f). The endogenous IAA level in the cuttings was 1.77 ± 1.30 μg g^{-1} fresh weights (Figure 2a). During May, among ten tree accessions, the branch cuttings from six tree accessions (60% of tree accessions) of *D. latifolia* resulted in adventitious rooting, with an average rooting ability of 11.33 ± 17.22 % among the cuttings treated with 5.0mM IAA (Figure 1b). Only two tree accessions resulted in profuse adventitious rooting (> 40%). The endogenous IAA ranged from a minimum of 1.83 μg g_{-1} fresh weight to a maximum of 5.40 μg g_{-1} fresh weight (Figure 2b). The above experiment on *D. latifolia* was repeated in June, and adventitious rooting was observed in 80% of the tree accessions with an average rooting ability of 9.33 ± 7.82 % among their cuttings (Figure 2c, 1d). The endogenous IAA level in the cuttings was 1.19 ± 0.85 μg g^{-1} fresh weight (Figure 2c).

A non-significant relationship was obtained between endogenous IAA level and rooting ability (%) in the branch cuttings of *D. sissoo* (Figure 3a) and *D. latifolia* (Figure 3b, 3c) in all the experiments.

DISCUSSION

Dalbergia latifolia and *D. sissoo* are endemic to India. These species have been harvested mainly for timber, and other multipurpose uses. *Dalbergia latifolia* has a peculiar timber quality with a straight trunk, attractive texture and high wood density that enhances its value in the furniture market. Unlike *D. latifolia*, *D. sissoo* does not possess high economic value quality timber and is utilised for furniture-making, fuel-wood and fodder. Both the species are highly preferred for agroforestry planting due to their nitrogen-fixing characteristics, thus enhancing soil fertility. Due to high commercial demand, these species have been over-exploited. Clonal multiplication of these species will help their conservation and support the agroforestry practices in the region. Earlier investigation has reported the successful multiplication of *D. sissoo* in clonal propagation

Table 2 Adventitious rooting (%) in the branch cuttings of *Dalbergia latifolia* treated with different auxins

Treatment	R_1		R_2		R_3		R_4		R_5		Mean	
	Callus (%)	Root (%)	Callus (%)	Root (%)	Callus (%)	Root (%)	Callus (%)	Root (%)	Callus (%)	Root (%)	Callus (%)	Root (%)
Control	10	-	35	5	35	5	60	-	50	-	38	02
IAA	75	-	55	40	50	20	55	-	30	-	53	12
IBA	70	-	55	45	30	10	25	-	45	-	45	11
NAA	15	-	60	10	80	-	50	-	20	-	45	02

R = replications (tree accessions), IAA = indole-3-acetic acid, IBA = indole-3-butyric acid, NAA = 1- naphthalene acetic acid

(a) (b) (c) (d) (e) (f)

Figure 1 Experiment on rooting ability of (a) *Dalbergia latifolia* in May, (c) *Dalbergia latifolia* in June, (e) *Dalbergia sissoo,* (b, d) rooted cuttings of *Dalbergia latifolia and* (f) rooted cuttings of *Dalbergia sissoo*

through branch cuttings (Ansari et al. 1995). Similar success could not be achieved in *D. latifolia* due to poor rooting constraints. Thus, in the present investigation, *D. sissoo* was taken as a model tree to compare the experimental results with *D. latifolia* in similar environmental conditions. Since these species are from the same genus, the effect of genetic differences would be minimal. Similar results were obtained in the present investigation, and within only 45 days of planting, profuse rooting (60.67 ± 23.61%) was recorded in *D. sissoo*. Hence its consideration as an ER species has been confirmed.

Among the commercially synthesised auxins applied exogenously to the branch cuttings, IAA (5.0 mM) was associated with the highest rooting percentage (12%) in *D. latifolia*. The IAA exists in plants in a natural form as an undissociated molecule at pH < 4.0 or anionic form at pH > 4.0. The ionised IAA exhibits basipetal polar transport through specific electrogenic carrier proteins (Lomax et al. 1995). Therefore, depleted lateral distribution is unavailable to the target cells of the pericyclic region (Jacobs & Gilbert 1983, Mitsuhashi-Kato et al. 1978). Dedifferentiation of cells to form adventitious roots depends upon local redistribution through the exogenous supply of auxin, due to discontinuation of vascularisation in shoot cuttings (Ansari et al. 2004). The local redistribution is achieved by moving undissociated IAA molecules as lipophiles across the plasma membrane through passive diffusion (Lomax et al. 1995). Exogenous auxin treatment to the base of cuttings enhances

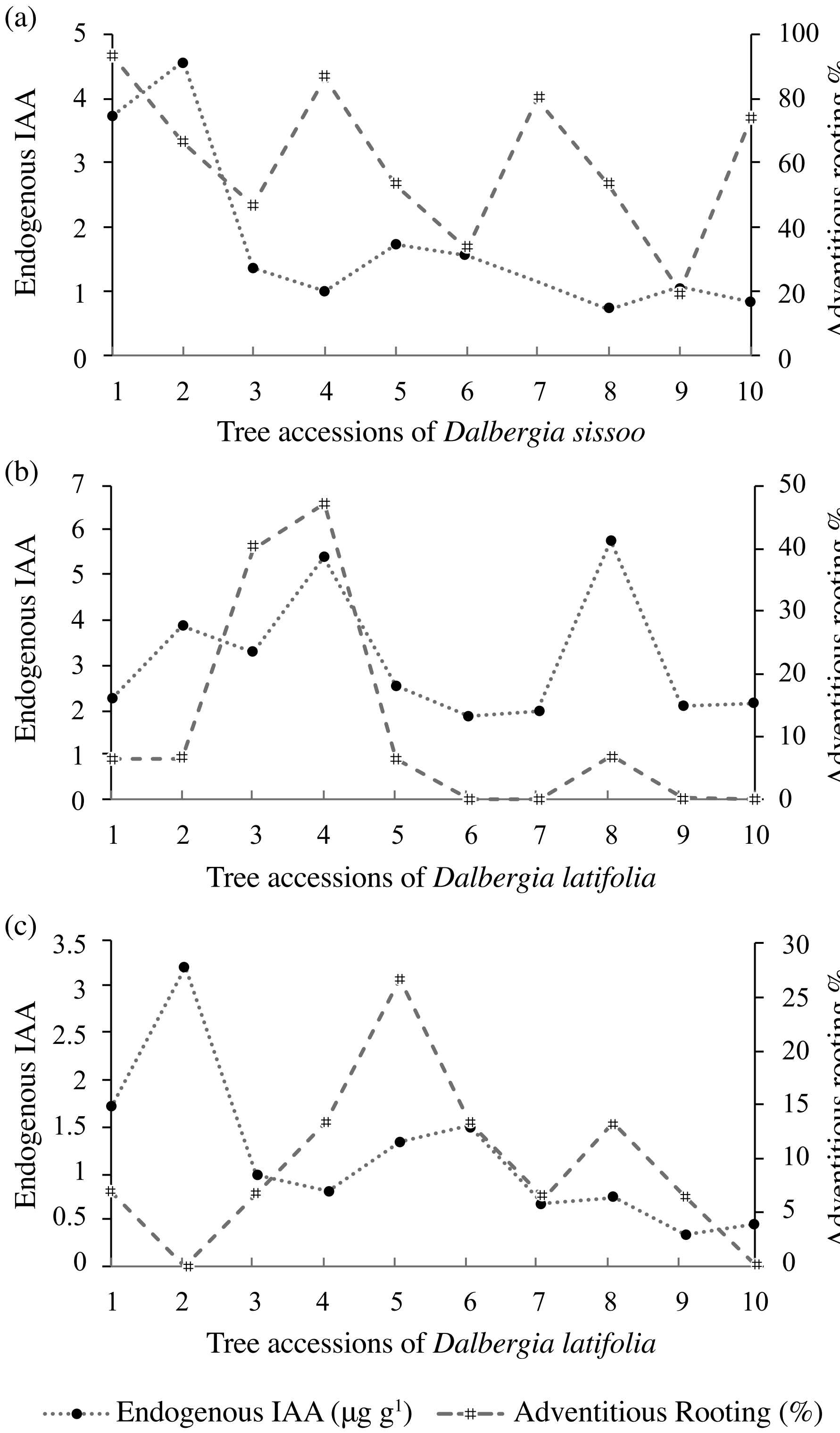

Figure 2 Endogenous IAA level and rooting ability among the tree accessions of *Dalbergia sissoo* estimated during March (a) and *Dalbergia latifolia* estimated during May (b) and June (c)

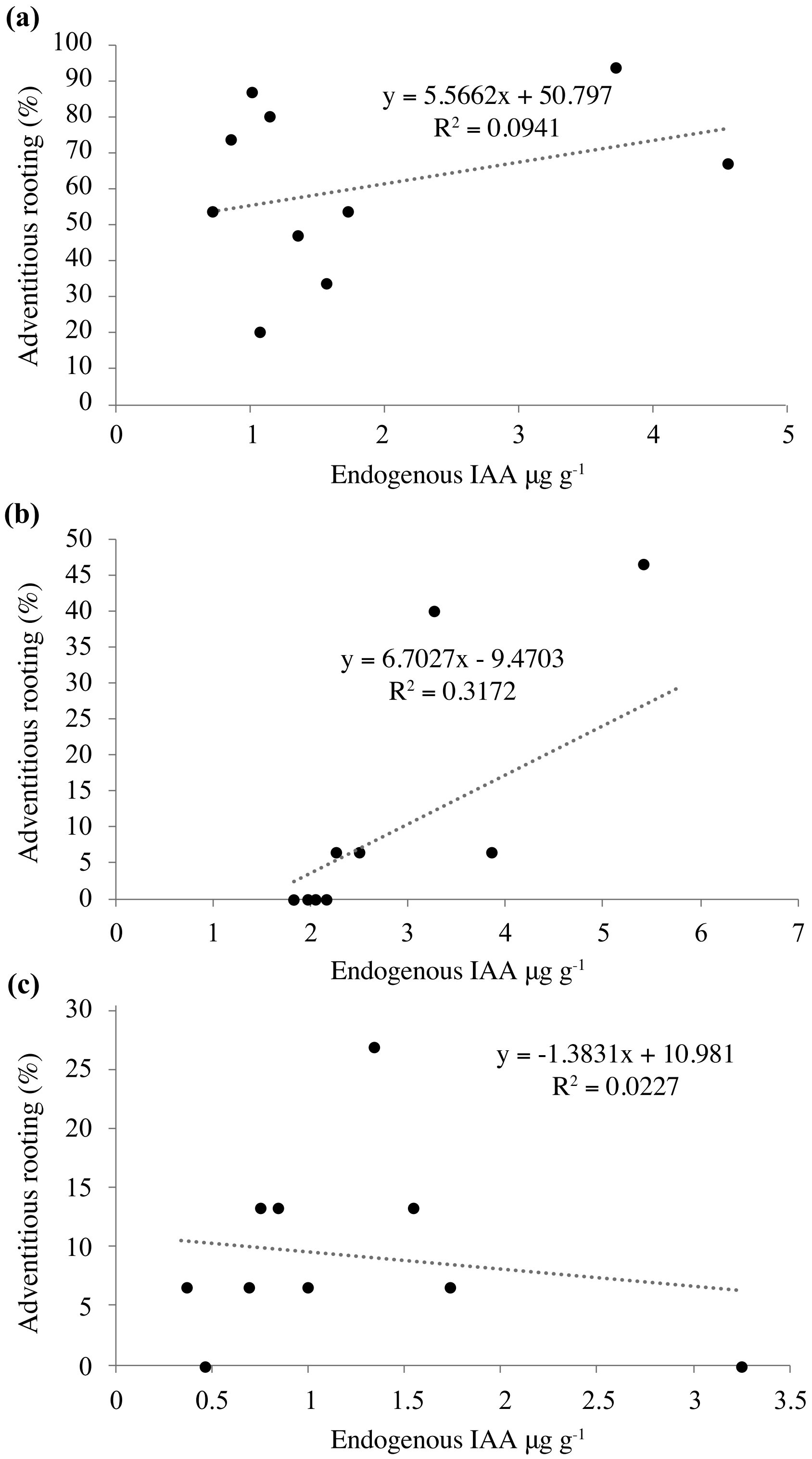

Figure 3 Relationship between endogenous IAA level and rooting ability (adventitious rooting, %) in tree accessions cutting from *Dalbergia sissoo* in March (a) and *Dalbergia latifolia* in May (b) and June (c)

the availability of IAA to overcome the loss due to oxidation of endogenous IAA (Fu et al. 2011). Negishi et al. (2014) found similar IAA levels in ER and DR clones of *Eucalyptus globulus,* but noted higher IAA-Asp level in DR genotype, and suggested that IAA conjugation plays an essential role in determining adventitious rooting capacity. More success in ER plants is due to the rapid auxin transport capacity (Nakhooda et al. 2011).

Genetic variation was observed in the phenology-associated rooting ability of tree accessions of *D. latifolia.* Inherent competency of meristamatic cells determines optimum regeneration potential at the active growth phase. In May, the branch cuttings from 60% of the tree accessions produced adventitious roots with a rooting ability of 11.33 ± 17.22%. In June, 80% of the tree accessions had adventitious root formation in their branch cuttings with reduced rooting ability (9.33 ± 7.82 %). In *D. latifolia,* during one month (from May to June), the endogenous auxin level in the branch cuttings slightly declined, and no significant correlation was obtained between endogenous IAA level and rooting ability (%), in the branch cuttings of both ER and DR species. The concentration of endogenous auxin increases during the initial hours after severance of cuttings, and decreases during adventitious root formation (Nag et al. 2001). Ford et al. (2002) reported decreased rooting capacity with a free IAA endogenous concentration reduction at the base of *Forsythia* cuttings. Cuttings collected during active physiological state resulted in optimum adventitious rhizogenesis (Gardea et al. 1994). Meristematic tissues, during this period, exhibit optimum potential for cell division. Carbohydrate storage and its mobilisation to roots have a significant role in cuttings' establishment (Haissig 1986,Veierskov 1988, Haissig et al. 1992). Histological origin of adventitious roots in different woody plants revealed anatomical variation among species regulating adventitious root formation (Lovell & White 1986). Differences in adventitious root formation between species or within the same species complex may be a consequence of genetic and anatomical variation among them (Friend et al. 1994). Nanda & Anand (1970) reported season-dependent induction of enzyme activity, and mobilisation of soluble sugars and endogenous auxin in *Populas nigra.*

No significant correlation resulted between endogenous auxin level and adventitious root formation. Auxin regulates plant growth in response to diverse developmental and environmental cues (Woodward & Bartel 2005, Teale et al. 2006). However, auxin is not needed during all the stages of adventitious root formation. Blakesley et al. (1991) describe the involvement of auxins in differentiation, cell division and growth of cells during the induction phase of adventitious root formation in ER and DR species, where the peak of IAA was observed soon after excision of cuttings, correlated with changes in peroxidase activity. Naturally synthesised IAA controls many plant growth and development processes by regulating gene activity, resulting in cell division, elongation and differentiation. Therefore, the regulation of hormone concentration is crucial for developing plants, which is regulated by biosynthesis, reversible inactivation, degradation and transport (Ludwig-Muller 2004).

Phenolic compounds may inhibit or promote adventitious rooting (Steffens & Rasmussen 2016). Peroxidase oxidizes free IAA, whereas auxin is partially protected in conjugated forms (Nordstrom et al. 1991, Nag et al. 2001). Over 95% of the total auxin in a plant can be found in the conjugated form, therefore, the formation of auxin conjugates is one of the critical regulatory pathways for the activation/inactivation of IAA (Hangarter & Good 1981, Cohen & Bandurski 1982, Bandurski et al. 1995). A significant difference in the rooting ability observed in the auxin treated and untreated cuttings also suggested the need for adequate amounts of exogenous IAA during the initial root induction phase (Quaddoury & Amssa, 2004).

CONCLUSION

Results indicates that the adventitious rooting potential varies from tree to tree within a species and is significantly not dependent on their endogenous IAA level. Findings did not support the concept that auxins have a direct role in root formation. Confirmation of findings on trees would require recent advancements in IAA extraction, and assay for wider acceptability. Enhancement of adventitious rooting by exogenous IAA treatment indicated that auxins are promoters. However, the role of endogenous IAA is indirect or supportive in various physiological processes responsible for adventitious rhizogenesis. Exogenous IAA treatment seemingly helped local redistribution and lateral supply of auxin to the target cells in the pericycle region for their organisation and growth into adventitious roots. Besides endogenous IAA, the adventitious rooting response of tissue is regulated by rooting inhibitors at the base of the cutting, sensitivity of cells to auxin signals, stored carbohydrates and minerals in cuttings, and lignification or sclerification of the stem. It was assumed that the inherent competency of tissues, at the time of severance and movement of IAA to the target cells, is crucial in determining rooting ability. The wood density might govern the timely distribution of IAA to the target cells at the time of root induction in hardwood species.

ACKNOWLEDGEMENTS

The authors are grateful to the Indian Council of Forestry Research and Education, Dehradun, India, for financial grant (ID: 131/TFRI-2008/ GEN-1(17).

REFERENCES

Ansari SA, Pramod Kumar & Mandal AK. 1995. Effect of position and age of cuttings and auxins on induction and growth of roots in *Dalbergia sissoo* Roxb. *The Indian Forester* 121: 201–206.

Ansari SA, Sanjay Singh & Abha Rani. 2004. Inorganic salts influence IAA ionization and adventitious rhizogenesis in *Pongamia pinnata*. *Journal of Plant Physiology* 161: 117–120.

Bandurski RS, Cohen JD, Solvin JP & Reinecke DM. 1995. Hormone biosynthesis and metabolism. Pp 35–57 in Davis PJ (ed) *Plant Hormone: Physiology, Biochemistry and Molecular Biology*. 2nd edition. Kluwer Academic Publishers, Boston.

Bisognin DA. 2011. Breeding vegetatively propagated horticultural crops. Crop breeding and applied biotechnology. https://doi.org/10.1590/S1984-70332011000500006.

Blakesley D, Weston GD & Hall JF. 1991. The role of endogenous auxin in root initiation. Part I. Evidence from studies on auxin application and analysis of endogenous levels. *Plant Growth Regulation*. 10: 341–354.

Blakesley D. 1994. Auxin metabolism and adventitious root initiation. Pp 143–154 in Davis TD & Haissig BE (eds) *Biology of Adventitious Root Formation*. Plenum Press, New York.

Cohen JD & Bandurski RS. 1982. The chemistry and physiology of the bound auxins. *Annual Review of Plant Physiology*. 33: 403–430.

Davis TD, Haissig BE & Sankhla N. 1988. Adventitious root formation in cuttings. Pp 315 in Dudley TR (ed) *Advances in Plant Sciences Series*. Volume II. Dioscorides Press, Portland, Oregon.

Ford YY, Bonham EC, Cameron RWF, Blake PS, Judd HL & Harrison-Murray RS. 2002. Adventitious rooting examining the role of auxin in an easy and a difficult-to-root plant. *Plant Growth Regulation*. 36: 149–159.

Friend AL, Coleman MD & Isebrands JG. 1994. Carbon allocation to root and shoot systems of woody plants. Pp 343 in Davis TD & Haissig BE (eds) *Biology of Adventitious Root Formation*. Plenum Press, London and New York.

Fu JH, Sun XH, Wang JD, Chu JF & Yan CY. 2011. Progress in quantitative analysis of plant hormones. *Chinese Science Bulletin* 56: 355–366.

Gardea AA, Moreno YM, Azarenko AN, Lombard PB, Daley IS & Criddle RS. 1994. Changes in metabolic properties of grape buds during development. *Journal of American Society of Horticulture Science* 119: 756–760.

Gasper T, Kevers C & Hausman JF. 1997. Indissociable chief factors in the inductive phase of adventitious rooting. Pp 55–63 in Altman A & Waisel Y (eds) *Biology of Root Formation and Development*. Plenum Press, New York.

Haissig BE. 1986. Metabolic processes in adventitious rooting of cuttings. Pp 144–189 in Jackson, MB (ed) *New Root Formation in Plants and Cuttings*. Martinus Nijhoff Publishers, Dordrecht.

Haissig BE, Davis TD & Riemenschneider DE. 1992. Researching the controls of adventitious rooting. *Physiologia Plantarum* 84: 310–317.

Hangarter RP & Good NE. 1981. Evidence that IAA conjugates are slow release sources of free IAA in plant tissues. *Plant Physiology*. 68: 1424–1427.

Hartmann HT, Kester DE, Davies JR & Geneve RL. 2002. *Plant Propagation: Principles and Practices*. 7th edition. Prentice Hall, Englewood Cliffs, New Jersey.

Heloir MC, Kevers C, Hausman J & Gasper T. 1996. Changes in the concentrations of auxins and polyamines during rooting of *in vitro* propagated walnut shoots. *Tree Physiology* 16: 515–519.

Howard BH. 1994. Manipulating rooting potential in stock plants before collecting cuttings. Pp 123–142 in Davis TD & Haissig BE (eds) *Biology of Root Formation*. Plenum Press, New York.

Jacobs M & Gilbert SF. 1983. Basal localization of the presumptive auxin transport carrier in pea stems. *Science* 220: 1297–1300.

Kevers C, Hausman JF, Fairre-Rampant O, Evers D & Gasper T. 1997. Hormonal control of adventitious rooting: progress and questions. *Journal of Applied Botany* 71: 71–79.

Libby WJ & Ahuja MR. 1993. Clonal forestry. Pp 1–8 in Ahuja MR & Libby WJ (eds) *Clonal Forestry II: Conservation and Application*. Springer, New York.

Liu J, Sheng L, Xu Y, Li J, Yang Z, Huang H & Xu L. 2014. WOX11 and 12 are involved in the first-step cell fate transition during de novo root organogenesis in Arabidopsis. *The Plant Cell* 26: 1081–1093.

Lomax TL, Muday GK & Rubery PH. 1995. Auxin transport. Pp 509–530 in Davies PJ (ed) *Plant Hormones: Physiology, Biochemistry and Molecular Biology*. Kluwer Academic Publishers, Dordrechtpp.

Lovell PH & White J. 1986. Anatomical changes during adventitious root formation. Pp 111–140 in Jackson MB (ed) *New Root Formation in Plants and Cuttings*. Martinus Nijhoff Publishers, Dordrecht.

Ludwig-Muller J. 2004. From auxin homeostasis to understanding plant pathogen and plant symbiont interaction: editor's research interests. *Journal of Plant Growth Regul*ation 23: 1–8.

Mitsuhashi-Kato M, Shibaoka H & Shimokoriyama M. 1978. Anatomical and physiological aspects of developmental processes of adventitious root formation in *Azukia* cuttings. *Plant Cell Physiology*19: 393–400.

Mullin TJ, Morgenstern EK, Park YS & Fowler DP. 1992. Genetic parameters from a clonally replicated test of black spruce (*Picea mariana*). *Canadian Journal of Forest Research.* 22: 24–36.

Nag S, Saha K & Choudhuri MA. 2001. Role of auxin and polyamines in adventitious root formation in relation to changes in compounds involved in rooting. *Journal of Plant Growth Regulation* 20: 182–194.

Nakhooda M, Watt MP & Mycock D. 2011. Auxin stability and accumulation during *in vitro* shoot morphogenesis influences subsequent root induction and development in *Eucalyptus grandis*. *Plant Growth Regulation* 65: 263–271.

Nanda KK & Anand VK. 1970. Seasonal changes in auxin effects on rooting of stem cuttings of *Populusnigra* and its relationship with mobilization of starch. *Physiologia Plantarum* 23: 99–107.

Negishi N, Nakahama K, Urata N, Kojima M, Sakakibara H & Kawaoka A. 2014. Hormone level analysis on adventitious root formation in *Eucalyptus globulus*. *New Forest* 45: 577–587.

Nehra NS, Becwar MR, Rottmann WH et al. 2005. Forest biotechnology: innovative methods, emerging opportunities. *In Vitro Cellular and Devlopmental Biology-Plants* 41: 701–717.

Nordstrom AC, Jacobs FA & Eliasson L. 1991. Effect of exogenous indole-3-acetic-acid and indole-3-butyric acid on internal levels of the respective auxins and their conjugation with aspartic acid during adventitious root formation in pea cuttings. *Plant Physiol*ogy 96: 856–861.

Pijut PM, Wowste KE & Michler CH. 2011. Promotion of adventitious root formation of difficult-to-root hardwood tree species. *Horticulture Review* 38: 213–251.

Quaddoury A & Amssa M. 2004. Effect of exogenous indole butyric acid on root formation and peroxidase and indole-3-acetic acid oxidase activities and phenolic contents in date palm offshoots. *Botanical Bulletin of Academic Sinica* 45: 127–131.

Rezende DGSP, De Rezende MDV & De ASSIS TF. 2013. *Eucalyptus* breeding for clonal forestry. Pp 393–424 in Fenning TM (ed) *Challenges and Opportunities for the World's Forests in the 21st Century*. Forestry Sciences Book Series Volume 81. Springer Science & Business Media, Dordrecht. doi:10.1007/978-94-007-7076-8_16.

Steffens B & Rasmussen A. 2016. The physiology of adventitious roots. *Plant Physiology* 170: 603–617.

Stoessl A & Venis MA. 1970. Determination of submicrogram levels of Indole-3- acetic acid. *Analytical Biochemistry* 34: 344–351.

Teale WD, Paponov IA & Palme K. 2006. Auxin in action: signaling, transport and the control of plant growth and development. *Nature Review of Molecular Cell Biology* 7: 847–859.

Thorpe TA, Harry IS & Kumar PP. 1991. Application of micropropagation to forestry. Pp 311–336 in Debergh PC & Zimmerman RH (eds) *Micropropagation, Technology and Application*. Kluwer Academic, Dordrecht. https://doi.org/10.1007/978-94-009-2075-0_21.

Veierskov B. 1988. Relation between carbohydrates and adventitious root formation in cuttings. Pp 70–78 in Davis TD et al. (eds) *Adventitious Root Formation in Cuttings*. Dioscorides Press, Portland.

Wilson PJ. 1994. The concept of a limiting rooting morphogen in woody stem cuttings. *Journal of Horticultural Sciences* 69: 91–600.

Woodward AW & Bartel B. 2005. Auxin: regulation, action and interaction. *Annals of Botany* 95: 707–735.

Wu HX. 2019. Benefits and risks using clones in forestry-a review. *Scandinavian Journal of Forest Research* 34: 352–359.

