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Vegetative propagation of *Azadirachta indica*: effect of auxin and rooting media on adventitious root induction in mini-cuttings

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Abstract
Azadirachta indica (Neem), a prodigious multipurpose tree, has immense potential to benefit mankind and to protect the environment. In order to investigate the effects of the auxins hormone and rooting media on root formation in mini-cuttings of *A. indica*, an experiment was conducted using mist system in poly house in summer 2012. The experiment was established on three different rooting media: sand, vermiculite and soil assembled with six concentrations (100; 250; 500; 750; 1,000 and 1,500 mg L^{-1}) of the auxins IBA (indole-3-butyric acid), IAA (indole-3-acetic acid) and NAA (α-naphthalene acetic acid) with randomized block design, each block containing four replicates. The basal long soak methods were used for treating cuttings. The data revealed significant effect of different auxins and rooting media on the Neem establishment. The rooted mini-cuttings were assessed for rooting percent, number of roots, root length and number of leaves. In present finding among all the auxins and rooting media, , IBA (250 mg L^{-1}) showed better results with sand in terms of rooting percent (80%), number of roots (70.63), root length (11.13) and number of leaves (5.25) per rooted mini-cuttings. The formation of healthy plants after hardening under outdoor conditions showed that *A. indica* could be successfully propagated by mini-cuttings techniques.
Key words: Mini-cuttings; IAA; IBA; NAA.

Introduction

Azadirachta indica A. Juss (Neem) is widely known as a versatile tree for urban re-greening, agroforestry systems, fuel and wood production and for a variety of other products including biopesticides. The Neem tree traditionally used in India over the centuries for wide range purposes is rapidly unfolding its potential for restoring degraded lands, protecting the environment in arid zone, fighting harmful pest and serving as a contraceptive. Neem can grow easily in degraded lands and farms without competing with food crops. In Rajasthan, Neem trees were used for social and agro forestry program. Rural people can easily get economic benefits through production of seeds and leaves within a short period of time. It is necessary to popularize the cultivation of Neem to get benefits and economic advantages in urban as well in rural areas. Neem improvement requires understanding of the genetic nature of wild populations, their development and utility of genetic variability. The effective exploration, identification, documentation and use of genetic resources of Neem are imperative for its efficient use (Kundu and Luukkanen 2003).

In recent breeding strategies of Neem differ considerably with the objectives of the tree improvement program. Neem can be bred for its medicine, pesticides, fungicides, nematicides, cosmetics, fodder and organic manure, timber and fuel wood, agro forestry species, shelterbelts, avenue trees, drought and disease resistance. (Kundu and Luukkanen 2003). There is a need to take up tree improvement, which is possible only by multiplication of superior genotypes through vegetative propagation. However, when propagated through various stem cuttings, there is a problem of rooting being delayed, sparse or erratic in certain species. In this regard, the idea of the mini-cutting propagation has faced the limitation that the rooting ability of stem-cuttings decreases with ontogenetic aging and the decline may be faster than reported in the literature.

In *Eucalyptus grandis* for example, the adventitious rooting competence decreased from the fourteenth node up (Paton and Willing 1974), while it took longer in the *E. deglupta*. Assis et al. (1992) observed that clones of *E. saligna*, *E. grandis* and *E. urophylla* that had equally high proportion of stem-cutting rooting *in vitro* showed differential levels of decline in the rooting percentage when managed in clonal hedges. This indicated that some factor related to clone growth, encompassing period between planting and cutting harvest (6 months), could be responsible for these differences. The rooting ability of mini-cutting is much higher than the stem-cuttings, although the benefits vary among the species and clones. In general, in a poor rooting species, on comparing clones from stem-cutting with clones from mini-cuttings, rooting may increase by 40%. The main reasons for such increase are related to higher levels of juvenility and optimal nutritional content of the tissues, which improves the rooting predisposition and speed of root initiation.

The role of plant growth regulators in inducing the adventitious root ability in mini-cutting has been successfully employed in *Eucalyptus cloeziana* (Almeida et al. 2007), *Eucalyptus benthamii* Maiden & Cambage × *Eucalyptus dunnii* Maiden (Brondani et al. 2008), *Eucalyptus globulus* × *Eucalyptus maidennii* (Schwambach et al. 2008) clones of *E. benthamii* × *E. dunnii* (Brondani et al. 2010a; 2010b), *Eucalyptus benthamii* (Brondani et al. 2012) and *Pinus pinaster* (Majada et al. 2011). Plants grown using mini-cutting technique possess largely all the intrinsic, physiological and biochemical efficiencies, thus facilitating them to acclimatize early and perform better under any given natural field conditions. Hence they are superior compared to conventional coppice plants. Mini-cutting is an important cloning technique in the forestry sector, mainly used for the production of selected tree mini-cuttings, allowing genetic characteristics to be maintained (Stape et al. 2001; Wendling and Xavier 2005; Schwambach et al. 2008; Wendling et al. 2010; Majada et al. 2011). The mini-cuttings come from axillary sprouts of plants and used for large scale production of plants (macropropagation) for the first time.

In plant propagation, the induction of roots is a process regulated by environmental and endogenous factors such as temperature, light, hormones (especially auxin), carbohydrates, mineral salts and other molecules. Phyto hormones have direct (i.e., involved in cell division or cell growth) or indirect (i.e., interacting with other hormones or molecules) effects on plants.

Auxins are a group of tryptophan-derived signals, which are involved in most aspects of plant development (Woodward and Bartel 2005) including controlling growth and development, early stages of embryogenesis, organization of apical meristem (phyllotaxy) and branching of the plant aerial parts (apical dominance), formation of main root, lateral and adventitious root initiation (Went and Thimann 1937). Auxin is synthesized mainly in young leaves and is actively transported to other tissues to coordinate growth and facilitate responses to environmental variations.

The retention of leaves or parts of leaves on the stem is necessary for the production of auxins and rooting cofactors that are translocated to the cutting base and promote rooting (Hartmann et al. 2011). Auxin is one of the major endogenous hormones known to be intimately involved in the process of adventitious rooting (Wiesman et al. 1988) and the physiological stages of rooting are correlated with changes in endogenous auxin concentrations (Heloir et al. 1996). High endogenous auxin concentration is normally associated with a high rooting rate at the beginning of the rooting process (Blažková et al. 1997; Caboni et al. 1997).

Auxins have been shown to be effective inducers of adventitious roots in many woody species (De Klerk et al. 1999) and are usually synthesized in the stem tip and tender leaves of aerial parts of plants and then transported to the action site (Ljung et al. 2001). When applying exogenous auxin on cuttings, the endogenous auxin concentration reaches a peak after wounding (Gaspar et al. 1996; Gatineau et al. 1997) coinciding with the initiation of the rooting process.

The widely used sources of growth hormones for rooting of cuttings are the IBA (indole-3-butyric acid), IAA (indole-3-acetic acid) and NAA (α-naphthalene acetic acid) and commercialized root promoters (root-growing powders). IAA was the first to be used to stimulate rooting of cuttings (Cooper 1935) and soon after another (IBA) auxin which also promoted rooting. IBA was discovered and was considered even more effective (Zimmerman and Wilcoxon 1935).

The application of exogenous plant growth regulators, as the auxins group, can increase the success of adventitious rooting in vegetative propagules collected from selected genotypes of *Eucalyptus* and the indole-3-butyric acid (IBA) is the most used (Wendling et al. 2000; Wendling and Xavier 2005; Almeida et al. 2007; Schwambach et al. 2008; Brondani et al. 2010a; Wendling et al. 2010).

The formation of adventitious roots in vegetative propagules can occur in two ways: (*i*) by direct organogenesis from differentiated cellular tissues (e.g., vascular cambium) or (*ii*) from callus tissue. Generally, the presence of callus occupying a region of vascular connection is problematic because the connection with the root is very fragile and the presence of callus can compromise the functionality of the root. Therefore, it is preferable to have a vascular connection with a direct origin from the vascular cambium, which influences the proper functionality of the root, influencing the development of plant (Li et al. 2009). Callus formation in the basal region of the vegetative propagules is directly associated with the induction of adventitious roots and is an important event for cell differentiation (Baltierra et al. 2004; Amri et al. 2010; Hunt et al. 2011).

The objective of the study was to investigate the effect of different auxin, their concentrations and rooting media on root induction in mini cuttings of *Azadiracta indica*.

Materials and methods

General characterization of experiment

This study was carried out at Arid Forest Research Institute, Jodhpur, Rajasthan (24°40’N and 71°15’ E) during March to June of 2012. In summer, the climate of region is generally hot and semi-arid. Summer (March to June) is severely hot and weary with temperature levels ranges between 36°C and 42°C. Though the temperature settles down to a more comfortable level during monsoon season.

Source and preparation of mini-cuttings

The selection of Candidate Plus Tree (CPT) based on General Growth, Phenology (leafing, beginning of leaf fall, initiation of leaf fall, initiation of flowering (summer and winter flowering), initiation of fruiting, fruiting period and maturation of fruiting), Resistance to disease and Pest, High biomass (crown diameter) Viability of seeds, Regeneration ability (Coppicing ability). The mini-cuttings were collected from selected mature tree of 15-25 year age, naturally growing at Forest Genetics and Tree Breeding Field at Jodhpur. The mini-stumps were propagated by conventional cutting methods (Stape et al. 2001; Schwambach et al. 2008; Wendling et al. 2010) from trees at two years and six months of age. The mini-cuttings were harvested using sterile pruning scissors in morning time. After harvesting, firstly these were screened for desired length (30-35cm by use scale) and diameter (<0.5cm) by using calibrated vernier caliper then kept in ice box (prevention from damage) for transportation to laboratory site. They were treated with aqueous solution of 0.1% Bavistin (Carbendazim 50% WP, Systemic fungicide, BASF India Limited, Bombay) for 15 min, subsequently washed with distilled water.

Since the discovery of natural plant rooting hormones (Thimann and Went 1934), for propagation, it has been intuitive to apply these substances to the basal end of cuttings to produce new roots. Six concentrations of aqueous rooting solutions, i.e., 100; 250; 500; 750; 1,000 and 1,500 mg L^{-1} IBA, IAA or NAA and was prepared in 1N of NaOH with autoclaved distilled water (v/v). Untreated cutting was considered as control (cuttings were dipped in distilled water serves as control) and was taken for each rooting media. The Basal Long Soak Methods were used for treating cuttings (Kroin 1992; 2011a; 2011b).

These cuttings were prepared according to the procedure described by Hartmann et al. (2011). After auxin treatment, the cuttings were sown in root trainer (250 cc - conical cells) containing different rooting media (sand, vermiculite and soil). The one third basal cut portion was inserted in different rooting media. Placing the cuttings in rooting medium is called as “sticking”. The medium has to be damp and the surface even. Holes should be punched into the medium to allow the insertion of the cuttings without damaging the cambium or removing the rooting hormone. After the cuttings were stuck, the rooting medium was pressed slightly around them. Four replication of five cutting in each were randomly assigned to each treatment.

Growth conditions

The cuttings were kept under intermittent mist (misting flow for 60 sec in 30 min of interval on a root trainer) for maintained relative humidity (60-80%) and temperature (25-30°C / 15-20°C day/night). The cuttings were regularly watered and treated with 0.1% Bavistin to avoid desiccation damage and attack of pathogens at every 15 d interval. The rooting experiment was run for 60 d. Then rooted cuttings were transferred to polythene bags (16 x 28 cm) containing soil+FYM (field yard manure) (5:1) and kept in poly house for 15 to 20 d. The polythene bags were moved daily in order to minimize misting variation. After this, polythene bags containing rooted mini-cutting were transferred to Agro shade house for hardening (Fig. 1D). In Agro-shade house, plants were manually irrigated by tap water once in a day. After 35-45 d of hardening, plants were planted in field and were manually irrigated by tap water once in a week. Experiment was completed within four month.

Analysis of rooting in mini-cuttings

The percentage of rooting was recorded by observing all mini-cuttings of each replication. Roots from five propagules of each replication were randomly collected for studying number of roots, root length (cm) and number of leaves.

Experimental design and statistical analyses

In present investigation, an attempt was made to study the rooting ability in mini-cuttings by the influence of auxins, i.e., IBA (indole-3-butyric acid) IAA (indole-3-acetic acid) and NAA (α-naphthalene acetic acid) and their different concentration in three rooting media, i.e., sand, vermiculite and soil. The data were analyzed through General liner Model (GLM) multi variance factor analysis using statistical Packages for social Sciences software (SPSS 8.0) for all the for studied parameters including rooting percentage, root number, root length and leaves number. Leaves number counted after completion of experiment. 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) at 0.05% probability was used to compare the means from main effects. Minimum of four replications with five samples (one cutting). 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 present investigation revealed a significant influence of auxin (IBA, IAA and NAA), their different concentration and rooting media (sand, vermiculite and soil) on adventitious root formation in mini-cuttings of *Azadirachta indica*.

Effect of auxin treatment in sand rooting media

In present finding, among the auxins IBA, IAA and NAA used, the IBA showed better results in combination with sand rooting media (Table 1). The data given in Table 1 revealed that mini-cuttings planted in sand rooting media and 250 mg L^{-1} IBA treatment gives significantly best results in terms of rooting percent (80%), number of roots (70.63), root length (11.13) and number of leaves (5.25) per rooted cuttings (Fig. 1A, B and C), followed by treatment of 500 mg L^{-1} IBA which has rooting percent (60%), number of roots (45.71), root length (5.64) and number of leaves (9.14).

In IAA treatment, the data showed better results in 750 mg L^{-1} IAA treatment with rooting percent (60%), number of roots (25.83), root length (8.0) and number of leaves (4.33) per rooted cuttings followed by treatment of 250 mg L^{-1} IAA which has rooting percent (50%), number of roots (30.00), root length (5.40) and number of leaves (4.60) respectively. In NAA treatment, the data showed better results in 500 mg L^{-1} NAA treatment with rooting percent (70%), number of roots (61.43), root length (6.14 cm) and number of leaves (8.43) per rooted cuttings followed by treatment of 750 mg L^{-1} NAA which has rooting percent (60%), number of roots (77.50), root length (4.50 cm) and number of leaves (3.00) respectively.

From ANOVA, interaction of auxin and their concentration in sand rooting media were found to have significant ($p \leq 0.05$) effect on number of roots, root length and number of leaves but not find significant effect on rooting percent (Table 2).

Table 1. Effect of different concentration of auxin (IBA, IAA and NAA) on rooting percentage, number of root, root length and number of leaves of *Azadirachta indica* mini-cuttings in sand.

Auxin (mg L^{-1})	Rooting (%)	Number of root	Root length (cm)	Number of leaves
Control (DW)	20.00 (26.56)^{d*}	8.00±0.00^{i}	2.50±0.29^{i}	3.00±0.00^{gh}
IBA				
100	40.00 (39.23)^{cd}	15.25±0.16^{ghi}	5.75±0.16^{de}	3.25±0.16^{fgh}
250	80.00 (69.75)^{a}	70.63±4.49^{a}	11.13±0.44^{a}	5.25±0.11^{d}
500	60.00 (51.05)^{bc}	45.71±1.16^{c}	5.64±0.12^{def}	9.14±0.94^{a}
750	60.00 (51.05)^{bc}	42.00±0.67^{c}	5.33±0.14^{defg}	7.17±0.68^{bc}
1,000	60.00 (51.05)^{bc}	26.67±0.71^{de}	4.75±0.17^{efgh}	3.83±0.32^{defgh}
1,500	50.00 (45.00)^{c}	15.40±0.16g^{hi}	4.20±0.13^{gh}	2.60±0.16^{h}
Mean	58.33 (51.19)	39.86	6.47	5.50
IAA				
100	40.00 (38.95)^{cd}	16.25±0.82^{fgh}	5.25±0.16^{defg}	3.50±0.19^{fgh}
250	50.00 (45.00)^{c}	30.00±1.05^{d}	5.40±0.16^{def}	4.60±0.34^{def}
500	50.00 (45.00)^{c}	27.00±0.82^{de}	8.00±0.70^{b}	4.80±0.13^{def}
750	60.00 (51.05)^{bc}	25.83±1.04^{de}	8.00±0.35^{b}	4.33±0.41^{defg}
1,000	50.00 (45.00)^{c}	20.00±2.36^{efg}	5.20±0.13^{defg}	5.20±0.13^{de}
1,500	60.00 (51.05)^{bc}	10.33±0.22^{hi}	4.00±0.35^{h}	3.67±0.41^{efgh}
Mean	51.67 (46.01)	21.52	6.00	4.35
NAA				
100	60.00 (51.05)^{bc}	11.33±0.81^{hi}	5.33±0.14^{defg}	4.50±0.23^{defg}
250	70.00 (63.98)^{ab}	23.57±1.43^{def}	4.71±0.19^{efgh}	3.86±0.38^{defgh}
500	70.00 (57.11)^{abc}	61.43±4.84^{b}	6.14±0.35^{cd}	8.43±0.51^{ab}
750	60.00 (50.77)^{bc}	77.50±3.11^{a}	4.50±0.15^{fgh}	3.00±0.17^{gh}
1,000	60.00 (50.77)^{bc}	40.00±2.30^{c}	6.33±0.28^{cd}	5.33±0.14^{d}
1,500	50.00 (44.25)^{c}	20.00±1.49^{efg}	7.00±0.84^{bc}	7.00±0.84^{c}
Mean	61.67 (52.99)	39.68	5.62	5.35
Total Mean	55.26 (48.82)	33.83	5.96	5.07

*Arc Sine values in parentheses. Values within a column followed by different letters are significantly different using Duncan's multiple range test ($p \leq 0.05$). Each value represents mean ± standard error. DW - distilled water; mg L^{-1} - parts per million.

Table 2. Analysis of variance for effects of different concentrations of the auxins (IBA, IAA and NAA) on rooting percentage, number of root, root length and number of leaves of *Azadirachta indica* mini-cuttings in sand.

Source of Variance	-------- Rooting (%) --------			------- Number of root -------			-- Root length (cm) --		- Number of leaves -	
	Df	F	*p* value	Df	F	*p* value	F	*p* value	F	*p* value
Auxin (A)	2	2.64	0.080	2	82.84	0.000	3.04	0.050	7.20	0.000
Concentration (C)	5	3.08	0.016	5	108.29	0.000	16.71	0.000	24.21	0.000
A x C	10	1.33	0.237	10	49.35	0.000	41.06	0.000	14.12	0.000
Error	57			193						

Df - Degree of freedom.

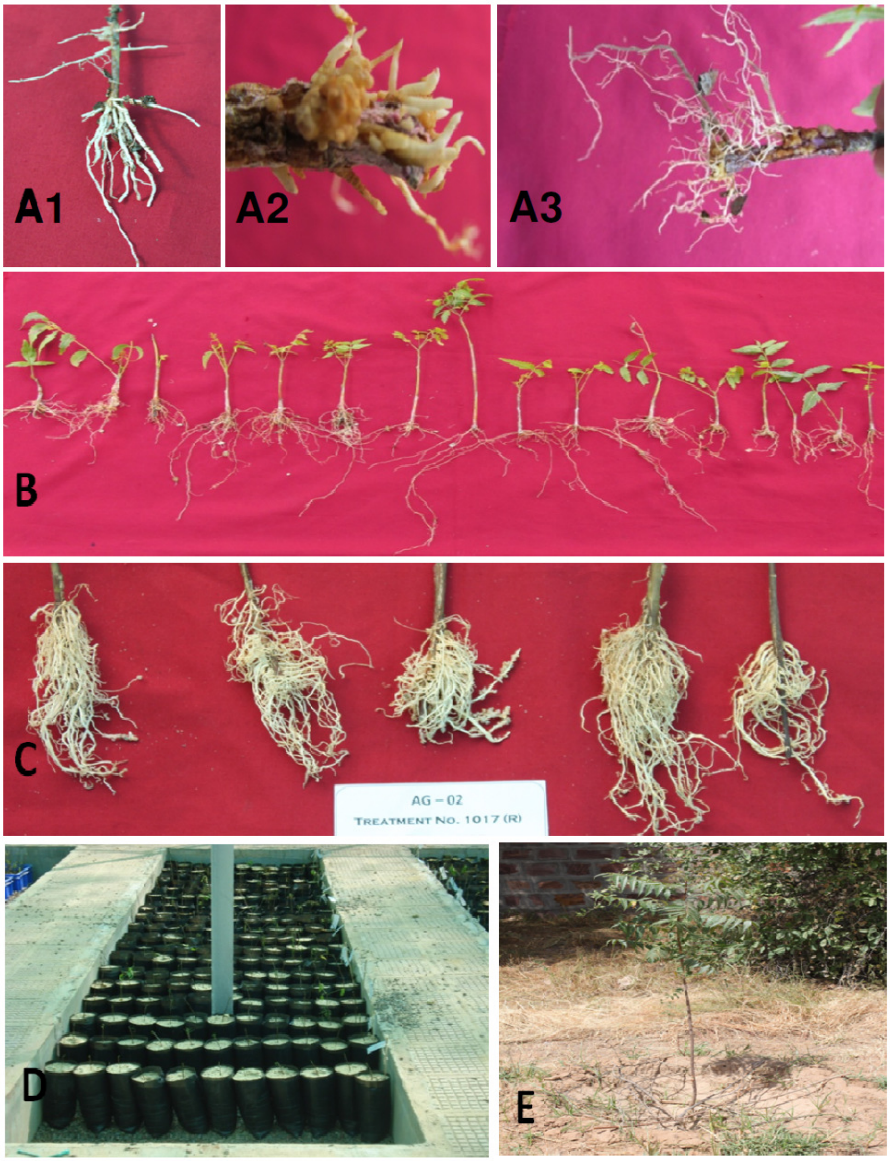

Figure 1. Details of Adventitious root formation in mini-cutting of *Azadirachta indica*. (A1-3) Development of root primordia and initiation of root formation from primordial. (B) Early rooting in mini cuttings. (C) High rate of rooting in mini-cuttings treated with 250 mg L^{-1} IBA in sand. (D) Plant produced from mini-cuttings in poly bags under agro shade house conditions. (E) Field trial of plant produced from mini-cutting.

Effect of auxin treatment in vermiculite rooting media

The data revealed that among all the different auxins (IBA, IAA and NAA) used, the IBA showed better results with combination vermiculite rooting media (Table 3). The 250 mg L^{-1} IBA treatment gives significantly best results in terms of rooting percent (80%), number of roots (56.88), root length (9.63 cm) and number of leaves (4.38) per rooted cuttings followed by treatment of 500 mg L^{-1} IBA which has rooting percent (80%), number of roots (38.63), root length (4.81 cm) and number of leaves (6.63). In IAA treatment, the data showed better results in 250 mg L^{-1} IAA treatment with rooting percent (50%), number of roots (28.00), root length (4.40 cm) and number of leaves (3.20) per rooted cuttings followed by treatment of 500 mg L^{-1} IAA which has rooting percent (40%), number of roots (26.25), root length (5.50 cm) and number of leaves (3.75) respectively. In NAA treatment, the data showed better results in 500 mg L^{-1} NAA treatment with rooting percent (60%), number of roots (51.67), root length (5.50 cm) and number of leaves (6.67) per rooted cuttings followed by treatment of 750 mg L^{-1}

NAA which has rooting percent (60%), number of roots (50.00), root length (4.00 cm) and number of leaves (3.17) respectively. From ANOVA, interaction of auxin and their concentration in vermiculite rooting media were found significant ($p \leq 0.05$) effect on rooting percent, number of roots, root length and number of leaves (Table 4). As described in earlier part of results, 250 mg L^{-1} concentration of auxin (IBA) gives better results in terms of rooting percent, number of roots, root length.

Effect of auxin treatment in soil rooting media

The data revealed that among all the different auxins (IBA, IAA and NAA) used, the IBA showed better results with combination soil rooting media (Table 5). The 250 mg L^{-1} IBA treatment gives significantly best results in terms of rooting percent (30%), number of roots (18.33), root length (5.67 cm) and number of leaves (4.33) per rooted cuttings followed by treatment of 1,000 mg L^{-1} IBA which has rooting percent (30%), number of roots (13.33), root length (5.00 cm) and number of leaves (5.33).

In IAA treatment, the data showed better results in 500 mg L^{-1} IAA treatment with rooting percent (40%), number of roots (5.75), root length (4.00 cm) and number of leaves (3.00) per rooted cuttings followed by treatment of 750 mg L^{-1} IAA which has rooting percent (30%), number of roots (11.67), root length (6.67 cm) and number of leaves (3.67) respectively. In NAA treatment, the data showed better results in 500 mg L^{-1} treatment with rooting percent (30%), number of roots (18.33), root length (7.00 cm) and number of leaves (5.00) per rooted cuttings followed by treatment of 100 mg L^{-1} NAA which has rooting percent (30 %), number of roots (5.67), root length (2.33 cm) and number of leaves (2.33) respectively. From ANOVA, interaction of auxin and their concentration in soil rooting media were found significant ($p \leq 0.05$) effect on number of roots, root length and number of leaves but not found significant ($p \leq 0.05$) effect on rooting percent (Table 6). As described in earlier part of results, 250 mg L^{-1} concentration of auxin (IBA) gives better results in terms of rooting percent, number of roots, root length.

Table 3. Effect of different concentration of auxin (IBA, IAA and NAA) on rooting percentage, number of root, root length and number of leaves of *Azadirachta indica* mini-cuttings in vermiculite.

Auxin (mg L^{-1})	Rooting (%)	Number of root	Root length (cm)	Number of leaves
Control (DW)	20.00 (26.56)^{d*}	7.50±1.44^{hi}	2.50±0.29^{hi}	2.00±0.00^{f}
IBA				
100	30.00 (32.90)^{cd}	10.00±0.00^{ghi}	1.87±0.12^{i}	2.00±0.00^{f}
250	80.00 (66.59)^{a}	56.88±3.47^{a}	9.63±0.31^{a}	4.38±0.13^{b}
500	80.00 (69.75)^{a}	38.63±1.37^{c}	4.81±0.18^{cd}	6.63±0.26^{a}
750	60.00 (51.05)^{b}	23.33±1.12^{de}	4.83±0.11^{cd}	6.33±0.33^{a}
1,000	50.00 (45.00)^{bc}	16.40±0.65^{fg}	3.70±0.13^{fg}	3.20±0.33^{de}
1,500	50.00 (45.00)^{bc}	13.40±0.69^{gh}	4.20±0.13^{def}	4.20±0.25^{bc}
Mean	58.33 (51.71)	30.94	5.41	3.67
IAA				
100	25.00 (29.73)^{d}	6.33±1.17^{i}	4.00±0.37^{ef}	3.00±0.00^{de}
250	50.00 (45.00)^{bc}	28.00±0.82^{d}	4.40±0.16^{def}	3.20±0.13^{de}
500	40.00 (39.23)^{bcd}	26.25±1.57^{d}	5.50±0.19^{c}	3.75±0.31^{bcd}
750	40.00 (39.23)^{bcd}	21.25±0.82^{def}	7.50±0.33^{b}	3.75±0.31^{bcd}
1,000	40.00 (39.23)^{bcd}	16.25±0.82^{fg}	4.50±0.19^{de}	3.50±0.19^{cd}
1,500	50.00 (45.00)^{bc}	8.80±0.25^{hi}	3.00±0.30^{gh}	3.40±0.27^{cd}
Mean	40.83 (39.57)	18.32	4.76	4.83
NAA				
100	40.00 (39.23)^{bcd}	8.50±0.33^{hi}	2.75±0.16^{h}	3.25±0.41^{de}
250	50.00 (45.00)^{bc}	17.00±0.82^{efg}	3.00±0.21^{gh}	3.40±0.27^{cd}
500	60.00 (50.77)^{b}	51.67±2.71^{b}	5.50±0.15^{c}	6.67±0.22^{a}
750	60.00 (51.05)^{b}	50.00±3.79^{b}	4.00±0.17^{ef}	3.17±0.11^{de}
1,000	50.00 (45.00)^{bc}	24.00±1.63^{d}	4.60±0.16^{de}	2.20±0.13^{f}
1,500	40.00 (38.95)^{bcd}	13.75±0.82^{gh}	3.25±0.41^{gh}	2.50±0.19^{ef}
Mean	50.00 (45.00)	30.13	3.97	4.01
Total Mean	48.16 (44.43)	26.74	4.70	4.01

*Arc Sine values in parentheses. Values within a column followed by different letters are significantly different using Duncan's multiple range test ($p \leq 0.05$). Each value represents mean ± standard error. DW - distilled water; mg L^{-1} - parts per million

Table 4. Analysis of variance for effects of different concentrations of the auxins (IBA, IAA and NAA) on rooting percentage, number of root, root length and number of leaves of *Azadirachta indica* mini-cuttings in vermiculite.

Source of Variance	----- Rooting (%) -----			----- Number of root -----			- Root length (cm) -		-- Number of leaves --	
	Df	F	p value	Df	F	p value	F	P	F	p value
Auxin (A)	2	12.17	0.000	2	33.73	0.000	34.94	0.000	31.37	0.000
Concentration (C)	5	8.32	0.000	5	99.72	0.000	65.45	0.000	58.30	0.000
A x C	10	2.89	0.005	10	38.68	0.000	68.64	0.000	17.31	0.000
Error	57			165						

Df - Degree of freedom.

Table 5. Effect of different concentration of auxin (IBA, IAA and NAA) on rooting percentage, number of root, root length and number of leaves of *Azadirachta indica* mini-cuttings in soil.

Auxin (mg L^{-1})	Rooting (%)	Number of root	Root length (cm)	Number of leaves
Control (DW)	10.00 (13.92)^{c*}	4.00±0.00^{ef}	2.00±0.00^{fg}	2.00±0.00^{e}
IBA				
100	10.00 (13.92)^{c}	3.00±0.00^{f}	1.00±0.00^{g}	2.00±0.00^{e}
250	30.00 (32.90)^{ab}	18.33±3.80^{b}	5.67±0.21^{bc}	4.33±0.21^{bc}
500	25.00 (29.73)^{ab}	13.00±1.22^{c}	5.00±0.00^{cd}	5.60±0.24^{a}
750	20.00 (26.56)^{abc}	16.00±2.31^{bc}	3.00±0.00^{ef}	2.50±0.29^{e}
1,000	30.00 (32.90)^{ab}	13.33±1.05^{c}	5.00±0.00^{cd}	5.33±0.21^{ab}
1,500	30.00 (32.90)^{ab}	6.00±0.37^{ef}	2.33±0.21^{f}	3.00±0.00^{de}
Mean	24.17 (28.15)	12.45	4.03	4.07
IAA				
100	30.00 (32.90)^{ab}	6.67±0.56^{ef}	2.33±0.21^{f}	2.67±0.21^{de}
250	30.00 (32.90)^{ab}	11.67±1.05^{cd}	4.33±0.21^{d}	2.67±0.21^{de}
500	40.00 (39.23)^{a}	5.75±0.16^{ef}	4.00±0.27^{de}	3.00±0.00^{de}
750	30.00 (32.90)^{ab}	11.67±0.56^{cd}	6.67±0.42^{ab}	3.67±0.56^{cd}
1,000	30.00 (32.90)^{ab}	6.67±1.05^{ef}	3.00±0.00^{ef}	2.33±0.21^{e}
1,500	30.00 (32.90)^{ab}	7.75±0.41^{def}	2.75±0.16^{f}	3.00±0.46^{de}
Mean	31.67 (33.95)	8.20	3.80	2.90
NAA				
100	30.00 (32.90)^{ab}	5.67±0.21^{ef}	2.33±0.21^{f}	2.33±0.21^{e}
250	25.00 (29.73)^{ab}	4.50±0.29^{ef}	2.00±0.00^{fg}	2.50±0.29^{e}
500	30.00 (32.90)^{ab}	18.33±1.05^{b}	7.00±0.97^{a}	5.00±0.00^{ab}
750	15.00(20.24)^{bc}	25.00±0.00^{a}	5.00±0.00^{cd}	5.00±0.00^{ab}
1,000	15.00 (20.24)^{bc}	6.50±0.29^{ef}	3.00±0.00^{ef}	2.50±0.29^{e}
1,500	20.00 (26.56)^{abc}	8.00±1.15^{de}	5.00±0.00^{cd}	4.50±0.29^{bc}
Mean	22.50 (27.10)	10.38	4.08	3.54
Total Mean	25.26 (28.90)	9.97	3.91	3.40

*Arc Sine values in parentheses. Values within a column followed by different letters are significantly different using Duncan's multiple range test ($p \leq 0.05$). Each value represents mean ± standard error. DW - distilled water; mg L^{-1} - parts per million

Table 6. Analysis of variance for effects of different concentrations of the auxins (IBA, IAA and NAA) on rooting percentage, number of root, root length and number of leaves of *Azadirachta indica* mini-cuttings in soil.

Source of Variance	------ Rooting (%) ------			-------- Number of root --------			- Root length (cm) -		- Number of leaves -	
	Df	F	*p* value	Df	F	*p* value	F	*p* value	F	*p* value
Auxin (A)	2	4.57	0.014	2	11.47	0.000	1.54	0.221	17.11	0.000
Concentration (C)	5	1.49	0.207	5	23.94	0.000	33.12	0.000	16.54	0.000
A x C	10	1.92	0.061	10	13.90	0.000	22.25	0.000	14.17	0.000
Error	57			178						

Df - Degree of freedom.

Discussion

The study carried out shows that the mini-cutting propagation techniques can be utilized for mass and rapid production of the *Azadirachta indica* in four month of experiment. The rooting capacity found in the present study indicates that the mini-cutting management and induction treatments imposed significantly influenced the rooting ability of *Azadirachta indica.* The high rate of rooting percentage (80%) with IBA application shows that *Azadirachta indica* can be successfully mass propagated under poly house conditions and shows 100% survival in the field conditions after one year (Fig. 1B-D). Mini-cuttings plants in field conditions were demonstrating the efficiency of our protocol. However, it is important to consider that 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.

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 mini cutting stage. It has been reported in woody tree species that the rooting potential of the cuttings is a juvenile characteristic and that the rooting capacity declines after maturation (Kibbler et al. 2004). It is because of this mature stem cuttings yield low rooting results.

IBA treatments were also significantly different for rooting percentage as exhibited by Table 1, 2 and 3. Sand followed by vermiculite and IBA hormone with 250 mg L^{-1} concentration was able to serve this purpose in *Azadirachta indica*. Maximum rooting percentage with 250 mg L^{-1} 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 mini cuttings that results in differentiation of root primordial and medium support to it by reduce damages during callus formation (Fig. 1A). Among the rooting media (sand, vermiculite and soil), sand shows the best results in treatment of 250 mg L^{-1} IBA (Table 2, 4 and 7). It is because sand provides a porous medium so root can be settled well without damaging. However the growth and development of adventitious root and leaves of mini-cutting was better with 250 mg L^{-1} IBA, 500 mg L^{-1} IBA, 750 mg L^{-1} IBA and 500 mg L^{-1} NAA in sand rooting media only.

Achievement of minimum rooting in control (distilled water) treatment rather than complete inhibition of rooting indicates that endogenous auxin along with some root inducing factors might occur naturally within the mini cuttings that may help for root primordia initiation. Overall better growth and development with IBA treatment could be because of its greater stability, transportability, ability to produce roots (Shagoo et al. 2007). Further, this could also be attributed to the fact that growth hormones determine cell elongation and cell division thereby promoting root length (Abidin and Baker 1984) and consequently resulting better overall growth of the cuttings. Earlier research work carried out suggested that it has been opined that 1,000 mg L^{-1} IBA induced adventitious rooting in mature Neem cuttings (Palanisamy and kumar 1996). The higher concentration of IBA (1,500 mg L^{-1}) adversely affected the rooting in comparison to untreated control. The present investigations have confirmed that use of 250 mg L^{-1} IBA is best for achieving rooting in mini-cuttings.

The application of auxin in the basal region of cuttings and mini-cuttings has been used worldwide to promote the adventitious rooting of woody species (Schwambach et al. 2008; Wendling et al. 2010; Hartmann et al. 2011; Hunt et al. 2011) and to influence the micro-cuttings survival and the rooting of species considered recalcitrant to rooting (Bennett et al. 2003; Schwambach et al. 2005). In the cases of auxin application, great variation was observed among the concentrations, formulations and forms of application of plant growth regulator (Wendling et al. 2000; Fogaça and Fett-Neto 2005; Wendling and Xavier 2005; Almeida et al. 2007; Schwambach et al. 2008; Wendling et al. 2010) as well as other factors considered intrinsic based on the genetics (Stape et al. 2001; Bennett et al. 2003; Corrêa and Fett-Neto 2004). It is known that IBA enhances the induction of adventitious roots in vegetative propagules, but depending on the management style and the genetic material, the concentrations should be adjusted to obtain better rooting rates (Corrêa and Fett-Neto 2004).

Adventitious rooting appears to be especially controlled by endogenous levels of auxins (Luckman and Menary 2002; Li et al. 2009; Zhu et al. 2010). Exogenous applications of auxins increase the rooting percentage due to their ability to act in plant tissues located near the region of contact with the plant growth regulator (Fogaça and Fett-Neto 2005; Husen and Pal 2007) and can also be related to the timing of IBA applications (Luckman and Menary 2002) and concentrations (Wendling et al. 2010). Therefore, the application of IBA increases the concentration of endogenous auxins, and the accumulation of the IBA in the basal region of the vegetative propagules acts as a metabolizing agent and signal to induce rooting (Husen 2008). During external contact with the cell, the IBA induces changes in the metabolism of enzymes, carbohydrates, RNA, DNA and proteins, and these changes in the rooting zone may inhibit or promote regeneration of adventitious roots, mainly during cell division and differentiation (Baltierra et al. 2004; Husen and Pal 2007; Komatsu et al. 2011).

Sivagnanam et al. (1989) reported that stem cuttings of Neem dipped into IAA and IBA rooted effectively in a mist propagator for 135 days. Tomar (1998) found that stem cuttings rooted effectively with IBA, but coppice shoots gave better results than the cuttings from the main woody stem. Palanisamy and Kumar (1997) found that maximum rhizogenesis in stem cuttings coincided with the emergence of new shoots, this being February in Jabalpur, India. The use of IBA at 1,000 mg L^{-1} increased the rooting percentage, number of roots and root biomass. The application of root promoting growth regulatory substances (auxins) is the most common treatment to enhance rooting in stem cuttings (Hartmann et al. 2011). In most tree species rooting ability of cuttings has been reported to increase from apical to basal part of the shoot which has been attributed to accumulation of carbohydrates at the base of shoot (Hartmann et al. 2011). It is also probably the single most effective treatment to achieve successful propagation. In addition to effects on cell differentiation, auxins promote starch hydrolysis and the mobilization of sugars and nutrients to the cutting base (Das et al. 1997), although increasing auxin concentrations did not result in respective increase in cutting dry mass (Mesén et al. 1997). However, behind this apparently ‘cure-all’ treatment, there lies a considerable body of evidence showing that auxin applications are interactive with other treatments (Palanisamy and Kumar 1997), types of material (Brennan and Mudge 1998), and environmental variables (Fett-Neto et al. 2001) affecting the rooting capacity of cuttings.

This high degree of interaction is probably the reason that the literature is full of apparently contradictory statements about the precise physiological role of auxins in the rooting process, a situation that cannot be resolved when authors do not present definitive information about either the physiological condition of their material or the propagation environment used. Typically cuttings treated with auxins root more rapidly and produce more roots and usually with a higher percentage of cuttings rooted. Usually, indole-3-butyric acid (IBA) is found to be the most effective root promoting auxin.

Conclusion

In plant propagation, multiplication at mass scale and minimize the cost a high survival rate with high percent of rooting is need for hour so as to, which is capable to fulfill the desired aim. Presently no such propagation methods of protocol is available for propagation of *Azadirachta indica* using cuttings. *Azadirachta indica* is important plant species and in demand for planting in every climatic conditions for multipurpose uses. In the present case vegetative propagation through mini cuttings found to be very promising methods, which may replace the existing stem cuttings which is slow with low success rate. Present experiment indicates the important role of determining the optimal rooting media and plant growth regulator in the process of vegetative propagation of mini-cuttings (Fig. 1A). The formation of healthy plants after successfully hardening under outdoor conditions showed that *Azadirachta indica* could be successfully propagated by using mini cutting techniques (Fig. 1E). Plants were successfully hardened and planted in the field. These plants showed a good survival in field condition.

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