Advances in Forestry Science
Original Article

Influence of cutting diameter, auxin and rooting substrate on adventitious rooting from hardwood cuttings of *Azadirachta indica* A. Juss (Neem)

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Received: 09 April 2015 / Accepted: 01 July 2015 / Published: 30 September 2015

Abstract
Azadirochta indica A. Juss (Neem) is a native Indian tree, well known for its medicinal features and have important to local economy. For Conservation of the genetic resources of neem, the effect of diameter (> 0.5; 0.5-1.5 and 1.5-2.5 cm), auxin, their different concentration in different rooting media on adventitious root formation (ARF) in hardwood cuttings of *Azadirachta indica* was studied. Three different rooting substrate (media) (sand, vermiculite and soil) were used and the experiment was established using three types of auxin (IBA, IAA and NAA) and 6 concentrations (100; 250; 500; 750; 1,000 and 1,500 mg L^{-1}). Significant effects of cuttings diameter, auxin, their concentration and rooting substrate on adventitious rooting from neem hardwood cuttings were observed. The rooting percentage, number of roots, root length and number of leaves were evaluated. Among the all diameter of hardwood cuttings used, 0.5-1.5 cm diameter showed maximum rooting. IBA (500 mg L^{-1}) resulted in higher rooting percentage (80%) with 6.82 sprouts, 53.06 roots, 7.13 cm root length and 7.0 leaves per rooted hardwood cuttings, when growing in sand rooting substrate. The determination of proper rooting protocols and the use of hardwood cuttings were proved important for propagation of *A. indica*.
Key words: Adventitious rooting; Cutting technique; Plant growth regulator; Rooting media.

Introduction

Azadirachta indica A. Juss (Neem) a member of Meliaceae family, is an evergreen tropical tree. Most of the parts such as leaves, bark, flower, fruit, seed and root have applications in the field of medicine (Muthu et al. 2010). It is an evergreen tree related to mahogany, growing in almost every state of India, South East Asian countries and West Africa (Muthu et al. 2010). It grows in drier areas and in all kinds of soil. It contains several thousands of chemicals which are terpenoids in nature and its high oil content of 39.7 to 60.0% (Ragit et al. 2011) A mature neem tree produces 30 to 50 kg fruit every year and has a productive life span of 150 to 200 years (Ragit et al. 2011). It has the ability to survive on drought and poor soils at a very hot temperature of 44°C and a low temperature of up to 4°C (Ragit et al. 2011).

The neem plants contain a variety of secondary metabolites including limonoids (e.g., azadirachtin, salanin), flavonoids, essential oil, etc. used as biopesticide against insects. The principal active ingredient, azadirachtin, is more concentrated in the kernel, which makes it the most effective part of the plant (Schmutterer 1990; Gahukar 2000). Neem holds the highly effective, non toxic and environment friendly means that control or eliminate pests that cause losses in agriculture production (Govindachari et al. 1992). Due to broad spectrum properties including low persistence in nature, low toxicity against non target organisms and systemic action, azadirachtin has received more attention as biopesticide and showed great potential against insects (Immaraju 1998; Prakash et al. 2002).

Recently, neem oil can be used as fuel in diesel engines directly and by blending it with methanol. The economic evaluation has also shown that the biodiesel production with a high calorific value matches diesel (Deepak et al. 2013). Its blends with diesel substituting nearly 35% of the later have been suggested for use, without any major engine modification and without any worthwhile drop in engine efficiency. The biodiesel production from *A. indica* oil on the basis of high yield and quality is very profitable (Karmakar et al. 2012). A broad variety of high free fatty acid content of vegetable oils is available in *A. indica*, which conveys great potential sources for biodiesel production in India (Anya et al. 2012; Deepak et al. 2013).

Neem offers a considerable potential for utilization in sustainable agriculture. When considered together, the various economic uses of neem suggest that the utilization of this tree can be a very attractive proposition. Breeding is done for many purposes such as higher fruit yield (including the uses as medicine, pesticides, cosmetics, fodder and organic manure) and other desired agronomic traits including timber and fuel wood, agro forestry species, shelterbelts, avenue trees (Kundu and Luukkanen 2003). Several studies have reported *A. indica* propagation by stem cuttings, they are Sivagnanam et al. (1989), Kijkar (1992), Pal et al. (1992), Pal et al. (1994), Palanisamy and Kumar (1996), Kamaluddin and Ali (1996), Gera et al. (1997), Palanisamy and Kumar (1997), Gera et al. (1998), Palanisamy et al. (1998), Puri and Swamy (1999), Parthiban et al. (1999), Singh and Chander (2001), Palanisamy and Kumar (2001), Palanisamy et al. (2003), Bhola (2004), Devarnavadagi et al. (2005), Gill et al. (2006), Ehiagbonare (2007), Reddy et al. (2007) and Gehlot et al. (2014a, 2014b). In cited reports, limited success to propagate *A. indica* using stem cuttings was recorded. In the present investigation, making use of earlier work done and other related researches; success has been achieved in standardization of macropropagation protocol through stem cuttings collected from mature trees. The present study was aimed to develop novel approach for clonal propagation of *A. indica* by the influence of cuttings diameter, auxin treatments and rooting substrates under greenhouse conditions, which are rapid and can provide uniform, high quality genetically predictable stocks in desired quantities, within a short period for plantation programs at mass level.

Material and methods

Selection of planting material and Preparation of stem cuttings

The study was carried out at greenhouse of Arid Forest Research Institute, Jodhpur, Rajasthan (24°40'N, 71°15' E) during July – September (Monsoon season) of 2011. The climate of this region is generally hot and semi-arid, but monsoon offers low to medium rainfall with various humid levels. The selection of Plus Tree or population variants (PT) based on vegetative characters (i.e., general growth, girth of the main stem at breast level, plant height and crown diameter) and reproductive characters (i.e., regeneration ability, initiation of leaf fall, initiation of new leaves, flowering initiation, number of flowers, fruiting initiation, number of fruits/bunch, fruiting period), seed traits (i.e., 100 seed weight (g), oil percentage, seed viability and azadirachtin percentage). The hardwood cuttings were collected from selected tree, naturally growing at Forest Genetics and Tree Breeding Field, Jodhpur (Fig. 1A). The hardwood cuttings were harvested using sterile pruning scissors in morning time. After harvesting, these were first screened for desired length (30-35 cm by using scale) and three diameter > 0.5 cm, 0.5-1.5 cm, 1.5-2.5 cm by using calibrated vernier caliper, then kept in wet cloth (for prevention from damage) for transportation to laboratory.

The hardwood cuttings were treated with aqueous solution of 0.1% Carbendazim (BASF®) for 10 minutes, subsequently washed with distilled water to remove excess fungicide. The selected cuttings were treated with freshly prepared aqueous solution of auxins at the Basal Long Soak method (Kroin 1992; 2011a; 2011b). The cuttings were dipped in distilled water (control) or treatments of IBA (indole-3-butyric acid, Duchefa Biochemi®), IAA (indole-3-acetic acid, Duchefa Biochemi®) and NAA (α-naphthalene acetic acid, Duchefa Biochemi®) individually at different concentration of 100; 250; 500; 750; 1,000 and 1,500 mg L^{-1}, respectively.

Twenty cuttings of each treatment were stuck in root container (250 cc) filled three nursery rooting substrates: sand, vermiculite and soil. To prevent any form of damage to the cambium of cuttings during insertion into rooting substrate, holes were made by a glass rod into substrate of root container. The cuttings were kept under intermittent mist (misting flow for 60 seconds at 30 minutes interval), maintained at 60-80% relative air humidity and 25-30°C/15-20°C day/night temperature. The cuttings were regularly irrigated and treated with 0.1% Bavistin to avoid desiccation damage and attack of pathogens respectively, at every 15 days interval.

The rooting experiments were run for 60 days, then rooted cuttings were transferred to polythene bags (16×28 cm) containing soil: FYM (farm yard manure) (5:1) and kept in greenhouse for 15 to 20 days. The polythene bags were moved daily in order to minimize misting variation. After this, polythene bags containing rooted hardwood cutting were transferred to shade house for hardening. In shade house, plants were manually irrigated by tap water once a day. After 35-45 days of hardening, plants were planted in the field and were manually irrigated by tap water once in a week (Figs. 1E, F and G).

Rooting analysis in hardwood cuttings

After completing of experiment, the cuttings from treatments were uprooted carefully without harming root and leaves with the help of running water. The number of leaves was recorded just before cutting uprooting of a treatment, whereas the rooting success, numbers of primary roots and roots length (cm) were recorded by evaluation the hardwood cuttings of each replication.

Data collection, experimental design and statistical analyses

The experiment was conducted as a completely randomized factorial arrangement (3×3×6×3), testing three cutting diameter, three auxins, six auxin concentrations and three substrates using four replicates with five explants per replicate. Resultant data were analyzed through General Linear Model (GLM) multivariance factor analysis and one way analysis using Statistical Packages for Social Sciences Software (SPSS 14.0) for all the evaluated parameters. The main effects and their interactions were studied for the test of significance. Means of control, significant factors and their interactions were compared using Duncan Multiple Range Test (DMRT) ($P<0.05\%$). The experiment was repeated thrice. Degree of variations was shown by Mean and standard error. Data given in percentages were subjected to arcsine √X) transformation (Snedecor and Cochran 1967) before statistical analysis.

Results

The results of experiments indicated the important role of cuttings diameter, auxin, their concentration and rooting medium in the process of adventitious root formation in hardwood cuttings of *A. indica* (Table 1).

Table 1. Summary of the analysis of variance (ANOVA) for effects of diameter (>0.5, 0.5-1.5 and 1.5-2.5 cm), auxin (IBA, IAA and NAA), their concentrations and rooting substrate (sand, vermiculite and soil) on rooting percentage, number of root, root length and number of leaves on hardwood cuttings of *Azadirachta indica*.

Source of variance	Dependent variable	df	F-value	p-value
Diameter				
	Rooting (%)	2	6.723	0.001
	No. of sprout	2	5.067	0.006
	No. of roots	2	4.268	0.014
	Root length (cm)	2	4.538	0.011
	No. of leaves	2	2.246	0.106
Auxin				
	Rooting (%)	2	8.120	0.000
	No. of sprout	2	57.870	0.000
	No. of roots	2	23.583	0.000
	Root length (cm)	2	2.930	0.054
	No. of leaves	2	7.069	0.001
Concentration				
	Rooting (%)	4	71.921	0.000
	No. of sprout	4	118.480	0.000
	No. of roots	4	435.358	0.000
	Root length (cm)	4	149.117	0.000
	No. of leaves	4	109.427	0.000
Rooting substrate				
	Rooting (%)	2	27.208	0.000
	No. of sprout	2	0.775	0.461
	No. of roots	2	17.391	0.000
	Root length (cm)	2	1.818	0.163
	No. of leaves	2	1.026	0.359
Diameter × Auxin × Concentration × Rooting substrate				
	Rooting (%)	32	0.596	0.963
	No. of sprout	32	0.470	0.995
	No. of roots	32	1.440	0.054
	Root length (cm)	32	0.503	0.991
	No. of leaves	32	0.200	1.000

No. = number.

Interactive effect of cuttings diameter and auxins treatment on hardwood cuttings in sand rooting media

In the present study three diameters (> 0.5, 0.5-1.5 and 1.5-2.5 cm) of hardwood cutting were used for auxin treatment in sand rooting media. Data revealed that there were highly significant effect ($P<0.01$) was found with auxin concentration on all the studied parameters, i.e., rooting percentage, number of sprouts, number of root, length of root and number of leaves on hardwood cuttings. Table 2 depicted that the rooting percentage varied from 15.0% (control) to 75.0% (500 mg L^{-1} IBA) followed by 70.0% (1,000 mg L^{-1} IBA), whereas number of sprout and roots were ranged from 1.00 and 3.00 to 6.50 and 44.60, respectively. The maximum lengths of roots (6.79 cm) were observed in treatment of 500 mg L^{-1} IBA, while lowest root length (1.30cm) was observed in control. The numbers of leaves were varied from 2.33 (in control and 100 mg L^{-1} NAA) to 5.93 observed in cuttings treated with 500 mg L^{-1} IBA. The significant variation in cuttings due to treatments is clearly observed when mean were subjected into DMRT analysis. Here maximum 8 groups for rooting percentage and minimum 3 for numbers of leaves were obtained for various treatments. The maximum grouping in rooting percentage suggested that a great significant variation is obtained due to the presence of different concentration of three distinct auxins in cutting thickness of >0.5 cm. It could be due to the endogenous level of auxins.

While the performance of hardwood cuttings having diameter of 0.5-1.5 cm revealed that this diameter is again significantly ($P<0.01$) affected by the using auxins doses. The rooting percentage was varied from 10.0% (control) to 80.0% (500 and 1,000 mg L^{-1} IBA) (Table 3).

Table 2. Effect of different auxin concentrations (IBA, IAA and NAA) on rooting percentage, number of sprout, number of root, root length (cm) and number of leaves in hardwood cuttings (>0.5 cm diameter) of *Azadirachta indica* in sand during monsoon season.

Auxin (mg L^{-1})	Rooting (%)	Number of sprout	Number of roots	Root length (cm)	Number of leaves
Control	15.00 (20.24)^{h}	1.00±0.00^{f}	3.00±0.00^{e}	1.30±0.12^{e}	2.33±0.33^{c}
IBA					
100	25.00 (29.73)^{fgh}	2.43±0.20^{def}	4.75±0.41^{e}	1.78±0.15^{ed}	2.50±0.19^{c}
250	35.00 (36.06)^{efg}	4.10±1.14^{bcd}	9.60±1.97^{e}	2.65±0.35^{cde}	3.20±0.25^{bc}
500	75.00 (60.27)^{a}	6.50±0.41^{a}	44.60±4.09^{a}	6.79±0.74^{a}	5.93±0.73^{a}
1,000	70.00 (57.11)^{ab}	5.58±0.59^{ab}	43.07±3.16^{a}	5.67±0.56^{ab}	5.92±0.86^{a}
1,500	60.00 (50.77)^{abcd}	5.93±0.43^{ab}	28.75±3.71^{bc}	3.50±0.66^{bcde}	3.16±0.56^{bc}
Mean	53.00 (46.76)	4.91	26.15	4.08	4.14
IAA					
100	20.00 (26.56)^{gh}	1.50±0.19^{ef}	4.86±0.26^{e}	1.60±0.19^{ed}	2.42±0.30^{c}
250	35.00 (36.06)^{efg}	3.00±0.30^{cde}	10.89±1.89^{e}	2.22±0.15^{ed}	3.33±0.29^{bc}
500	65.00 (54.22)^{abc}	5.14±0.63^{ab}	38.85±4.69^{ab}	4.77±0.78^{abc}	5.75±0.99^{a}
1,000	60.00 (50.77)^{abcd}	4.60±0.56^{bc}	38.33±2.71^{ab}	5.67±0.73^{ab}	4.69±0.76^{abc}
1,500	40.00 (39.23)^{def}	4.43±0.54^{bc}	23.13±5.97^{cd}	3.63±0.89^{bcde}	3.13±0.72^{bc}
Mean	44.00 (41.37)	3.73	23.21	3.58	3.86
NAA					
100	45.00 (42.12)^{cdef}	1.71±0.18^{ef}	4.00±0.37^{e}	1.70±0.16^{ed}	2.33±0.17^{c}
250	50.00 (45.00)^{bcde}	2.91±0.31^{cde}	14.60±1.18^{de}	2.10±0.18^{ed}	2.60±0.34^{c}
500	65.00 (53.94)^{abc}	4.79±0.65^{abc}	30.00±2.58^{bc}	5.69±0.75^{ab}	5.54±0.78^{ab}
1,000	55.00 (47.89)^{abcde}	4.50±0.51^{bc}	29.23±3.48^{bc}	5.09±0.91^{ab}	5.36±0.99^{ab}
1,500	55.00 (48.17)^{abcde}	4.00±0.36^{bcd}	24.82±3.54^{cd}	3.82±0.77^{bcd}	2.45±0.53^{c}
Mean	54.00 (47.42)	3.58	20.53	3.68	3.66
Total mean	48.13 (43.63)	4.33	25.49	4.03	4.09

*Arc sine values in parentheses. Values within the column followed by different letters significantly different at $P\leq0.05$ level as determined using Duncan's multiple range test. A value represents mean ± standard error.

Table 3. Effect of different auxin concentrations (IBA, IAA and NAA) on rooting percentage, number of sprout, number of root, root length (cm) and number of leaves in hardwood cuttings (0.5-1.5 cm diameter) of *Azadirachta indica* in sand during monsoon season.

Auxin (mg L^{-1})	Rooting (%)	Number of sprout	Number of roots	Root length (cm)	Number of leaves
Control	10.00 (13.92)^{e}	2.00±0.00^{de}	3.50±0.29^{d}	1.30±0.12^{e}	2.00±0.00^{e}
IBA					
100	20.00 (26.56)^{d}	2.56±0.18^{cde}	4.43±0.57^{d}	2.14±0.14^{de}	2.57±0.20^{de}
250	35.00 (35.78)^{cd}	5.20±0.50^{ab}	12.40±1.56^{d}	2.95±0.41^{cde}	3.40±0.40^{de}
500	80.00 (63.44)^{a}	6.82±1.76^{a}	53.06±2.70^{a}	7.13±0.77^{a}	7.00±0.67^{a}
1,000	80.00 (63.44)^{a}	6.69±0.41^{a}	50.25±2.15^{a}	6.69±0.60^{a}	6.44±0.70^{ab}
1,500	70.00 (57.11)^{ab}	6.65±0.45^{a}	31.57±3.30^{bc}	5.14±0.75^{abc}	3.79±0.67^{cde}
Mean	57.00 (61.58)	5.58	30.34	4.81	4.64
IAA					
100	30.00 (32.90)^{cd}	2.13±0.23^{de}	4.10±0.58^{d}	2.00±0.00^{de}	2.50±0.19^{de}
250	40.00 (39.23)^{c}	4.20±1.04^{bcd}	12.80±2.06^{d}	2.30±0.15^{de}	3.00±0.30^{de}
500	70.00 (57.11)^{ab}	5.64±0.27^{ab}	38.93±4.23^{b}	6.64±0.86^{a}	6.21±0.87^{ab}
1,000	50.00 (45.00)^{bc}	5.31±0.59^{ab}	36.50±3.01^{bc}	4.93±0.80^{abc}	5.93±0.80^{abc}
1,500	40.00 (38.95)^{c}	4.00±0.44^{bcde}	31.70±3.59^{bc}	3.80±0.44^{bcd}	4.10±0.90^{bcde}
Mean	46.00 (42.64)	5.32	24.81	3.93	4.35
NAA					
100	35.00 (36.06)^{cd}	1.75±0.16^{e}	4.00±0.65^{d}	1.89±0.11^{de}	2.71±0.18^{de}
250	50.00 (45.00)^{bc}	3.73±0.86^{bcde}	8.27±1.09^{d}	2.18±0.12^{de}	2.64±0.20^{de}
500	70.00 (57.11)^{ab}	5.15±0.60^{ab}	38.54±4.06^{b}	5.93±0.65^{ab}	6.00±0.83^{abc}
1,000	65.00 (53.94)^{ab}	4.85±0.41^{abc}	36.71±4.06^{bc}	5.69±0.67^{ab}	4.86±0.75^{abcd}
1,500	60.00 (51.05)^{b}	4.08±0.42^{bcde}	28.08±2.68^{c}	5.25±0.77^{ab}	3.33±0.68^{de}
Mean	56.00 (48.63)	3.91	23.12	4.19	3.91
Total mean	50.31 (44.79)	4.83	29.40	4.67	4.59

*Arc sine values in parentheses. Values within the column followed by different letters significantly different at $P\leq0.05$ level as determined using Duncan's multiple range test. A value represents mean ± standard error.

The maximum numbers of sprouting was 6.82 (500 mg L^{-1} IBA) and minimum 1.75 (100 mg L^{-1} NAA), closely followed by 2.00 (control). However the lowest numbers of root, root length and number of leaves was observed 3.50, 1.30 and 2.00, respectively in control while these were highest noticed in cuttings treated with 500 mg L^{-1} IBA (53.06, 7.13 and 7.00), respectively. DMRT analysis suggested maximum 5 groups (for rooting percentage, root length and numbers of sprouts and numbers of leaves) and the cuttings on the basis of number of roots were classified into 4 groups. For this size of cuttings the variation due to the various auxins doses are limited (Figs. 1B, C and D).

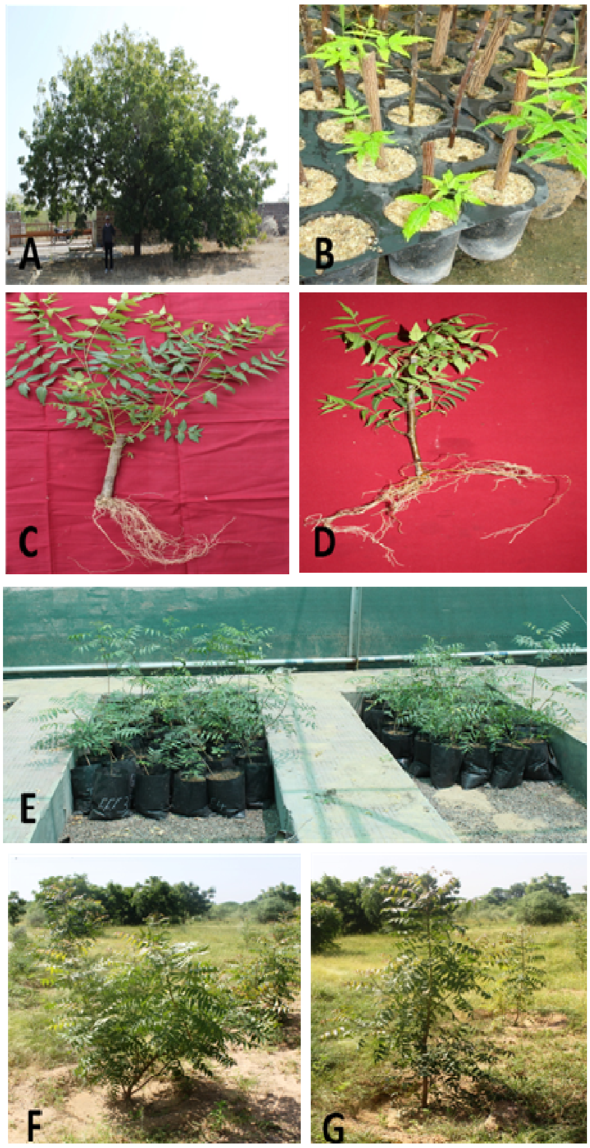

Figure 1. Macropropagation of *Azadirachta indica* (Neem) through hardwood cuttings. (A) Selected plus tree (population variant) in natural conditions. (B) Sprouting in hardwood cuttings in sand rooting substrate. (C-D) Rooting in hardwood cuttings with treatment of 500 mg L^{-1} IBA in sand rooting substrate. (E) Plant produced from hardwood cuttings. (F-G) Field plantation and growth of plants produced from hardwood cuttings.

The cuttings of 1.5-2.5cm thickness were significantly affected ($P< 0.01$) by the various concentrations of auxins (Table 3). Cuttings treated with 500 mg L^{-1} IBA were produced maximum values for studied parameters in term of rooting percentages (80.0%), number of sprouts (6.57) and roots (50.19), root length (7.00 cm) and number of leaves (7.0) while these were found minimum in control 15.0% (rooting percentages), 1.67 (number of sprouts), 3.0 (numbers of root), 1.10 cm (length of root) and 2.00 (numbers of leaves). The clusters of results indicate that for these size of cuttings the highest 8 clusters obtained for numbers and length of roots whereas lowest 3 clusters are obtained for number of leaves (Table 4).

The study of main effects revealed that, the effect of auxin independently was found highly significant ($P< 0.001$) for rooting percentage, sprout number, roots number, while found significant ($P< 0.01$ and $P< 0.03$, respectively) effect on root length and leaves number. DMRT analyses described that 1.5 cm cuttings diameter had significant higher rooting percent, number of sprout, number of roots, root length and number of leaves than > 0.5 cm and 1.5-2.5 cm diameter of hardwood cuttings. The effect of auxin concentration showed highly significant ($P< 0.001$) effect on rooting percent, number of sprout, number of roots, root length and number of leaves.

DMRT analyses described that 0.5-1.5 cm diameter of cuttings had significant higher rooting percent, number of sprout, number of roots, root length and number of leaves than > 0.5 cm and 1.5-2.5 cm diameter of hardwood cuttings. Two factors ANOVA (Table 5) described that, the interaction of diameter and auxin was not found significant effect on rooting percent, number of sprout, number of roots, root length and number of leaves. Two factors ANOVA described that the interaction of diameter and auxin concentration was found highly significant ($P< 0.001$) for number of sprout, but not significant effect on rooting percent, number of roots, root length and number of leaves. Two factors ANOVA described the interaction of auxin and their concentration was found highly significant ($P< 0.001$) effect on rooting percent and number of roots, while not found significant effect on number of sprout, root length and number of leaves. Three factors ANOVA described that, the interaction of diameter, auxin and concentration was not found significant effect on rooting percent, number of sprout, number of roots, root length and number of leaves (Table 5).

The effect of diameter was found highly significant ($P< 0.001$) effect on rooting percent, while not found significant on number of sprout, number of roots, root length and number of leaves. DMRT analyses described that 0.5-1.5 cm diameter of cuttings had significant higher rooting percent, number of sprout, number of roots, root length and number of leaves than > 0.5 cm and 1.5-2.5 cm diameter of hardwood cuttings.

Interactive effect of cuttings diameter and auxins on hardwood cuttings in vermiculite rooting media

With this study we tried to identify the variation effect of various auxins doses within hardwood cuttings (> 0.5, 0.5-1.5 and 1.5-2.5 cm diameter) in vermiculite rooting media. Table 6 showed the performance of cutting (> 0.5 cm thickness) in association with various doses of auxins in vermiculite rooting media. These treatments are significantly ($P< 0.01$) differ with various auxins concentration. The maximum percentages of rooting were registered by two auxin (500 mg L^{-1} IBA and IAA) which have 65.0% and the minimum rooting percentages were observed in cutting with no hormone treatment (control). The numbers of sprouts were varied from 1.71 (100 mg L^{-1} IAA) to 7.15 (500 mg L^{-1} IBA). The maximum numbers of root, length of root and numbers of leaves were observed 36.15, 6.10 and 6.00, respectively in treatment of 500 mg L^{-1} IBA, while these were minimum in controlled which have 2.50, 1.25 and 2.50, respectively. Produced data of DMRT analyses indicates that maximum clusters were obtained 7 (numbers of sprouts) groups while minimum were 2 (rooting percentage).

Table 7 exposed the performance of cutting (0.5-1.5 cm thickness) in association with different doses of three auxins in vermiculite media. Percentages of rooting in uprooted cuttings, numbers of sprouts and leaves were varied from minimum 10.00%, 2.50 and 2.00, respectively (observed in control treatment) to maximum 80.00%, 44.56 and 7.31, respectively (500 mg L^{-1} IBA). While the numbers of sprouts and root length were observed minimum in 100 mg L^{-1} NAA (1.83 and 1.87cm) and these were maximum in 500 mg L^{-1} IBA (7.11 and 7.00). Duncan's multiple range test classified these values in maximum 6 clusters (for numbers of sprouts and root length).

Table 4. Effect of different auxin concentrations (IBA, IAA and NAA) on rooting percentage, number of sprout, number of root, root length (cm) and number of leaves in hardwood cuttings (1.5-2.5 cm diameter) of *Azadirachta indica* in sand during monsoon season.

Auxin (mg L^{-1})	Rooting (%)	Number of sprout	Number of roots	Root length (cm)	Number of leaves
Control	15.00 (20.24)^{e}	1.67±0.33^{e}	3.00±0.00^{h}	1.10±0.06^{h}	2.00±0.00^{c}
IBA					
100	25.00 (29.73)^{de}	2.25±0.16^{de}	5.33±0.44^{h}	2.17±0.24^{fgh}	2.89±0.20^{c}
250	30.00 (32.90)^{de}	3.30±0.37^{cd}	16.82±1.79^{fg}	3.36±0.54^{defg}	3.45±0.25^{ab}
500	80.00 (63.44)^{a}	6.57±0.47^{a}	50.19±2.60^{a}	7.00±0.70^{a}	7.00±0.68^{a}
1,000	80.00 (63.44)^{a}	6.00±0.37^{a}	49.59±2.22^{a}	6.59±0.62^{a}	6.00±0.75^{a}
1,500	70.00 (57.11)^{ab}	5.42±0.57^{ab}	28.80±3.23^{cde}	4.27±0.58^{bcdef}	3.20±0.43^{ab}
Mean	57.00 (49.32)	4.71	30.15	4.69	4.51
IAA					
100	30.00 (32.90)^{de}	2.00±0.00^{de}	3.88±0.40^{h}	1.78±0.15^{gh}	2.75±0.16^{c}
250	45.00 (42.12)^{bcd}	2.89±0.39^{cde}	15.10±1.99^{fg}	3.01±0.58^{efgh}	3.10±0.28^{ab}
500	65.00 (54.22)^{abc}	5.41±0.53^{ab}	43.54±4.12^{ab}	6.36±0.61^{ab}	6.08±0.75^{a}
1,000	45.00 (41.83)^{bcd}	5.08±0.54^{ab}	39.57±3.41^{b}	5.85±0.77^{abc}	5.43±0.74^{a}
1,500	45.00 (41.83)^{bcd}	4.38±0.46^{bc}	26.89±3.68^{de}	4.00±0.53^{cdef}	3.33±0.58^{ab}
Mean	46.00 (42.58)	3.95	27.79	4.20	4.14
NAA					
100	40.00 (38.95)^{cd}	2.00±0.00^{de}	3.38±0.42^{h}	1.75±0.16^{gh}	2.50±0.19^{c}
250	55.00 (48.17)^{abc}	2.20±0.25^{de}	11.73±1.65^{gh}	2.51±0.29^{efgh}	2.82±0.30^{c}
500	65.00 (54.22)^{abc}	5.18±0.52^{ab}	38.15±3.97^{bc}	6.00±0.72^{abc}	5.85±0.64^{a}
1,000	65.00 (54.22)^{abc}	5.00±0.53^{ab}	36.46±3.89^{bcd}	5.46±0.55^{abcd}	5.08±0.70^{ab}
1,500	60.00 (51.05)^{abc}	3.55±0.43^{cd}	24.33±2.58^{ef}	4.42±0.67^{bcde}	2.75±0.45^{c}
Mean	57.00 (49.32)	3.59	22.81	4.03	3.80
Total mean	50.94 (45.40)	4.30	29.02	4.60	4.37

*Arc sine values in parentheses. Values within the column followed by different letters significantly different at $P \leq 0.05$ level as determined using Duncan's multiple range test. A value represents mean ± standard error.

Table 5. Analysis of variance (ANOVA) for effects of different auxin concentrations (IBA, IAA and NAA) and diameter (>0.5, 0.5-1.5 and 1.5-2.5 cm) on rooting percentage, number of sprout, number of root, root length and number of leaves in hardwood cuttings of *Azadirachta indica* in sand during monsoon season.

Source of variance	Rooting (%)	Number of sprout	Number of roots	Root length (cm)	Number of leaves
Auxin					
df	2.00	2.00	2.00	2.00	2.00
F - value	13.29	23.99	17.82	4.05	3.39
p - value	0.00	0.00	0.00	0.01	0.03
Concentration					
DF	4.00	4.00	4.00	4.00	4.00
F - value	53.48	47.89	232.36	72.68	50.55
p - value	0.00	0.00	0.00	0.00	0.00
Diameter					
df	2.00	2.00	2.00	2.00	2.00
F - value	6.10	1.79	1.26	0.86	0.25
p - value	0.00	0.16	0.28	0.42	0.77
Diameter × Auxin					
df	4.00	4.00	4.00	4.00	4.00
F - value	0.07	0.44	0.25	0.22	0.60
p - value	0.99	0.77	0.91	0.93	0.99
Diameter × Concentration					
df	8.00	8.00	8.00	8.00	8.00
F - value	1.02	2.95	1.13	0.36	0.72
p - value	0.42	0.00	0.23	0.94	0.67
Auxin × Concentration					
df	8.00	8.00	8.00	8.00	8.00
F - value	8.66	0.41	3.53	0.94	0.34
p - value	0.00	0.91	0.00	0.48	0.95
Auxin × Concentration × Diameter					
df	16.00	16.00	16.00	16.00	16.00
F - value	0.78	0.35	0.60	0.29	0.23
p - value	0.71	0.99	0.88	0.99	0.99

Table 8 showed the effect of auxins on rooting percentages, numbers of sprouts, number of roots, root length and number of leaves in hardwood cutting of (1.5-2.5 cm thickness) in vermiculite rooting media. The performance of hardwood cutting in term of rooting percentage, number of sprouts, number of root and root length were ranged from 10.00%, 1.67, 2.33 and 1.13 cm (control) to 80.00%, 6.13, 44.13 and 6.53cm, respectively (500 mg L^{-1} IBA) while the minimum number of leaves were observed 2.00 (in control as well as in 100 mg L^{-1} NAA) and maximum were 6.50 (again in IBA 500 mg L^{-1}).The clusters of results indicate that for these size of cuttings the highest 7 clusters obtained for numbers of roots whereas lowest 3 clusters are obtained for number of leaves.

The study of main effects revealed that (Table 9), the effect of auxin was found highly significant ($P< 0.001$) for number of sprout and number of roots, while found significant effect on rooting percent, root length and number of leaves. DMRT analyses described that 0.5-1.5 cm diameter of cuttings had significant higher rooting percent, number of sprout, number of roots, root length and number of leaves than > 0.5 cm and 1.5-2.5 cm diameter of hardwood cuttings. The effect of auxin concentration showed highly significant ($P< 0.001$) effect on rooting percent, number of sprout, number of roots, root length and number of leaves. DMRT analyses described that 0.5-1.5 cm diameter of cuttings had significant higher rooting percent, number of sprout, number of roots, root length and number of leaves than > 0.5 cm and 1.5-2.5 cm diameter of hardwood cuttings. The effect of diameter was found significant effect on number off sprouts, while not found significant on rooting percent, number of roots, root length and number of leaves. DMRT analyses described that 0.5-1.5 cm diameter of cuttings had significant higher rooting percent, number of sprout, number of roots, root length and number of leaves than > 0.5 cm and 1.5-2.5 cm diameter of hardwood cuttings. Two factors ANOVA described that, the interaction of diameter and auxin was not found significant effect on rooting percent, number of sprout, number of roots, root length and number of leaves. Two factors ANOVA described that, the interaction of diameter and auxin concentration was not significant effect on number of sprout, rooting percent, number of roots, root length and number of leaves. Two factors ANOVA described the interaction of auxin and their concentration was found highly significant (*P*< 0.001) effect on number of roots, while not found significant effect on rooting percent, number of sprout, root length and number of leaves. Three factors ANOVA described that, the interaction of diameter, auxin and concentration was not found significant effect on rooting percent, number of sprout, number of roots, root length and number of leaves (Table 9).

Table 6. Effect of different auxin concentrations (IBA, IAA and NAA) on rooting percentage, number of sprout, number of root, root length (cm) and number of leaves in hardwood cuttings (> 0.5 cm diameter) of *Azadirachta indica* in vermiculite during monsoon season.

Auxin (mg L^{-1})	Rooting (%)	Number of sprout	Number of roots	Root length (cm)	Number of leaves
Control	10.00 (13.92)^{c}	2.50±0.29^{efg}	2.50±0.29^{d}	1.25±0.14^{d}	2.50±0.29^{c}
IBA					
100	35.00 (36.06)^{ab}	2.71±0.29^{defg}	4.29±0.52^{cd}	2.14±0.14^{cd}	3.14±0.34^{bc}
250	60.00 (51.05)^{a}	4.17±0.94^{cde}	12.42±1.73^{c}	2.30±0.20^{cd}	2.92±0.26^{c}
500	65.00 (54.22)^{a}	7.15±0.49^{a}	36.15±3.00^{a}	6.10±0.82^{a}	6.00±0.91^{a}
1,000	60.00 (47.90)^{a}	6.33±0.61^{ab}	33.73±3.51^{a}	6.08±0.68^{a}	5.42±0.79^{ab}
1,500	55.00 (48.17)^{a}	4.72±0.38^{bcd}	32.46±2.74^{a}	3.82±0.77^{abc}	4.18±0.76^{abc}
Mean	55.00 (47.48)	5.01	23.81	4.09	4.33
IAA					
100	35.00 (35.78)^{ab}	1.71±0.29^{g}	3.14±0.51^{cd}	2.31±0.46^{cd}	3.00±0.38^{c}
250	50.00 (45.00)^{ab}	3.20±0.57^{cdefg}	11.40±1.18^{cd}	2.80±0.42^{bcd}	3.10±0.35^{bc}
500	65.00 (54.22)^{a}	5.31±0.65^{abc}	35.75±3.09^{a}	5.20±0.85^{a}	4.69±0.65^{abc}
1,000	60.00 (50.77)^{a}	4.80±0.57^{bcd}	35.66±4.42^{a}	5.08±0.72^{a}	4.75±0.73^{abc}
1,500	50.00 (45.00)^{ab}	4.58±0.62^{bcde}	28.70±3.11^{ab}	4.67±0.91^{ab}	3.50±0.81^{bc}
Mean	52.00 (46.15)	3.92	22.93	4.01	3.81
NAA					
100	25.00 (26.58)^{bc}	2.00±0.00^{fg}	3.80±0.73^{cd}	1.84±0.16^{cd}	2.60±0.24^{c}
250	50.00 (44.72)^{ab}	3.90±0.62^{cdef}	6.20±1.08^{cd}	2.60±0.40^{bcd}	2.90±0.35^{c}
500	60.00 (50.77)^{a}	4.00±0.26^{cdef}	29.00±1.71^{ab}	5.42±0.76^{a}	4.33±0.61^{abc}
1,000	50.00 (45.00)^{ab}	3.91±0.63^{cdef}	28.80±3.45^{ab}	5.08±0.69^{a}	3.56±0.84^{bc}
1,500	40.00 (39.23)^{ab}	3.88±0.73^{cdef}	21.33±2.48^{b}	5.08±0.61^{a}	3.50±0.54^{bc}
Mean	45.00 (41.26)	3.54	17.83	4.00	3.38
Total mean	48.13 (43.02)	4.38	23.19	4.18	3.96

*Arc sine values in parentheses. Values within the column followed by different letters significantly different at $P \le 0.05$ level as determined using Duncan's multiple range test. A value represents mean ± standard error.

Table 7. Effect of different auxin concentrations (IBA, IAA and NAA) on rooting percentage, number of sprout, number of root, root length (cm) and number of leaves in hardwood cuttings (0.5-1.5 cm diameter) of *Azadirachta indica* in vermiculite during monsoon season.

Auxin (mg L^{-1})	Rooting (%)	Number of sprout	Number of roots	Root length (cm)	Number of leaves
Control	10.00 (13.92)^{d}	2.50±0.29^{def}	2.50±0.29^{e}	2.10±0.52^{f}	2.00±0.00^{e}
IBA					
100	35.00 (36.06)^{bcd}	2.57±0.20^{def}	3.86±0.51^{e}	2.29±0.18^{ef}	2.86±0.14^{e}
250	45.00 (38.68)^{bc}	5.70±0.62^{abc}	12.89±1.70^{de}	2.50±0.35^{ef}	3.22±0.36^{de}
500	80.00 (63.44)^{a}	7.11±1.87^{a}	44.56±3.82^{a}	7.00±0.70^{a}	7.31±0.59^{a}
1,000	75.00 (60.27)^{ab}	7.00±0.37^{a}	44.13±3.04^{a}	6.27±0.69^{ab}	6.91±0.72^{ab}
1,500	50.00 (45.00)^{abc}	6.33±0.43^{ab}	32.20±4.43^{bc}	4.90±0.69^{abcd}	3.60±0.58^{cde}
Mean	57.00 (48.69)	5.74	27.53	4.59	4.78
IAA					
100	35.00 (32.35)^{cd}	2.14±0.26^{ef}	4.00±0.44^{e}	2.46±0.47^{ef}	2.86±0.14^{e}
250	50.00 (45.00)^{abc}	4.40±1.02^{bcd}	10.80±1.71^{e}	2.85±0.60^{def}	2.90±0.28^{e}
500	60.00 (51.34)^{abc}	6.45±0.61^{ab}	37.50±3.60^{ab}	6.25±0.59^{ab}	5.83±0.81^{ab}
1,000	55.00 (48.17)^{abc}	5.92±0.36^{abc}	32.00±3.27^{bc}	5.73±0.71^{ab}	5.40±0.77^{abc}
1,500	50.00 (44.72)^{abc}	3.90±0.48^{cdef}	22.20±1.31^{cd}	4.30±0.67^{bcde}	2.60±0.48^{e}
Mean	50.00 (44.32)	4.56	21.30	4.32	3.92
NAA					
100	30.00 (32.61)^{cd}	1.83±0.17^{f}	3.67±0.71^{e}	1.87±0.13^{ef}	2.50±0.22^{e}
250	50.00 (45.00)^{abc}	3.80±0.94^{cdef}	7.70±1.34^{e}	2.66±0.31^{ef}	3.30±0.21^{de}
500	65.00 (54.22)^{abc}	5.00±0.64^{abc}	36.66±4.22^{ab}	5.75±0.73^{ab}	5.15±0.67^{bcd}
1,000	60.00 (51.05)^{abc}	4.83±0.44^{abcd}	30.15±3.32^{bc}	5.23±0.61^{abc}	5.08±0.76^{bcd}
1,500	45.00 (41.83)^{abc}	4.11±0.48^{bcdef}	25.78±3.34^{cde}	3.56±0.60^{cdef}	3.11±0.54^{de}
Mean	50.60 (44.94)	3.91	20.79	3.81	3.83
Total mean	49.69 (43.98)	5.00	25.92	4.57	4.45

*Arc sine values in parentheses. Values within the column followed by different letters significantly different at $P \le 0.05$ level as determined using Duncan's multiple range test. A value represents mean ± standard error.

Table 8. Effect of different auxin concentrations (IBA, IAA and NAA) on rooting percentage, number of sprout, number of root, root length (cm) and number of leaves in hardwood cuttings (1.5-2.5 cm diameter) of *Azadirachta indica* in vermiculite during monsoon season.

Auxin (mg L^{-1})	Rooting (%)	Number of sprout	Number of roots	Root length (cm)	Number of leaves
Control	10.00 (13.92)^{e}	1.67±0.33^{c}	2.33±0.67^{g}	1.13±0.09^{d}	2.00±0.58^{c}
IBA					
100	30.00 (32.90)^{cde}	2.57±0.30^{de}	5.14±0.26^{g}	2.43±0.30^{cd}	2.43±0.20^{c}
250	40.00 (38.95)^{bcd}	3.88±0.55^{bcd}	11.38±2.09^{efg}	2.94±0.52^{acd}	3.63±0.38^{bc}
500	80.00 (63.44)^{a}	6.13±0.40^{a}	44.13±3.81^{a}	6.53±0.58^{a}	6.50±0.75^{a}
1,000	75.00 (63.43)^{a}	5.33±0.65^{ab}	36.27±3.13^{ab}	6.13±0.61^{a}	5.47±0.62^{ab}
1,500	45.00 (42.12)^{abcd}	4.82±0.62^{abc}	30.33±4.81^{bc}	4.22±0.88^{abc}	3.22±0.70^{bc}
Mean	54.00 (48.16)	4.55	25.45	4.45	4.25
IAA					
100	30.00 (29.46)^{cde}	2.17±0.17^{de}	5.00±0.26^{g}	2.20±0.59^{cd}	2.50±0.22^{c}
250	40.00 (38.95)^{bcd}	2.50±0.27^{de}	9.63±2.04^{fg}	2.25±0.16^{cd}	3.00±0.33^{bc}
500	55.00 (47.89)^{abc}	4.90±0.48^{abc}	35.80±3.36^{ab}	4.60±0.85^{abc}	4.50±0.92^{abc}
1,000	50.00 (44.72)^{abcd}	4.64±0.64^{abc}	28.73±3.55^{bcd}	4.38±1.00^{abc}	4.27±0.80^{abc}
1,500	40.00 (35.51)^{bcd}	4.38±0.50^{bc}	19.00±2.88^{def}	4.36±0.81^{abc}	2.13±0.64^{c}
Mean	43.00 (39.31)	3.72	19.63	3.56	3.28
NAA					
100	20.00 (23.41)^{cde}	2.00±0.00^{de}	4.50±0.50^{g}	1.55±0.26^{cd}	2.00±0.41^{c}
250	40.00 (39.23)^{bcd}	2.50±0.27^{de}	7.88±1.63^{g}	2.13±0.23^{cd}	2.75±0.41^{c}
500	70.00 (57.11)^{ab}	4.29±0.41^{bc}	30.87±2.34^{bc}	5.73±0.80^{ab}	4.43±0.70^{abc}
1,000	55.00 (47.89)^{abc}	3.27±0.32^{cde}	31.79±3.17^{bc}	4.43±0.45^{abc}	4.09±0.77^{abc}
1,500	40.00 (38.95)^{bcd}	3.25±0.49^{cde}	21.13±1.85^{cde}	3.63±0.80^{bcd}	2.63±0.60^{c}
Mean	45.00 (41.32)	3.06	19.23	3.49	3.18
Total mean	45.00 (41.12)	4.00	24.68	4.21	3.92

*Arc sine values in parentheses. Values within the column followed by different letters significantly different at $P \leq 0.05$ level as determined using Duncan's multiple range test. A value represents mean ± standard error.

Table 9. Analysis of variance (ANOVA) for effects of different auxin concentrations (IBA, IAA and NAA) and diameter (> 0.5, 0.5-1.5 and 1.5-2.5 cm) on rooting percentage, number of sprout, number of root, root length and number of leaves in hardwood cuttings of *Azadirachta indica* in vermiculite during monsoon season.

Source of variance	Rooting (%)	Number of sprout	Number of roots	Root length (cm)	Number of leaves
Auxin					
df	2.00	2.00	2.00	2.00	2.00
F - value	1.94	24.60	14.97	0.81	2.54
p - value	0.14	0.00	0.00	0.44	0.08
Concentration					
df	4.00	4.00	4.00	4.00	4.00
F - value	19.90	30.47	167.24	49.98	32.93
p - value	0.00	0.00	0.00	0.00	0.00
Diameter					
df	2.00	2.00	2.00	2.00	2.00
F - value	0.69	3.58	0.41	0.84	0.88
p - value	0.50	0.02	0.66	0.43	0.41
Diameter × Auxin					
df	4.00	4.00	4.00	4.00	4.00
F - value	0.65	0.20	1.42	1.78	0.80
p - value	0.62	0.93	0.22	0.13	0.52
Diameter × Concentration					
df	8.00	8.00	8.00	8.00	8.00
F - value	0.60	1.38	1.05	0.51	1.47
p - value	0.77	0.20	0.40	0.85	0.16
Auxin × Concentration					
df	8.00	8.00	8.00	8.00	8.00
F - value	0.55	1.41	1.55	0.40	0.80
p - value	0.81	0.19	0.13	0.91	0.59
Auxin × Concentration × Diameter					
df	16.00	16.00	16.00	16.00	16.00
F - value	0.38	0.95	0.79	0.73	0.66
p - value	0.98	0.51	0.70	0.76	0.83

Interactive effect of cuttings diameter and auxins treatment on hardwood cuttings in soil rooting media

In the present investigation, three diameters (> 0.5, 0.5-1.5 and 1.5-2.5 cm) of hardwood cutting were used for auxin treatment in soil. Among the different cutting diameters used, cutting having 0.5-1.5 cm diameter showed significant results in terms of rooting (60%), number of sprout (6.66), number of roots (43.10), root length (6.67 cm) and numbers of leaves (7.58) followed by cutting having 1.5-2.5 cm diameter showed results in terms of rooting (60%), number of sprout (7.14), number of roots (36.78), root length (7.38 cm) and numbers of leaves (5.87) and cutting having > 0.5 cm diameter showed results in terms of rooting (60%), number of sprout (6.40), number of roots (37.55), root length (5.90 cm) and numbers of leaves (6.73). When we study auxin effect and their concentration on individual cutting diameter in sand rooting substrate, the results revealed that, in > 0.5 cm cutting diameters showed maximum rooting (60%), number of sprout (6.40), number of roots (37.55), root length (5.90 cm) and numbers of leaves (6.73) was observed, when cuttings treated with 500 mg L^{-1} IBA, followed by rooting (55%), number of sprout

(5.66), number of roots (37.27), root length (5.75 cm) and numbers of leaves (5.80) was observed, when cuttings treated with 1,000 mg L^{-1} IBA.

The outcomes in other auxin used for cutting treatments of revealed that 500 mg L^{-1} IAA showed rooting (50%), number of sprout (5.38), number of roots (34.60), root length (5.25 cm) and numbers of leaves (4.90), whereas 500 mg L^{-1} NAA showed rooting (50%), number of sprout (4.38), number of roots (35.17), root length (4.64 cm) and numbers of leaves (4.36) respectively (Table 10).

The effect of auxin and their concentration on 0.5-1.5 cm cuttings diameter in sand rooting substrate, concluded that maximum rooting (60%), number of sprout (6.66), number of roots (43.10), root length (6.67 cm) and numbers of leaves (7.58) was observed, when cuttings treated with 500 mg L^{-1} IBA, followed by rooting (60%), number of sprout (6.30), number of roots (39.92), root length (6.56 cm) and numbers of leaves (7.00) was observed, when cuttings treated with 1,000 mg L^{-1} IBA. The outcomes in other auxin used for cutting treatments revealed that 500 mg L^{-1} IAA showed rooting (50%), number of sprout (6.16), number of roots (28.33), root length (5.60 cm) and numbers of leaves (5.58), whereas 500 mg L^{-1} NAA showed rooting (60%), number of sprout (5.00), number of roots (39.11), root length (5.83 cm) and numbers of leaves (5.66) respectively (Table 11).

Table 10. Effect of different auxin concentrations (IBA, IAA and NAA) on rooting percentage, number of sprout, number of root, root length (cm) and number of leaves in hardwood cuttings (> 0.5 cm diameter) of *Azadirachta indica* in soil during monsoon season.

Auxin (mg L^{-1})	Rooting (%)	Number of sprout	Number of roots	Root length (cm)	Number of leaves
Control	5.00 (7.60)^{e}	2.00±0.00^{ef}	3.00±0.00^{d}	1.75±0.14^{d}	2.00±0.00^{e}
IBA					
100	20.00 (19.97)^{de}	2.16±0.17^{ef}	4.17±0.54^{d}	1.83±0.17^{d}	2.33±0.21^{de}
250	30.00 (29.74)^{bcd}	3.83±0.60^{bcdef}	9.00±2.13^{d}	2.85±0.47^{bcd}	3.00±0.37^{bcde}
500	60.00 (50.77)^{a}	6.40±0.54^{a}	37.55±3.53^{a}	5.90±0.82^{a}	6.73±0.78^{a}
1,000	55.00 (47.89)^{ab}	5.66±0.50^{ab}	37.27±3.84^{a}	5.75±0.73^{a}	5.80±1.09^{ab}
1,500	45.00 (42.12)^{abc}	5.27±0.75^{abc}	25.40±5.41^{abc}	5.00±1.11^{ab}	3.56±0.97^{bcde}
Mean	42.00 (38.09)	4.66	22.68	4.26	4.29
IAA					
100	20.00 (26.56)^{cd}	1.75±0.25^{f}	3.75±0.85^{d}	1.80±0.20^{d}	3.00±0.00^{bcde}
250	40.00 (39.23)^{abcd}	3.00±0.38^{def}	8.13±1.20^{d}	2.38±0.18^{bcd}	3.00±0.33^{bcde}
500	50.00 (44.72)^{abc}	5.38±0.91^{abc}	34.60±3.38^{ab}	5.25±0.94^{ab}	4.90±0.84^{abcd}
1,000	40.00 (38.95)^{abcd}	5.20±0.80^{abc}	28.13±7.35^{abc}	4.43±1.09^{abc}	4.87±0.83^{abcd}
1,500	35.00 (36.06)^{abcd}	5.14±0.59^{abcd}	15.00±2.80^{cd}	4.38±0.96^{ab}	3.14±0.51^{bcde}
Mean	37.00 (37.10)	4.09	17.92	3.65	3.78
NAA					
100	25.00 (29.73)^{bcd}	1.80±0.20^{f}	4.20±0.58^{d}	1.84±0.16^{d}	2.60±0.24^{de}
250	40.00 (39.23)^{abcd}	3.38±0.53^{cdef}	5.63±0.63^{d}	2.13±0.13^{cd}	2.88±0.30^{cde}
500	50.00 (45.00)^{abc}	4.38±0.56^{abcd}	35.17±5.07^{ab}	4.64±0.65^{abc}	4.36±0.86^{abcde}
1,000	50.00 (45.00)^{abc}	4.00±0.71^{bcde}	30.44±3.36^{ab}	5.09±1.08^{ab}	5.58±0.87^{abc}
1,500	40.00 (38.67)^{abcd}	3.75±0.41^{bcdef}	22.75±2.48^{bc}	4.80±0.73^{abc}	2.50±0.46^{de}
Mean	41.00 (39.53)	3.46	19.64	3.70	3.58
Total mean	37.81 (36.33)	4.24	22.58	4.12	4.10

*Arc sine values in parentheses. Values within the column followed by different letters significantly different at $P \leq 0.05$ level as determined using Duncan's multiple range test. A value represents mean ± standard error.

Table 11. Effect of different auxin concentrations (IBA, IAA and NAA) on rooting percentage, number of sprout, number of root, root length (cm) and number of leaves in hardwood cuttings (0.5-1.5 cm diameter) of *Azadirachta indica* in soil during monsoon season.

Auxin (mg L^{-1})	Rooting (%)	Number of sprout	Number of roots	Root length (cm)	Number of leaves
Control	5.00 (7.60)^{d}	1.00±0.00^{h}	2.00±0.00^{g}	1.00±0.00^{h}	2.00±0.00^{f}
IBA					
100	20.00 (26.56)^{c}	2.40±0.24^{fgh}	3.40±0.87^{fg}	2.20±0.20^{fgh}	2.20±0.20^{f}
250	40.00 (39.23)^{abc}	3.63±0.46^{def}	8.38±1.60^{efg}	3.51±0.73^{cdefg}	3.25±0.25^{def}
500	60.00 (50.77)^{a}	6.66±0.38^{a}	43.10±4.14^{a}	6.67±0.74^{a}	7.58±0.67^{a}
1,000	60.00 (50.77)^{a}	6.30±0.65^{ab}	39.92±3.62^{ab}	6.56±0.77^{a}	7.00±0.75^{ab}
1,500	45.00 (41.83)^{ab}	5.44±0.63^{abcd}	28.00±3.07^{bcd}	5.00±0.60^{abcd}	3.22±0.60^{def}
Mean	45.00 (41.83)	4.88	24.56	4.78	4.65
IAA					
100	25.00 (29.73)^{ab}	2.00±0.32^{fgh}	3.80±0.37^{dfg}	2.04±0.29^{gh}	2.80±0.20^{ef}
250	50.00 (45.00)^{ab}	4.40±1.02^{bcde}	9.10±1.62^{efg}	2.96±0.59^{defg}	3.20±0.25^{def}
500	50.00 (45.00)^{ab}	6.16±0.42^{ab}	28.33±5.03^{bcd}	5.60±0.72^{abc}	5.58±0.90^{abc}
1,000	50.00 (45.00)^{ab}	5.80±0.36^{ab}	16.70±2.59^{de}	4.20±0.20^{bcdef}	4.70±0.56^{cde}
1,500	30.00 (29.74)^{ab}	3.66±0.61^{abcd}	16.00±5.13^{def}	4.50±0.50^{abcde}	3.00±0.89^{ef}
Mean	41.00 (38.89)	4.40	14.78	3.86	3.86
NAA					
100	25.00 (29.73)^{ab}	1.60±0.24^{gh}	3.20±0.66^{fg}	1.84±0.16^{gh}	2.40±0.24^{ef}
250	45.00 (41.83)^{ab}	3.00±0.29^{efg}	8.78±1.66^{efg}	2.46±0.17^{efgh}	3.11±0.26^{def}
500	60.00 (51.05)^{ab}	5.00±0.47^{abcd}	39.11±5.23^{ab}	5.83±0.68^{ab}	5.66±0.76^{abc}
1,000	50.00 (44.72)^{ab}	4.75±0.64^{abcde}	32.25±4.90^{abc}	5.00±0.62^{ab}	5.33±0.76^{bcd}
1,500	45.00 (41.83)^{ab}	3.90±0.48^{cdef}	22.20±1.31^{cd}	4.30±0.67^{bcdef}	2.60±0.48^{ef}
Mean	43.00 (33.46)	3.65	21.10	3.88	3.82
Total mean	41.25 (38.77)	4.56	22.23	4.38	4.38

*Arc sine values in parentheses. Values within the column followed by different letters significantly different at $P \leq 0.05$ level as determined using Duncan's multiple range test. A value represents mean ± standard error.

The effect of auxin and their concentration on 1.5-2.5 cm cutting diameter in sand rooting substrate, concluded that maximum rooting (60%), number of sprout (7.14), number of roots (36.78), root length (7.38 cm) and numbers of leaves (5.87) was observed, when cuttings treated with 500 mg L^{-1} IBA, followed by rooting (45%), number of sprout (6.14), number of roots (33.50), root length (5.67 cm) and numbers of leaves (5.75) was observed, when cuttings treated with 1,000 mg L^{-1} IBA. The outcomes in other auxin used for cutting treatments revealed that 500 mg L^{-1} IAA showed rooting (40%), number of sprout (6.13), number of roots (19.43), root length (4.29 cm) and numbers of leaves (5.00), whereas 1,000 mg L^{-1} NAA showed rooting (40%), number of sprout (5.63), number of roots (20.88), root length (3.29 cm) and numbers of leaves (5.00) respectively (Table 12).

The study of main effects revealed that, the effect of auxin was found highly significant ($P< 0.001$) for number of sprout and number of roots, while found significant effect on rooting percent, root length and number of leaves. DMRT analyses described that 1.5 cm diameter of cuttings had significant higher rooting percent, number of sprout, number of roots, root length and number of leaves than 0.5 cm and 2.5 cm diameter of hardwood cuttings. The effect of auxin concentration showed highly significant ($P< 0.001$) effect on rooting percent, number of sprout, number of roots, root length and number of leaves. DMRT analyses described that 1.5 cm diameter of cuttings had significant higher rooting percent, number of sprout, number of roots, root length and number of leaves than 0.5 cm and 2.5 cm diameter of hardwood cuttings. The effect of diameter was found highly significant ($P< 0.001$) effect on rooting percent, while not found significant on number of sprout, number of roots, root length and number of leaves. DMRT analyses described that 1.5 cm diameter of cuttings had significant higher rooting percent, number of sprout, number of roots, root length and number of leaves than 0.5 cm and 2.5 cm diameter of hardwood cuttings. Two factors ANOVA described that, the interaction of diameter and auxin was not found significant effect on rooting percent, number of sprout, number of roots, root length and number of leaves. Two factors ANOVA described the interaction of diameter and auxin concentration was not found significant effect on rooting percent, number of sprout, number of roots, root length and number of leaves. Two factors ANOVA described the interaction of auxin and their concentration was found highly significant ($P< 0.001$) effect on number of roots, while not found significant effect on rooting percent, number of sprout, root length and number of leaves. Three factors ANOVA described that, the interaction of diameter, auxin and concentration was highly significant ($P< 0.001$) effect on number of roots, while not found significant effect on rooting percent, number of sprout, root length and number of leaves (Table 13).

Discussion

Rooting is one of the most important events in development of plant. For the successful rooting of *A. indica* cuttings, physiological and morphological effect seems to be the most important. Physiological states of the cuttings play a significant role in determination of success of rooting. The size of planting stock is important for initial survival and establishment of cuttings. The carbohydrate and hormonal content were found to be related to the size of the cutting (length and diameter) and to the original location of the cutting on the stock plant. Basal cuttings of the shoot produced longer and heavier roots than that from tops, while the diameter of the cutting also strongly affects poplar rooting ability (Desrochers and Thomas 2003). The formation of adventitious roots required a high energy process, which involves cell division, in which predetermined cells switch from their morphogenetic path to act as mother cells for the root premordia; hence need more reserve food material for root initiation (Aeschabacher et al. 1994). The most probable reason for good sprouting and rooting of 1.5-2.5 cm cuttings of *A. indica* might be due to more reserved food material, level of inducing factors and quantity of permanent tissues as compared to higher diameter cuttings. The cuttings of 1.5-2.5 cm diameter have more stored food material as well as, it have more permanent tissues and lower amount of root initiation factors resulting the sprouting and rooting capacity reduced gradually from 0.5-1.50 cm to > 0.50 cm cuttings.

Cuttings rooting in many species have also been found to be dependent of the cutting thickness (Hartmann et al. 2011). Fielding (1969) reported that shoot caliper had little effect on rooting percentages, but there was a strong positive relationship between cutting diameter and subsequent growth of rooted cuttings transplanted to nursery beds. Dickman et al. (1980) demonstrated that large diameter cuttings survived better and produced taller shoots than small diameter cuttings. In *Pinus caribea* var. *hondurensis*, *Pinus tecunumanii* cuttings having a diameter of at least 2 mm rooted better than thinner cutting stock (Haines et al. 1992). Diameter/length ratios in plum cuttings have shown that a smaller ratio (thinner, longer shoots) was accompanied by improved rooting (Howard and Ridout 1991b). No discernible patterns were evident from efforts to quantify the effect of cutting length and diameter on rooting by calculating diameter/length ratios similar to those developed by Howard and Ridout (1991a). Rana and Sood (2012) reported that, in both seasons (rainy and spring), large sized (1.25-2.50 cm diameter). *Ficus roxburghii* cuttings resulted in higher growth of all the studied parameters than that of small sized (< 1.25 cm diameter) cuttings, except the number of shoots in both the seasons and number of lateral roots in rainy season.

The effect of hormonal treatments (IBA, IAA and NAA) was also significant on all the studied parameters in both the study seasons except in the case of number of shoots in rainy season where the influence was not-significant. The interaction large size × IBA resulted in significantly better growth of the studied parameters in both seasons except for the number of lateral roots, number of shoots, shoot length and number of leaves in rainy season. The biomass of the roots was significantly higher in the 2.0 cm diameter cuttings than that of the 1.0 cm or 0.5 cm diameter and this is consistent with the results obtained in *Dalbergia sissoo* (Ansari et al. 1995). In addition, to reserve food material other inducing factor, such as growth regulator, plays an important role on adventitious rooting in plant (Hartmann and Kester 1981). Efficiency for root induction depends upon the presence of endogenous level of auxin. Jacobs (1979) reported that the endogenous auxin content was higher in the shoot tip portion and decrease as the distance increase from the apices in the same plant. In *A. india* the endogenous auxin level in the branch is presumably in the order of 0.50 > 0.50-1.5 cm > 1.5-2.5 cm, but the permanent tissue and reserve material in order of 2.5-1.5 cm > 1.5-0.5 cm > 0.5 resulting in the best combination of auxin and reserve material are found in 0.5-1.5 cm cuttings. Stem cuttings which have appropriate level of auxins and carbohydrates are especially suitable for adventitious rooting. A number of researchers have shown that rooting is facilitated when the carbohydrate reserve food material is in abundance (Haissig 1974).

Table 12. Effect of auxin concentrations (IBA, IAA and NAA) on rooting percentage, number of sprout, number of root, root length (cm) and number of leaves in hardwood cuttings (1.5-2.5 cm diameter) of *Azadirachta indica* in soil during monsoon season.

Auxin (mg L^{-1})	Rooting (%)	Number of sprout	Number of roots	Root length (cm)	Number of leaves
Control	0.00 (1.28)^{d}	0.00 ±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{f}	0.00±0.00^{d}
IBA					
100	25.00 (29.73)^{ab}	2.25±0.25^{cd}	3.00±0.71^{e}	1.80±0.20^{def}	2.75±0.25^{abcd}
250	30.00 (32.61)^{ab}	2.83±0.40^{cd}	10.50±2.19^{bcde}	2.08±0.20^{def}	2.83±0.31^{abcd}
500	60.00 (47.89)^{a}	7.14±0.55^{a}	36.78±3.71^{a}	7.38±0.78^{a}	5.87±1.29^{a}
1,000	45.00 (41.83)^{ab}	6.14±0.55^{ab}	33.50±4.07^{a}	5.67±0.73^{ab}	5.75±0.86^{ab}
1,500	35.00 (35.78)^{ab}	6.08±0.45^{ab}	18.86±2.03^{bc}	3.00±0.72^{cde}	4.00±0.65^{abc}
Mean	39.00 (37.56)	4.89	20.53	3.99	4.24
IAA					
100	20.00 (26.56)^{BC}	2.00±0.00^{d}	5.25±0.48^{de}	1.75±0.25^{ef}	2.25±0.48^{cd}
250	35.00 (35.78)^{abc}	2.50±0.38^{cd}	7.25±1.32^{cde}	2.13±0.13^{def}	3.00±0.33^{abcd}
500	40.00 (38.95)^{abc}	6.13±0.58^{ab}	19.43±4.26^{bc}	4.29±0.89^{bcd}	5.00±1.20^{abc}
1,000	40.00 (35.51)^{abc}	5.14±0.80^{ab}	18.14±5.18^{bcd}	3.75±0.37^{bcde}	3.60±0.87^{abc}
1,500	25.00 (29.73)^{abc}	4.20±0.66^{bc}	12.80±2.94^{bcde}	3.00±0.84^{cde}	3.00±0.33^{abcd}
Mean	40.00 (33.31)^{abc}	3.99	12.57	2.98	3.37
NAA					
100	15.00 (20.32)^{c}	2.00±0.00^{d}	3.33±0.88^{e}	1.73±0.27^{ef}	2.33±0.67^{cd}
250	30.00 (32.90)^{abc}	3.00±0.67^{cd}	8.44±1.82^{bcde}	2.11±0.20^{def}	2.66±0.29^{bcd}
500	45.00 (42.12)^{ab}	5.66±0.69^{ab}	16.50±5.13^{bcd}	5.22±1.16^{abc}	5.71±1.23^{abc}
1,000	40.00 (35.51)^{abc}	5.63±0.63^{ab}	20.88±4.56^{b}	3.29±0.61^{cde}	5.00±1.08^{abc}
1,500	35.00 (35.78)^{abc}	2.71±0.36^{cd}	16.57±3.15^{bcd}	2.57±0.43^{ef}	2.85±0.63^{abcd}
Mean	33.00 (33.32)	5.00	13.14	2.98	3.71
Total mean	32.50 (32.64)	4.38	17.10	3.51	3.87

*Arc sine values in parentheses. Values within the column followed by different letters significantly different at $P \leq 0.05$ level as determined using Duncan's multiple range test. A value represents mean ± standard error.

Table 13. Analysis of variance (ANOVA) for effects of different auxin concentrations (IBA, IAA and NAA) and diameter (>0.5, 0.5-1.5 and 1.5-2.5 cm) on rooting percentage, number of sprout, number of root, root length and number of leaves in hardwood cuttings of *Azadirachta indica* in soil during monsoon season.

Source of variance	Rooting (%)	Number of sprout	Number of roots	Root length (cm)	Number of leaves
Auxin					
df	2.00	2.00	2.00	2.00	2.00
F - value	1.53	13.15	3.65	0.68	1.68
p - value	0.21	0.00	0.02	0.50	0.18
Concentration					
df	4.00	4.00	4.00	4.00	4.00
F - value	18.38	48.07	76.98	36.09	30.98
p - value	0.00	0.00	0.00	0.00	0.00
Diameter					
df	2.00	2.00	2.00	2.00	2.00
F - value	3.78	0.28	2.65	2.64	0.95
p - value	0.02	0.75	0.07	0.07	0.38
Diameter × Auxin					
df	4.00	4.00	4.00	4.00	4.00
F - value	0.16	0.29	0.01	1.61	0.60
p - value	0.95	0.87	1.00	0.16	0.83
Diameter × Concentration					
df	8.00	8.00	8.00	8.00	8.00
F - value	0.39	2.19	1.56	1.70	1.64
p - value	0.92	0.20	0.13	0.09	0.11
Auxin × Concentration					
df	8.00	8.00	8.00	8.00	8.00
F - value	1.03	1.21	3.83	0.92	1.15
p - value	0.41	0.29	0.00	0.49	0.32
Auxin × Concentration × Diameter					
df	16.00	16.00	16.00	16.00	16.00
F - value	0.66	0.83	3.00	0.81	0.35
p - value	0.82	0.65	0.00	0.66	0.99

Auxin-induced effect on rooting of cuttings is presumed to be mediated through its effect in mobilizing the reserve food material by enhancing the activity of hydrolytic enzymes (Nanda 1975). On the other hand Kesari et al. (2009) reported that mature stem cuttings which have high level of auxins and carbohydrates are especially suitable for adventitious rooting in *Pongamia pinnata*. Similarly, result was obtained by Singh et al. (2009), they reported that sprouting percentage increased with increasing stem diameter they also observed maximum sprouting in stem diameter of 2.0 cm and above (38.8%), whereas minimum was observed in 0.5 cm (16.10%) in *Commiphora*. The good response of 0.5-1.5 cm cutting size is might be due to the presence of intermediate compound and suitable physiological conditions of cutting. Cutting having diameter of 0.5-1.5 cm presented fast sprouting, due to the presence of reserve material (e.g., fructose, glucose and sucrose), but it does not able to produce best rooting, because to the lack of sufficient concentration of auxins. As compared to the cutting of 0.5-1.5 cm to > 0.5 cm diameter, does not sprouted fast and also not gives sufficient rooting suggests that the level of carbohydrate and other root-inducing factors (i.e., co-factor of adventitious rooting) may be low for rooting in small cuttings 0.5 cm diameter).

The formation of adventitious roots is a high energy requiring process, which involves cell division, in which predetermined cells switch from their morphogenetic path to act as mother cells for the root primordia; hence need more reserve food material for root initiation (Aeschabacher et al. 1994). Apart from reserve food material, other inducing factors such as growth regulator play an important role for adventitious root formation in plants. Efficiency for root induction depends upon the presence of endogenous level of auxins, which is perhaps more in hardwood cutting stage (Hartmann et al. 2011). On the basis of results it has been cleared that hormone (e.g., auxin group) promotes the cutting tissue to produce more number of roots than control. The all studied parameters i.e. rooting percent, number of sprouts, number of roots, root length and no. of leaves was found best with treatment of IBA as compared to IAA and NAA. The maximum rooting was obtained on 500 mg L^{-1} treatment of IBA in hardwood cuttings planted in sand, vermiculite and soil rooting media.

Sivagnanam et al. (1989) reported that stem cuttings of neem dipped into IAA and IBA rooted effectively in mist propagator in 135 days. Leaf less hardwood cuttings of mature neem (15-20 cm long and 1-2 cm in diameter) collected during summer months, treated with 100 mg L^{-1} IBA for 24 hour took about 10-12 weeks to root, but percent rooting was very low. Pal et al. (1994) studied on cheap non-auxinic chemicals for rooting hardwood cuttings of Neem. They study the effect of IBA and non-auxinic chemicals (potassium permanganate, cobalt chloride and ascorbic acid) on the rooting. The results revealed that rooting was found in hardwood cuttings (1-2 cm in diameter and 15-20 cm long), when cutting treated with 100 mg L^{-1} IBA solution for 24 hour by basal dip methods and planted under high humidity conditions with shade exhibited rooting after 10-12 weeks. Singh and Chander (2001) reported that hardwood, semi hardwood and softwood (22.5 cm long and 0.8 cm to 1.25 cm diameter) of neem with IBA, IAA, 2,4-D (i.e., 0; 500 and 1,000 mg L^{-1}) and their combination solution for 5 minutes. They observed maximum rooting in softwood and semi hardwood cuttings treated with 500 mg L^{-1} IBA. Palanisamy and Kumar (2001) reported that branch cuttings (25 cm long ,1.0-1.5 cm of diameter) of neem were taken from 20 year old and treated with water (control), 1,000; 2,000 and 3,000 mg L^{-1} IBA, IAA and thiamine solution for 30 second. The 1,000 mg L^{-1} IBA induced 80% rooting and luxuriant root system during leaf fall season.

Palanisamy and Kumar (1997) stated that in *A. indica* the cuttings from distal end gave better rooting than proximal end supporting our result. Among different environmental factors, temperature, light intensity and relative humidity are the main factors which influence the growth and development of plants considerably (Ozturk and Serdar 2011). The variation in rooting and shooting response may be attributed to the physiological condition of the plant cuttings. Cellular activities during root initiation require availability of sugars which are synthesized due to activity of various hydrolytic enzymes (Nanda 1975). The activity of these enzymes might have been at the highest level during monsoon and post-monsoon months. The failure of cuttings to produce good root system in non-monsoon months may be due to a high rate of metabolism, low enzymatic activity and increased inhibitor – promoter ratio (Nanda 1975).

Similar to our results, Kesari et al. (2009) find relatively poor rooting with IAA treated stem cuttings of *Pongamia pinnata* in comparison to IBA. The production of more ethylene which is known to inhibit the root production and could also be due to the higher metabolic turnover. IBA is less sensitive than IAA to non-biological degradation such as photo-oxidation (Epstein and Ludwig-Muller 1993; De Klerk et al. 1997).

Among three rooting substrate (sand, vermiculite and soil), highest rooting (80%) was on obtained in sand and vermiculite rooting media respectively. Maximum rooting percentage with 500 mg L^{-1} IBA in sand and vermiculite medium is probably due to the reason that optimum concentration of IBA might be responsible to increase the cambial growth at the base of hardwood cuttings that results in differentiation of root primordial and medium support to it, reducing damages during callus formation (Hartmann et al. 2011). Sand provides a porous medium so root can be settled well without damaging. In present finding rooting response of hardwood cuttings of *A. indica* in different rooting media showed that sand was best substrate followed by vermiculite and 500 mg L^{-1} IBA produced best rooting. The high root formation could be attributed probably due to appropriate water holding capacity and good aeration of the rooting substrate. Aeration also plays a very significant role in numbers of root initiation and as well as on root elongation (Khayyat et al. 2007). EL-Naggar and El-Nasharty (2009) reported that potting media as well as nutritional requirements are the most important factors affecting growth of ornamental plants. Khayyat et al. (2007) observed that the type of rooting substrate and their characteristics are of most importance for the quality of rooted cuttings.

Different portion of a single branch vary in their rooting and sprouting response depending on the seasonal, physiological conditions and age factor, thus their response will differ under same environmental conditions. Propagation techniques of hardwood cuttings by using auxins provide an opportunity for the rapid and large scale production of plant with cost effective purpose especially in tree species which are difficult to produce by traditional methods.

Conclusion

The results revealed that the percent of rooting was higher and increased in 0.5-1.5 cm diameter, IBA (500 mg L^{-1}) treatment and in sand rooting. Hardwood cuttings plants in field conditions demonstrated the efficiency of our protocol (Figs. 1E, F and G). However, it is important to consider that plant growth regulator not only influence percentage of rooting, it also may accelerate the onset of the rooting process and increase the number and quality of roots.

Acknowledgements

Authors are thankful to the Director, AFRI, Jodhpur (Rajasthan), India for providing necessary facilities during the course of work. Authors are also grateful to the State Forest Department, Gujarat, India for financial support of the work. AG is also grateful to Prof. Gilvano Ebling Brondani, Federal University of Mato Grosso/UFMT, Cuiabá-MT, Brazil for ameliorating manuscript with valuable suggestions.

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