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Role of Anthraquinones as a Marker of Juvenility and Maturity in Response to Adventitious Rooting of *Tectona grandis*

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ABSTRACT

Adventitious root formation in woody plants is highly influenced by the process of physiological ageing. There are increasing efforts to identify markers for distinguishing between mature and juvenile stages of trees. However, it appears to be very little information on this aspect at the chemical and molecular levels. The aim of the work was to identify potential chemical marker of juvenile and mature state; and accomplish maximum rooting in teak (*Tectona grandis* Linn. f.) leafy shoot cuttings. Experiments with teak leafy shoot cuttings of three different physiological ages 30- and 15-year-old (mature); and 2-month-old (juvenile) were conducted in mist propagation system. Maturity of donor teak plants decreased rooting ability. Cuttings obtained from 2-month-old donors rooted more profusely in comparison to 15- and 30-year-old donors. Indole-3-butyric acid treatment at 4000 mg L^{-1} significantly increased rooting percentage and produced highest number of roots. High Performance Thin Layer Chromatography (HPTLC) analysis of anthraquinones (AQs; $C_{14}H_8O_2$) was utilized to detect qualitative and quantitative differences in AQs in stem tissues from 2-month, 15- and 30-year-old donor plants. The HPTLC analysis showed that AQs varied from 2.8 to 18.3% in cuttings derived from 2-month-old donor plants, while the variations were 4.9 to 27.3% and 11.8 to 43.4% in those from 15- and 30-year-old donor plants, respectively. Altogether, data support that AQs could be a reliable marker for maturity vis-à-vis juvenility in teak.

Key words: Maturation, juvenility, cutting, auxin, adventitious rooting, marker, anthraquinones

INTRODUCTION

The loss of ability to form adventitious rooting in various types of cuttings is one of the most dramatic effects of maturation and remains a hindrance for the clonal multiplication of superior genotypes of forest trees species. Reduced/loss of ability of adventitious rooting in mature shoots can be attributed to physiological aging and many clarifications for the diminishing rooting ability of cuttings obtained from mature stock plants are presented (McGowran *et al*., 1998; De Klerk *et al*., 1999; Greenwood, 1995; Greenwood *et al*., 2001; Diaz-Sala *et al*., 2002; Vidal *et al*., 2003; Husen and Pal, 2006; 2007a; Osterc *et al*., 2009; Husen, 2011; 2012). Physiologically juvenile donor plants are prone to develops strong and ample Adventitious Root Formation (ARF) and/or axillary bud growth, which has been explained in *Backhousia citriodora* (Kibbler *et al*., 2003), *Abies fraseri* (Rosier *et al*., 2005), *Dalbergia sissoo* (Husen, 2008a), *Tectona grandis* (Husen and Pal, 2007a),

Grewia optiva (Husen, 2012). It has been generally reported that physiological maturation increased with chronological age (cyclophysis). Conversely, the same plant may possess zones (the top of a plant, outer part of a plant) which act more physiologically mature than others (plant basis, inner part of plants). As a result, specific portion of chronologically adult plant can react like juvenile parts (Husen and Pal, 2000, 2007b; Osterc *et al*., 2009).

Morphological, physiological, biochemical and histological activities have previously been used to describe phase changes and topophysis in woody species, in order to assess the juvenility of plant material and ARF (Borchert, 1976; Haffner *et al*., 1991; Greenwood, 1995; Husen and Pal, 2006; Husen, 2008b; Huang *et al*., 2012). McGowran *et al*. (1998) evaluated morphological descriptors of plant juvenility (angle of shoots to the horizontal, shoot length, stem length, leaf area) in *Quercus robur* and *Quercus petraea*; Nicolini and Chanson (1999) described the differences in tree architecture in juvenile and mature stages of *Fagus sylvatica* trees and leaf morphology was an important factor in research on genomic DNA methylation in *Acacia mangium* (Baurens *et al*., 2004). The level of nuclear DNA methylation was higher in juvenile than mature shoots of *Sequoiadendron giganteum* (Monteuuis *et al*., 2008). In contrast, the level of nuclear DNA methylation is higher in mature than in juvenile or rejuvenated shoots of *Sequoia sempervirens* (Huang *et al*., 2012). Baurens *et al*. (2004) suggested that phase change phenomena happened at the meristematic level during shoot elongation; and such inconsistency may be due to the nature of tissues used (Fraga *et al*., 2002; Monteuuis *et al*., 2008). Three ratios among five polyphenols were associated with rejuvenation in walnut (Jay-Allemand *et al*., 1988). Subsequently, hydrojuglone glucoside and myricitrin were found as good markers of physiological age in walnut (Claudot *et al*., 1992). Enhanced activities of enzymes associated with the synthesis of polyphenols such as phenylalanine-ammonia-lyase and chalcone synthase were found in juvenile material (Claudot *et al*., 1993). In *Hedera helix*, mature phase is differentiated by a lack of dihydroflavonol reductase activity, which results in a lack of anthocyanins in mature tissues (Murray and Hackett, 1991). In addition, Valdes *et al*. (2003, 2004) explained the changes in hormonal status during maturation in *Pinus radiata* and *Pinus pinea*, where the mature phase appears to be characterized by inactivation of one or more enzymes involved in the biosynthesis of active polyphenols and flavonoids. Husen (2008b) have reported that anthraquinones in coppice shoots obtained from etiolated and non-etiolated stock plants showed that etiolation restored some chemical features of juvenile shoots in *Tectona grandis*. The position on the plant apparently plays a role in the accumulation of the major anthocyanin in senescing leaves of *Acer palmatum* and the level of cyanidin 3-glucoside was used as a quantitative marker of the maturation process (Schmitzer *et al*., 2009).

Adventitious rooting in cutting/or shoots occurs in four steps: cell dedifferentiation, induction, root primordia development and root emergence (De Klerk *et al*., 1999). Various explanations have shown that at each step, auxins (endogenous or exogenously applied) play a central role (Blazkova *et al*., 1997; Vidal *et al*., 2003; Osterc *et al*., 2009; Husen, 2008a, 2012) and in general, IBA and NAA have been recommended for promoting ARF in cutting propagation in many shrub (Husen and Mishra, 2001; Husen, 2003) or tree (Kaul, 2008; Husen, 2008a; 2012). Teak (*Tectona grandis* Linn. f.) is the principal timber tree species in the Indian subcontinent and southeastern Asian countries. Teak distribution is mainly determined by climate, geology and soil. Accordingly, it has been introduced to the other parts of the worlds (Pandey and Brown, 2000). Teak timber demand in the market is increasing for several luxury applications including quality furniture, shipbuilding and decorative building components; however its supplies from natural

forests have declined during the last 2-3 decades (Tewari, 1992; Pandey and Brown, 2000). Numerous clones of teak timber combine qualities such as strength, durability and resistance to termites and currently it became an important component of many plantation programmes in throughout the tropical world (Tewari, 1992; Pandey and Brown, 2000). Teak plants can be raised using either sexual (seeds) (Tewari, 1992) or asexual (vegetative) propagation methods (Husen and Pal, 2006). The seed production in clonal seed orchards is very poor and most of the plantations are raised from unselected seed source resulting in low productivity, moreover, clonal multiplication of superior genotypes cannot be achieved by seed; therefore, vegetative propagation is used to produce progeny plants, which are genetically identical to a single source plant. Like many other tree species, ARF in teak cuttings is also affected by maturation (Husen and Pal, 2006; Husen, 2011). However, no reports have attempted to investigate ageing (2-month, 15- and 30-year-old plants) markers in terms of ARF; therefore, the objective of this study was to gain deeper insight into the identification of markers in relation to rooting response of teak shoot cuttings.

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 (FRI), Uttrakhand, Dehra Dun, India. FRI is located at 30°20'40"N, 77°52'12"E at 640 m above mean sea level. For 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 to encourage bud sprouting and new shoot formation while in case of 2-month-old seedlings the main shoots were used for anthraquinones estimation and making cuttings. The open portion of stem was coated with chuapatia pest, comprising mixture of 1 g of copper carbonate and 1 g of red lead in 1 L blue copper to avoid infection.

Anthraquinones estimation: For HPTLC analysis of anthraquinones, 1.0 g dried shoot material (2-month, 15- and 30-year-old plants) was extracted with 5.0 mL of methanol for 5 min at 70°C over a rotary flash evaporator. The contents were cooled to room temperature and filtered through Whatman paper No. 3; then the dried extract was weighed and a 1.0% solution was prepared using methanol. The extracted plant material was analyzed on HPTLC pre-coated silica gel plates Merck 60F254 (Merck KgaA, Darmstadt, Germany). The solvent was toluene: ethylacetate (90:10). The sample was applied (20.0 μL) through Camag Automatic TLC Sampler III (Camag Scientific Inc., Muttenz, Switzerland) on different tracts and the development mode was Camag Twin Trough chamber (Camag Scientific Inc., Muttenz, Switzerland). The detection of spots and photography of TLC plate were done with the help of a Desaga video documentation system (Camag Scientific Inc., Muttenz, Switzerland) under visible, UV-366 nm and UV-254 nm. Further, details are given in Table 1. The components were derivatized by spraying with anisaldehyde H_2SO_4 (Wagner and Bladt, 1996); anthraquinones ($C_{14}H_8O_2$) are the derivatives of anthracene.

Table 1: Scanner settings for anthraquinones detection and analysis

Parameter	Value	Parameter	Value
Plate size (width×height)	11×10 cm	Slit dimension	5.0×0.45 mm
Application position Y	14.5 mm	Data step resolution	100 μm
Position of solvent front	94.0 mm	Display scaling	1000 AU
Scan start position Y	11.0 mm	Measurement mode	Absorption/reflection
Scan end position Y	95.0 mm	Scanning speed	5 mm sec^{-1}
Scan start position X	10.5 mm	Optical filter	Second order
Distance between tracks X	11.0 mm	Zeroing mode	Normal
No. of tracks	3	Y position for 0 adjustment	11.0 mm, track: 1
Lamp	Deuterium	Offset	10%
Monochromator bandwidth	20 mm	Sensitivity	Automatic (33)
Wavelength	281 nm	High voltage of PM	295 V

Collection and cutting preparations: The new shoots which grew on the pruned branches were harvested (at that time shoots were about 2-months old) 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 trials each shoot was made into mono-nodal leafy soft-wood cuttings. Each nodal shoot cutting retained about 25.0 cm^2 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) IBA treatments. As described above the shoots were collected from three different age groups of donor plants. Indole-3-butyric acid (IBA) was used with two different concentration, i.e. at 2,000 and 4,000 mg L^{-1}. IBA was applied in their powder formulation (IBA×talcum powder), which also contained 0.05% fungicide, i.e., bavastin. Other sets of cuttings from each age group of donors were taken and treated with talcum powder containing bavastin as controls.

Rooting environment: 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 IBA treatments and kept inside a mist chamber where the relative humidity was maintained at 85±2% with maximum and minimum day and night temperature at 32±1 and 26±1°C, respectively. The automatic day/night misting cycle was set at 60/30 sec, with 1 h delay between successive cycles.

Rooting assessments: The cuttings were carefully removed from the rooting medium and observations were recorded. The rooting ability (percentage of rooting and number of roots grown per cutting) of cuttings per donor plant was assessed 40 days after planting.

Statistics: Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS) windows® software package. The experimental design was a randomized complete block with a 3×3 factorial arrangement of treatments. There were 5 replications (blocks). Treatments were donor plant age (2, 15 and 30) and IBA concentration (0, 2000 and 4000 ppm). There were 10 individual cuttings per treatment (subsamples). Data were subjected to two-way analysis of variance (ANOVA) to determine the significant difference among the age of donor plants and IBA treatments. Means were compared by using Tukey's test at significance level $p<0.05$ (the same letters indicate that means within a row or column are not significantly different at $p>0.05$ level).

RESULTS

Rooting response: After 40 days, the rooting abilities showed a great variation among the donor plants (Table 2). Cuttings with juvenile character (2-month old) tended to show higher rooting rate (84%). However cuttings obtained from mature donors (15- and 30-year old) showed significantly lower rooting value (40 and 26%). Similarly, number of roots/cutting was recorded more in 2-month-old donor in comparison to 15- and 30-year-old donors. Exogenous application of IBA has a significant positive effect of ARF (Table 3). With increasing concentration of IBA 2000 to 4000 mg L^{-1}, there was an increase in rooting percentage. Therefore, IBA 4000 mg L^{-1} gave highest percent rooting (69%) in comparison to IBA 2000 mg L^{-1} and control which gave 57 and 24% rooting, respectively. In addition, number of roots/cutting was also more with IBA 4000 mg L^{-1} (3.33) in comparison to IBA 2000 mg L^{-1} and control which produced 2.19 and 1.05, respectively.

The two factor interaction (age of donor plants x IBA treatments) was significant for percent rooting and number of roots/cutting at the $p<0.01$ level (Table 4). Mono-nodal leafy soft-wood cuttings obtained juvenile donor, i.e. from 2-month old and treated with IBA 4000 mg L^{-1} have shown highest rooting percentage (93%) and number of roots/cutting (4.59). On the other hand, the control cuttings from the 15- and 30-year-old donors have not given any response. However, cuttings obtained from the 15- and 30-year-old donors and treated with IBA 4000 mg L^{-1} was more efficient and have enhanced rooting ability in comparison to IBA 2000 mg L^{-1} and control (Fig. 1, 2).

Table 2: Effect of age of donor plants on rooting of softwood cuttings in *Tectona grandis*

	Rooting parameters	
Age of donor plants	Rooting (%)	Roots/cutting
2 month	84.69[a]	3.81[a]
15 year	40.19[b]	2.09[b]
30 year	26.30[c]	0.66[c]

Values followed by the same letter are not significant at $p<0.05$ level according to Tukey's test

Table 3: Effect of IBA treatments on rooting and sprouting of softwood cuttings in *Tectona grandis*

	Rooting parameters	
IBA treatments (mg L^{-1})	Rooting (%)	Roots/cutting
Control	24.57[a]	1.05[a]
IBA 2000	57.06[b]	2.19[b]
IBA 4000	69.55[c]	3.33[c]

Values followed by the same letter are not significant at $p<0.05$ level according to Tukey's test

Table 4: ANOVA result on the effect of age of donor plants, IBA treatments and their combination on rooting traits

	Age of donor plants (A)			IBA treatments (T)			A×T		
Parameters	MSS	p<0.05	p<0.01	MSS	p<0.05<0.01	p<0.01	MSS	p<0.05	p<0.01
Rooting (%)	13958.53	-	**	8086.99	-	**	828.33	-	**
Roots/cutting	19.42	-	**	37.22	-	**	2.78	-	**

*Significance at $p<0.01$

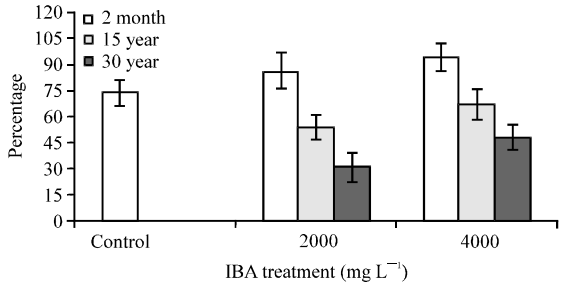

Fig. 1: Effect age of donor plant and IBA treatment on rooting percentage, Values are Mean±SE of five replicates

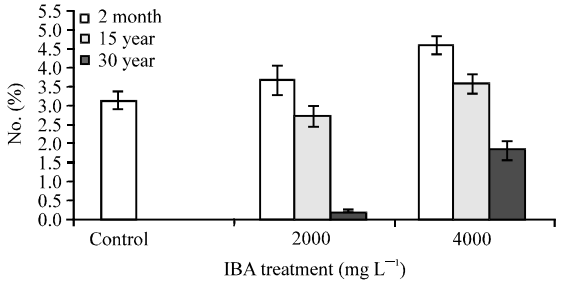

Fig. 2: Effect age of donor plant and IBA treatment on number of roots per cutting, Values are Mean±SE of five replicates

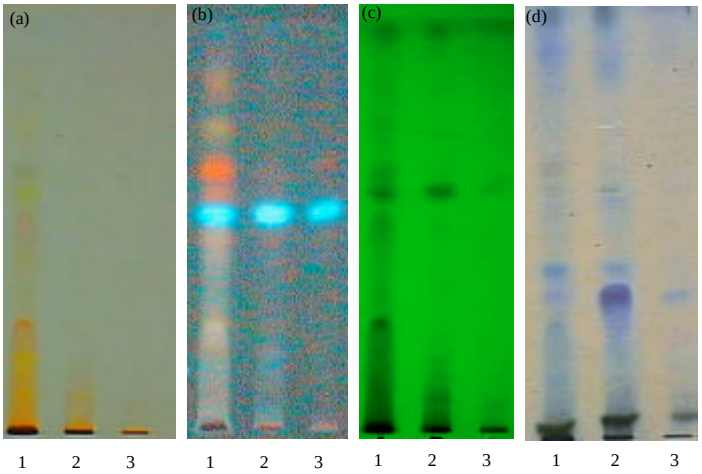

Fig. 3(a-d): The HPTLC fingerprinting profiles of anthraquinones in shoot cuttings of *Tectona grandis* obtained from 1: 2-month, 2: 15-year and 3: 30-year-old donor plants at (a) Visible light (b) Under UV_{366} (c) Under UV_{254} and (d) Visible light after Derivatization with anisaldehyde H_2SO_4

Qualitative and quantitative changes in anthraquinones: The HPTLC fingerprinting profile of AQs in shoots obtained from 2-month, 15- and 30-year-old plants are shown in Table 5 and Fig. 3. Based on a comparison of Rf values of different spots, eleven different compounds belonging

Table 5: HPTLC finger printing profiles studies of anthraquinones in shoot cuttings of various age groups of *Tectona grandis* donor plants

Rf			Visible light before derivatization		Visible light after derivatization			UV_{366}			UV_{254}			Compound (%)		
2-month	15-year	30-year	2-month	15-year	2-month	15-year	30-year	2-month	15-year	30-year	2-month	15-year	30-year	2-month	15-year	30-year
0.06	0.08	0.07	Brown	Yellow	Yellow	Black	Black	Black	Red	Yellow	Black	Black	Black	2.81	19.16	43.41
0.19	0.23	0.50	Light yellow	Yellow	Yellow	Blue	Blue	Light yellow	White	White	Black	Black	Black	2.85	4.99	11.81
0.27	0.36	0.60	Light yellow	Yellow	Yellow	Blue	Blue	Light yellow	White	Blue	Black	Black	Black	16.86	7.55	20.52
0.33	0.49	0.71	Light yellow	Yellow	Yellow	Blue	Blue	Blue	Light yellow	White	Red	Black	Black	Black	7.35	11.91	24.26
0.49	0.58		Light yellow	Yellow		Blue	Blue		Light yellow	Blue		Black	Black		10.80	27.34	
0.58	0.63		Light yellow	Yellow		Blue	Blue		Light yellow	Yellow		Black	Black		18.30	9.60	
0.63	0.83		Red	Yellow		Blue	Blue		Blue	Red		Black	Black		13.34	5.24	
0.72	0.98		Yellow	Yellow		Blue	Blue		Red	Yellow		Black	Black		5.65	14.23	
0.83			Yellow			Blue			Red			Black			3.55		
0.85			Brown			Blue			Yellow			Black			3.59		
0.99			Yellow			Blue			Yellow			Black			14.89		

to the group AQ could be distinguished in shoots of 2-month old plants. On the other hand, only eight and four different compounds could be distinguished in shoots of 15- and 30-year-old plants, respectively. Only one spot with an Rf value of 0.49 was common in shoots of 2-month and 15-year-old plants. Under visible light without any chemical spraying, in 2-month-old plants five spots appeared light yellow, three yellow, two brown; and only one red color at 0.63 Rf in shoot extracts. However, in case of 15- and 30-year-old plants shoot extracts, all spots appeared yellow in color. When spots were observed under visible light after spraying with anisaldehyde H_2SO_4 the lower most one spots from each type of donors appeared black in color, while all other spots appeared blue. All spots appeared black in color when they were observed under UV light at 254 nm. The difference between the spots in 2-month, 15- and 30-year-old plants shoot extracts was more conspicuous when they were observed under UV light at 366 nm (Fig. 3). The percentage of different compounds varied from 2.8 to 18.3% in shoot cuttings extracts derived from 2-month-old donor plants, while the variations were 4.9 to 27.3% and 11.8 to 43.4% in those from 15- and 30-year-old donor plants, respectively (Table 5).

DISCUSSION

The present investigation showed that ARF declined with increasing age of donor plants. As an evident, cuttings obtained from juvenile 2-month-old donor have shown the highest rooting performances then their mature counterparts; and perhaps it was due to that is physiologically younger propagation material than those of mature donors. Similarly, numerous studies cuttings from juvenile 2-month-old donor plants rooting in higher percentages then their mature counterparts, perhaps it was due to that is physiologically younger propagation material than those of mature donors. Similarly, numerous studies with several plant species have shown that ARF decreases with increasing age of donor plants (Husen and Pal, 2006, 2007b; Osterc *et al*., 2009; Husen, 2012). This decrease in ARF due to a decrease in required endogenous auxins content or root promoters, accumulation of root inhibitors, decreased sensitivity of tissue to auxins with maturity of donor plants and/or due to decreased rate of net photosynthesis and carbohydrate metabolism (Hackett and Murray, 1993; Greenwood and Hutchison, 1993; Haissig and Davis, 1994; Husen and Pal, 2006, 2007b; Husen, 2012).

IBA treatments improved ARF in both juvenile and mature cuttings of all age group of donor plants. Enhanced rooting ability due to IBA application in teak and many other tree species have been reported by several investigators (Husen and Pal, 2006, 2007a; Husen, 2008, 2012). However, in the present studies, the promotive effect varied with IBA concentrations. IBA 4000 mg L^{-1} produced the best rooting percentage and number of roots/cutting and hence it was recommended for mature and juvenile cuttings of teak.

The HPTLC fingerprinting profiles of AQs in shoot extracts obtained from various age groups of donors showed that 2-month-old-donor has some chemical features of juvenile shoots associated with enhanced rooting. Similarly, relationship between the contents of various secondary metabolites including AQs and the maturity of trees has been reported by several investigators (Haffner *et al*., 1991; Haissig and Davis, 1994; Schmitzer *et al*., 2009; Husen, 2008b). Many others, such as the differences in concentration and ratio of leaf polyamines have been suggested as markers of maturity in *Corylus avellana* (Rey *et al*., 1994) and Bon (1988) detected a 16 kD membrane-associated protein in shoot apices of juvenile *Sequoiadendron giganteum*, which was absent in apices of mature trees. Further, a comparison of in vitro shoots derived from basal and crown shoots of the same mature *Castanea sativa* tree revealed protein differences

(Amo-Marco *et al*., 1993). Mellerowich *et al*. (1995) reported that the dormant mature cambium contained more extranuclear RNAs than the juvenile one in *Larix laricina*. A juvenility-specific protein was also identified in *Prunus avium* using antibodies (Hand *et al*., 1996). Fernandez-Lorenzo *et al*. (1999) analyzed 24 polyphenols as potential markers for differentiating juvenile and mature plant material in chestnut cultures and a juvenile-phase-dependant accumulation of anthocyanin was observed in a number of woody perennials, including species of *Betula* (Brand and Lineberger, 1992) and *Malus* (Noiton *et al*., 1992). In addition to these, it was reported that there were remarkable differences in AQs in coppice shoots obtained from etiolated and non-etiolated stock plants showed that etiolation restored some chemical features of juvenile shoots associated with enhanced rooting (Husen, 2008b). Similarly, the present study showed that AQs could be used as a reliable and specific marker of maturity and juvenility in teak.

In conclusion, the results presented in this investigation suggested that exogenous IBA application could produce good rooting response even in case of mature donor plants of teak. IBA 4000 mg L^{-1} was suggested for all age groups of donor plants. On the other hand, notable variation in AQs chemical features are observed in juvenile and mature shoot extracts and these changes suggested that AQs could be used as a reliable marker for phase change in teak.

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REFERENCES

Amo-Marco, J.B., N. Vidal, A.M. Vietez and A. Ballester, 1993. Polypeptide markers differentiating juvenile and adult tissues in Chestnut. J. Plant Physiol., 142: 117-119.

Baurens, F.C., J. Nicolleau, T. Legavre, J.L. Verdeil and O. Monteuuis, 2004. Genomic DNA methylation of juvenile and mature *Acacia mangium* micropropagated *in vitro* with reference to leaf morphology as a phase change marker. Tree Physiol., 24: 401-407.

Blazkova, A., B. Sotta, H. Tranvan, R. Maldiney and M. Bonnet *et al*., 1997. Auxin metabolism and rooting in young and mature clones of *Sequoia sempervirens*. Physiol. Plant., 99: 73-80.

Bon, M.C., 1988. J 16: An apex protein associated with juvenility of *Sequoiadendron giganteum*. Tree Physiol., 4: 381-387.

Borchert, R., 1976. The concept of juvenility in woody plants. Acta. Hortic., 56: 21-36.

Brand, M.H. and R.D. Lineberger, 1992. *In vitro* rejuvenation of *Betula* (Betulaceae): Morphological evaluation. Am. J. Bot., 79: 618-625.

Claudot, A.C., A. Drouet and C. Jay-Allemand, 1992. Tissue distribution of phenolic compounds in annual shoots from adult and rejuvenated hybrid walnut trees. Plant Physiol. Biochem., 30: 565-572.

Claudot, A.C., C. Jay-Allemand, E.A. Magel and A. Drouet, 1993. Phenylalanine ammonia-lyase, chalcone synthase and polyphenolic compounds in adult and rejuvenated hybrid walnut tree. Trees: Struct. Funct., 7: 92-97.

De Klerk, G.J., W. Van der Krieken and J.C. De Jong, 1999. The formation of adventitious roots: New concepts, new possibilities. *In vitro* Cell Dev. Biol. Plant, 35: 189-199.

Diaz-Sala, C., G. Garrido and B. Sabater, 2002. Age-related loss of rooting capability in *Arabidopsis thaliana* and its reversal by peptides containing the Arg-Gly-Asp (RGD) motif. Physiol. Plant., 114: 601-607.

Fernandez-Lorenzo, J.L., A. Rigueiro and A. Ballester, 1999. Polyphenols as potential markers to differentiate juvenile and mature chestnut shoot cultures. Tree Physiol., 19: 461-466.

Fraga, M.F., M. Canal and R. Rodriguez, 2002. Phase-change related epigenetic and physiological changes in *Pinus radiata* D. Don. Planta, 215: 672-678.

Greenwood, M.S., 1995. Juvenility and maturation in conifers: Current concepts. Tree Physiol., 15: 433-438.

Greenwood, M.S. and K.W. Hutchison, 1993. Maturation as a Developmental Process. In: Clonal Forestry I: Genetics and Biotechnology, Ahuja, M.R. and W.J. Libby (Eds.). Springer-Verlag, New York, ISBN-13: 978-3540525011, pp: 14-33.

Greenwood, M.S., X. Cui and F. Xu, 2001. Response to auxin changes during maturation-related loss of adventitious rooting competence in loblolly pine (*Pinus taeda*) stem cuttings. Physiol. Plant., 111: 373-380.

Hackett, W.P. and J.R. Murray, 1993. Maturation and Rejuvenation in Woody Species. In: Micropropagation of Woody Plants, Ahuja, M.R. (Ed.). Kluwer Academics Publishers, The Netherlands, pp: 93-105.

Haffner, V., F. Enjalric, L. Lardet and M.P. Carron, 1991. Maturation of woody plants: A review of metabolic and genomic aspects. Ann. Sci. For., 48: 615-630.

Haissig, B.E. and T.D. Davis, 1994. A Historical Evaluation of Adventitious Rooting Research to 1993. In: Biology of Adventitious Root Formation, Davis, T.D. and B.E. Haissig (Eds.). Plenum Press, New York and London, pp: 275-331.

Hand, P., R.T. Besford, C.M. Richardson and S.D. Peppitt, 1996. Antibodies to phase related proteins in juvenile and mature *Prunus avium*. Plant Growth Regul., 20: 25-29.

Huang, L.C., L.J. Hsiao, S.Y. Pu, C.I. Kuo and B.L. Huang *et al*., 2012. DNA methylation and genome rearrangement characteristics of phase change in cultured shoots of *Sequoia sempervirens*. Physiol. Plant, 145: 360-368.

Husen, A. and M. Pal, 2000. Analytical studies on the effects interaction with respect to position, season and auxin on adventitious root formation in stem cuttings of mature teak (*Tectona grandis* Linn. f.). Ann. For., 8: 253-261.

Husen, A. and V.K. Mishra, 2001. Effect of IBA and NAA on vegetative propagation of *Vitex negundo* L. through leafy stem cuttings from hedged shoots during rainy season. Indian Perf., 45: 83-87.

Husen, A., 2003. Effect of IBA and NAA treatments on rooting of *Rauvolfia serpentina* Benth. ex Kurz shoot cuttings. Ann. For., 11: 88-93.

Husen, A. and M. Pal, 2006. Variation in shoot anatomy and rooting behaviour of stem cutting in relation to age of donor plants in teak (*Tectona grandis* Linn. F). New For., 31: 57-73.

Husen, A. and M. Pal, 2007a. Effect of branch position and auxin treatment on clonal propagation of *Tectona grandis* Linn. f. New For., 34: 223-233.

Husen, A. and M. Pal, 2007b. 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. New Forests, 33: 309-323.

Husen, A., 2008a. Clonal propagation of *Dalbergia sissoo* Roxb. and associated metabolic changes during adventitious root primordium development. New For., 36: 13-27.

Husen, A., 2008b. Stock-plant etiolation causes drifts in total soluble sugars and anthraquinones and promotes adventitious root formation in teak (*Tectona grandis* L. f.) coppice shoots. Plant Growth Regul., 54: 13-21.

Husen, A., 2011. Rejuvenation and adventitious rooting in coppice-shoot cuttings of Tectona grandis as affected by stock-plant etiolation. Am. J. Plant Sci., 2: 370-374.

Husen, A., 2012. Changes of soluble sugars and enzymatic activities during adventitious rooting in cuttings of *Grewia optiva* as affected by age of donor plants and auxin treatments. Am. J. Plant Physiol., 7: 1-16.

Jay-Allemand, C., D. Cornu and J.J. Macheix, 1988. Biochemical attributes associated with rejuvenation of walnut tree. Plant Physiol. Biochem., 26: 139-144.

Kaul, K., 2008. Variation in rooting behavior of stem cuttings in relation to their origin in *Taxus wallichiana* Zucc. New For., 36: 217-224.

Kibbler, H., M.E. Johnston and R.R. Williams, 2003. Adventitious root formation in cuttings of *Backhousia citriodora* F. Muell1. Plant genotype, juvenility and characteristics of cuttings. Sci. Hortic., 102: 133-143.

McGowran, E., G.C. Douglas and M. Parkinson, 1998. Morphological and physiological markers of juvenility and maturity in shoot cultures of oak (*Quercus robur* and *Q. Petraea*). Tree Physiol., 18: 251-257.

Mellerowich, E.J., R.T. Riding and M.S. Greenwood, 1995. Nuclear and cytoplasmic changes associated with maturation in the vascular cambium of *Larix laricina*. Tree Physiol., 15: 443-449.

Monteuuis. O., S. Doulbeau and J.L. Verdeil, 2008. DNA methylation in different origin clonal offspring from a mature *Sequoiadendron giganteum* genotype. Tree-Struct. Funct., 22: 779-784.

Murray, J.R. and W.P. Hackett, 1991. Dihydroflavonol reductase activity in relation to differential anthocyanin accumulation in juvenile and mature phase *Hedera helix* L. Plant Physiol., 97: 343-351.

Nicolini, E. and B. Chanson, 1999. The short shoot, an indicator of beech maturation (*Fagus sylvatica* L.). Can. J. Bot., 77: 1539-1550.

Noiton, D., J.H. Vine and M.G. Mullins, 1992. Endogenous indole-3-aceticacid and abscisic-acid in apple microcuttings in relation to adventitious root-formation. Plant Growth Regul., 11: 63-67.

Osterc, G., M. Stefancic and F. Stampar, 2009. Juvenile stockplant materail enhances root development through higher endogenous auxin level. Acta Physiol. Plant., 31: 899-903.

Pandey, D. and C. Brown, 2000. Teak: A global overview. Unasylva, 51: 3-13.

Rey, M., A.F. Tiburcio, C. Diaz-Sala and R. Rodriguez, 1994. Endogenous polyamine concentration in juvenile, adult and *in vitro* reinvigorated hazel. Tree Physiol., 14: 191-200.

Rosier, C.L., J. Frampton, B. Goldfarb, F.C. Wise and F.A. Blazich, 2005. Stumping height, crown position and age of parent tree influence rooting of stem cuttings of fraser fir. HortScience, 40: 771-777.

Schmitzer, V., F. Stampar, R. Veberic and G. Osterc, 2009. Phase change modifies anthocyanin synthesis in *Acer palmatum* Thunb. (Japanese maple) cultivars. Acta Physiol. Plant., 31: 415-418.

Tewari, D.N., 1992. A Monograph on Teak (*Tectona grandis* Linn. f.). International Book Distributors, Dehra Dun, India, ISBN-13: 9788170891819, Pages: 479.

Valdes, A.E., B. Fernandez and M.L. Centeno, 2003. Alterations in endogenous levels of cytokinins following grafting of *Pinus radiata* support ratio of cytokinins as an index of ageing and vigour. J. Plant Physiol., 160: 1407-1410.

Valdes, E.A., B. Fernandez and M.L. Centeno, 2004. Hormonal changes throughout maturation and ageing in *Pinus pinea*. Plant Physiol. Biochem., 42: 335-340.

Vidal, N., G. Arellano, M.C. San-Jose, A.M. Vieitez and A. Ballester, 2003. Developmental stages during the rooting of *in vitro*-cultured *Quercus robur* shoots from material of juvenile and mature origin. Tree Physiol., 23: 1247-1254.

Wagner, H. and S. Bladt, 1996. Plants Drug Analysis: A Thin Layer Chromatography Atlas. 2nd Edn., Springer, Berlin, pp: 306-364.