



# Aeroponics for adventitious rhizogenesis in evergreen haloxeric tree *Tamarix aphylla* (L.) Karst.: influence of exogenous auxins and cutting type

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**Abstract** *Tamarix aphylla* (L.) Karst., a drought resistant halophyte tree, is an agroforestry species which can be used for reclamation of waterlogged saline and marginal lands. Due to very low seed viability and unsuitable conditions for seed germination, the tree is becoming rare in Indian Thar desert. Present study concerns the evaluation of aeroponics technique for vegetative propagation of *T. aphylla*. Effect of various exogenous auxins (indole-3-acetic acid, indole-3-butyric acid, naphthalene acetic acid) at different concentrations (0.0, 1.0, 2.0, 3.0, 5.0, 10.0 mg l<sup>-1</sup>) was examined for induction of adventitious rooting and other morphological features. Among all three auxins tested individually, maximum rooting response (79%) was observed with IBA 2.0 mg l<sup>-1</sup>. However, stem cuttings treated with a combination of auxins (2.0 mg l<sup>-1</sup> IBA and 1.0 mg l<sup>-1</sup> IAA) for 15 min resulted in 87% of rooting response. Among three types of stem cuttings (apical shoot, newly sprouted cuttings, mature stem cuttings), maximum rooting (~ 90%) was observed on mature stem cuttings. Number of roots and root length were significantly higher in aeroponically rooted stem cuttings as compared to stem cuttings rooted in soil conditions. Successfully rooted and sprouted plants were transferred to polybags with 95% survival rate. This is the first report on aeroponic culture of *Tamarix aphylla* which can be utilized in agroforestry practices, marginal land reclamation and physiological studies.

**Keywords** Adventitious rooting · Aeroponics · Farash · Haloxeric tree · Stem cuttings · *Tamarix aphylla*

## Abbreviations

IAA Indole-3-acetic acid  
IBA Indole-3-butyric acid  
NAA Naphthalene acetic acid  
AR Adventitious root

## Introduction

*Tamarix aphylla* (L.) Karst., commonly known as ‘Farash’ is an evergreen haloxeric tree of family Tamaricaceae with native range from Central Sahara towards Middle East, Pakistan, India and Afghanistan (Bhandari 1990). It is a fast growing tree which is adaptable to extreme drought, frost and salinity conditions with low transpiration rate (Davenport et al. 1982; Mahmoud et al. 2015). Therefore, it can be propagated in sandy arid, calcareous, saline sodic and waterlogged soils as agroforestry species (Jha and Sarma 2009). Its active anti-erosion protection strategy due to its surface cracks and deep root system, make it resistant against wind-sand erosion (Han et al. 2013). *T. aphylla* is also planted as a shade tree along road side and canals. Its tender branches and leaves have high fodder value and favored by camels and goats (Marwat et al. 2011). Branches are used as fuel and wood is used for furniture making. Galls obtained from different parts of the tree are used for tanning the leather (Orwa et al. 2009). Galls on flowers are used as an astringent and bark is used to cure skin diseases in traditional medicines (Marwat et al. 2011).

*Tamarix aphylla* naturally propagates through seeds. For seed germination in natural conditions, seed dispersal and flooding/raining have to coincide. If suitable conditions for

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germination are unavailable, seeds quickly lose their viability (Lemmans et al. 2012). Such suitable conditions are generally not met in extreme drought conditions of Thar Desert. Thus, being an ecologically significant and native tree of Indian Thar desert, *T. aphylla* is becoming rare in natural habitat (Bhandari 1990). Therefore, alternative means of its propagation are necessary.

Propagation of trees through sexual means i.e. seeds causes variability from mother plant. Furthermore, seed dormancy and low viability are the other bottlenecks in sexual means of propagation. In contrary, propagation of trees by vegetative means, produce true to type plants which possess the superior traits of mother plants (Kesari et al. 2009). Formation of AR is the prerequisite for the vegetative propagation of trees or woody plants. It is a complex genetic phenomenon controlled by the interaction between environmental and endogenous factors especially auxins (Pacurar et al. 2014). Vegetative propagation of plants through aeroponics, in which plant stem and roots remain suspended in air, provides better root hair development in plants (Laurent et al. 1997). Aeroponics system have been used for various aspect of research in plant sciences i.e. growth studies, mineral nutrition, water use efficiency of roots, effect of root atmosphere, nitrification by bacteria in roots, plant pathological studies, mycorrhizal studies, exudation from roots, mechanical impedance to roots, effect of root temperature on growth, screening for root mutants, culture of tissues and organs and structural modifications in roots (Weathers and Zobel 1992; Barak et al. 1996; Waisel 2002; Eshel and Grunzweig 2012). Moreover, aeroponics technique is beneficial in terms of nutrient solution recirculation, less water and energy inputs per unit growing area (Chiipanthenga et al. 2012).

The paper is concerned with the potential of aeroponics system for clonal propagation of *T. aphylla* and effect of different root promoting auxins on adventitious rooting in this valuable halophyte tree. Analysis of quantitative and qualitative feature in terms of root number and root length as well as effect of age of stem cuttings on percent rooting and subsequent survival was also carried out.

## Materials and methods

### Establishment of aeroponics system

An aeroponics unit made up of styrofoam chamber and lined with black polysheet was used for adventitious rooting in *T. aphylla* stem cuttings. For misting in aeroponic chamber, there were nozzles (50  $\mu$ ) of high pressure which were evenly spaced and fixed into PVC pipes (50 mm). For water pumping, the pipeline was connected to a motor (Crompton Greaves) of 0.5 hp with affixed filters. Water was pumped at

60 psi pressure and misting lasted for 60 s with 800 s pause. To control the misting at required time interval, a digital timer was linked to the pump. Water of storage reservoir was renewed after every 1 week. Solar powered generator was used for electricity supply to the system. Aeroponic unit was kept in green house at a maintained temperature (30–32 °C) and relative humidity (60%) while temperature within mist chamber (rhizospheric zone) was 28–30 °C with 80–90% of relative humidity

### Plant material selection and stem cutting preparation

Preliminary assays evaluated 10 different individuals of a population of *T. aphylla* for cutting rooting on aeroponics with and without auxin and rooting responses were very similar for all genotypes. Therefore, we chose to conduct detailed experiments with a single individual mature tree. A 20 years old morphologically healthy and sexually mature tree growing at premises of a temple in Jodhpur (26°16'10.2"N and 73°01'08.0"E) was selected for the collection of plant material throughout the experiment. Plant material was collected during March–April in the form of stem cuttings containing apical shoot cuttings, newly sprouted stem cuttings and mature stem cuttings. Leaflets were removed and uniform cuttings of 15–16 cm length were prepared. The cuttings were treated with 0.1% (w/v) Bavistin (Carbendazim powder from BASF India Limited), a broad spectrum systemic fungicide, for 10 min followed by 2–3 times washing with distilled water.

### Adventitious rooting under aeroponics conditions

Different concentrations (1.0, 2.0, 3.0, 5.0 or 10.0 g l<sup>-1</sup>) of auxins (IAA, IBA and NAA) were used either alone or in combination for adventitious rooting in stem cutting under aeroponic conditions. Auxins were dissolved in 1 N NaOH and pH was adjusted to 5.8 after final makeup. The basal ends of cuttings (1.0 cm) were dipped in different types and concentrations of auxins for 15 min at room temperature. Stem cuttings with no auxin treatments served as control. After auxin treatments, stem cuttings were inserted on holes in Styrofoam platform in such a way that lower half of cuttings remain in the aeroponic chamber. After AR formation, rooted cuttings were removed from aeroponic system and transferred to polybag containing field soil: soilrite (3:1).

### Rooting in stem cutting under soil conditions

In order to compare the performance of cuttings under aeroponics rooting, stem cuttings were also rooted in soil conditions. For this, auxin treated shoots were inserted in polybags containing field soil: soilrite (3:1) and kept in

same green house conditions as described above for aeroponic conditions. During this period, observations regarding percent survival after transplant were recorded. Severely rotted cuttings with no response were defined as dead. Watering was carried out on alternative days during this period.

### Statistical analysis

Completely randomized block design was used to set up the experiment and each experiment was conducted thrice with 15 replicates per treatment. Data was scored in terms of rooting percentage, number of roots, and root length after 15 days of insertion on aeroponic chamber as well as in soil. Results are shown as mean  $\pm$  SD. Statistical analysis of data was done by one way analysis of variance (ANOVA) and Duncan multiple range test (DMRT) was applied to find the significant difference between means at  $P < 0.05$  using SPSS V.17 (SPSS, Chicago, USA).

### Results and discussion

The technique of aeroponics provides a tool for large scale root cultivation with high quality and purity, which could be used in commercial scale production of bioactive molecules of pharmaceutical interest (Hayden 2006). It is a valuable system for biomass production which works under controlled environment and has many advantages i.e. limited consumption and recycling of water, large scale production in limited space, independence of yield from seasonal variations, production of pathogen free root material and improved root biomass yield with phytochemical stability (Pagliarulo and Hayden 2002). The technique ensures the accelerated growth and maturation of plants (Mirza et al. 1998) as well as improvement in quantity and quality of root biomass production with consistency (Kumari et al. 2016).

Vegetative propagation of trees provides the benefit of producing superior planting stock having genetic resemblance with elite mother plant (Davies et al. 1982). It could be a well-adapted method for propagation of halophyte species which are restricted to narrow ecological limits considering production of seeds and their germination (Batanouny 1996). Vegetative propagation of many horticultural (Wendling et al. 2013; Georget et al. 2017) and agroforestry (Baccarin et al. 2015; De Almeida et al. 2017) species proved beneficial for large scale propagation of true to type germplasm. For this, selection of elite or candidate plus tree is a crucial step (Sidhu 1996). Therefore, a mature adult tree of *T. aphylla* was selected on the basis of plant height, girth of main trunk, canopy size as well as sexual maturity (Fig. 1a). This candidate plus tree was used

as the source of plant material for aeroponics culture. Similar studies for the selection of elite trees were also reported by Rao et al. (2001) and Kesari et al. (2009).

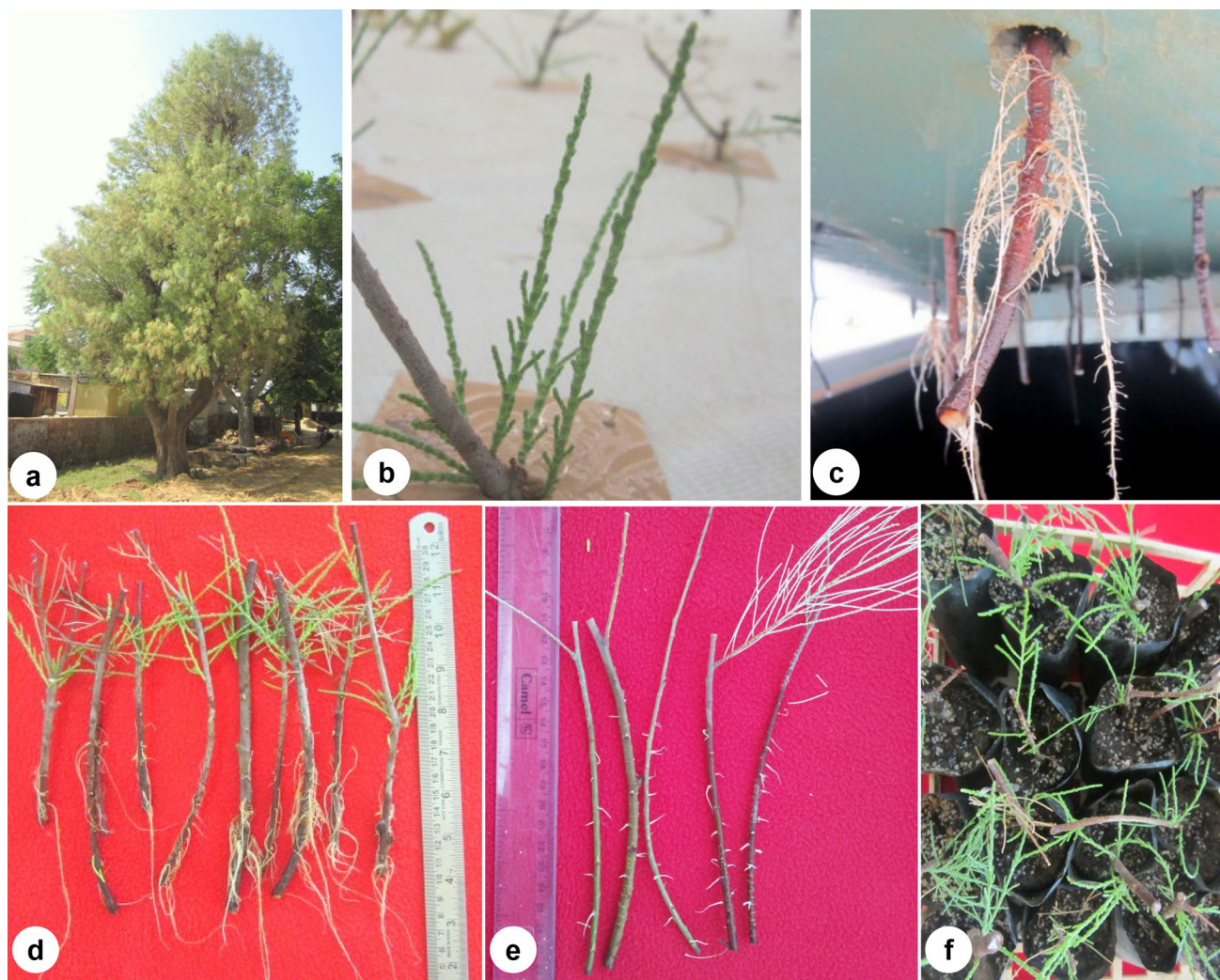
### Effect of different types of stem cuttings on rooting

In the present study, suitability of aeroponics system was evaluated for the AR development in stem cuttings of a haloxeric tree, *T. aphylla*. Different types of stem cuttings used for the AR development showed variation in percent rooting and transplant survival rate. Mature stem cuttings had highest rooting percentage and survival rate followed by newly sprouted stem cuttings and apical shoot cuttings (Fig. 2). Adventitious rooting with thick stem cuttings was also reported in *Jatropha curcus* (Severino et al. 2011), and *Pongamia pinnata* (Kesari et al. 2009). In *T. aphylla*, adventitious roots develop from lenticels which are produced on branches by differentiated parts of phellogen (Ginzburg 1967). Occurrence of higher rooting percentage in mature stem cuttings could be due to the higher number of lenticels, from which adventitious roots are developed. In contrast, stem cuttings taken from newly sprouted shoots and apical shoot cuttings took more time for initial response of AR formation. Time required for induction of roots is associated with ease of propagation (Mehandru et al. 2014). Comparatively delayed response of rooting could be associated with deterioration and limited development of tissues which negatively affect the survival of cuttings even when the rooting occurs (Saranga and Cameron 2007).

### Adventitious root formation in aeroponics

Among the endogenous factors affecting formation of AR, auxins play a significant role in terms of auxin content, polar transport, auxin regulated signaling and its interaction with other hormones. When auxin is applied exogenously, it enters through the cut surface and involve in a new auxin transport route (Guan and De Klerk 2000; da Costa et al. 2013). Uptake of auxin by cells could be through influx carriers (Delbarre et al. 1996) or pH trapping mechanism (Rubery and Sheldrake 1973). Basal ends of stems cuttings accumulate auxins in response to wound injury which regulate the divisions of the first root initials during AR formation in stems (Mehandru et al. 2014). Different types and concentrations of auxins regulate the differentiation of phloem ray parenchyma into root primordial cells, thus, with the availability of suitable auxins, differentiating cells become competent for organogenic signals (Sabatini et al. 1999; Blakesley and Chaldecott 1997). With the exogenous auxin treatment, the process of differentiation and root induction could be triggered (Praveen et al. 2009). In addition to increasing the adventitious root development





**Fig. 1** Adventitious rhizogenesis in *T. aphylla*. **a** Mature candidate plus tree of *T. aphylla*. **b** Sprouted shoots in stem cuttings. **c** Suspended roots in aeroponic chamber. **d** Stem cuttings rooted

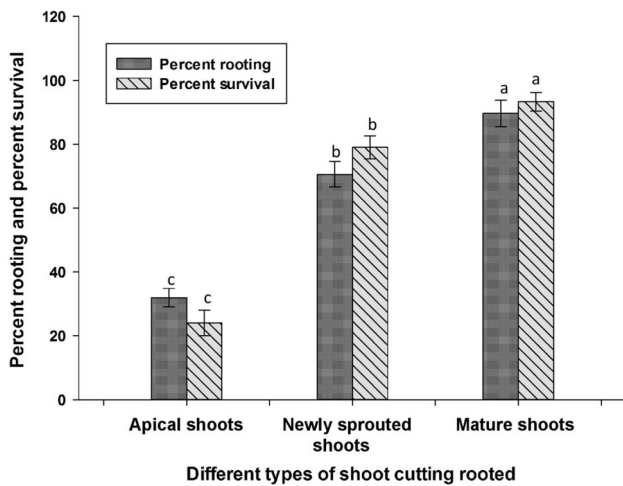
under aeroponic growth chamber. **e** Stem cuttings rooted in soil. **f** Successfully rooted plants in polybags

rate, auxins also enhance the number of roots per cutting (Kesari et al. 2009).

In the present experiment, first observation of AR development and shoot induction in auxin treated stem cuttings was recorded after about 10 days and 15 days of insertion on aeroponic chamber respectively (Fig. 1b, c). Stem cuttings used as control (without auxin treatment), exhibited 41.6% rooting with  $4.10 \pm 0.92$  of average number of roots (Table 1).

All the tested auxins (IAA, IBA, and NAA) affected the AR development significantly. Among all three auxins with different concentrations used, highest percent (79.0%) rooting was observed in stem cuttings treated with  $2.0 \text{ mg l}^{-1}$  IBA. IBA is considered best auxin for general use due to its non-toxicity to plants over a wide concentration range as compared to IAA or NAA (Hartmann et al. 2011). Efficacy of IBA could also be due to its higher

stability and slow conjugation rate resulting into availability of free IBA over a longer period of time than NAA or IAA (Krisantini et al. 2006). IBA could be converted into IAA through the peroxisomal  $\beta$ -oxidation of IBA in higher plants (Woodward and Bartel 2005; da Costa et al. 2013). Recent studies show that IBA uses the transporters which are different from those used by IAA for movement (Bellini et al. 2014). In addition, it remains non-degraded and is not converted to IAA during the long distance transport (Strader and Bartel 2011) which shows that an independent transport system works for the movement of inactive precursor to the specific site of action thus avoiding the auxin responses during the transport (Bellini et al. 2014). The effectiveness of IBA in adventitious root induction has been reported in many plant species (Teklehaimanot et al. 1996; Henrique et al. 2006; Mehandru et al. 2014). Among different independent concentrations



**Fig. 2** Effect of different type of stem cuttings on percent rooting in aeroponics and transplant survival. Mean values followed by different letters are significantly different according to DMRT test at  $P < 0.05$

of IAA and NAA, best responses were observed with  $3 \text{ mg l}^{-1}$  IAA (47.3%) and  $5 \text{ mg l}^{-1}$  NAA (20.3%). The average number of roots per stem cutting ranged from zero

( $10.0 \text{ mg l}^{-1}$  NAA) to  $9.41 \pm 0.96$  ( $2 \text{ mg l}^{-1}$  IBA). Highest value of average root length ( $8.24 \pm 0.66$ ) was observed with  $2.0 \text{ mg l}^{-1}$  IBA (Table 1). Higher concentrations of all three auxins inhibited rooting response. However, maximum inhibition was observed with NAA ( $10.0 \text{ mg l}^{-1}$ ), where neither shoot induction nor rooting response occurred. Callus formation was not observed at any concentration of auxins from cut ends of treated shoots. Similar observations were also reported by Li et al. (2010) during sand culture of *Tamarix chinensis*.

Combination of auxins was also tested for inducing the rooting response. IBA with other auxins exerted a synergistic effect on root induction. A combination of IBA ( $2.0 \text{ mg l}^{-1}$ ) and IAA ( $1.0 \text{ mg l}^{-1}$ ) was found to be the optimum hormone concentration with 87% of rooting response and  $11.5 \pm 0.98$  number of roots (Table 1; Fig. 1d). IAA as a major endogenous auxin controls the root system architecture and different developmental stages of plant root (Saini et al. 2013). Synergism of IBA and other auxins was also reported by Henselova (2002) and Kesari et al. (2009) in woody plant species.

**Table 1** Effect of auxin concentrations on AR formation in *T. aphylla* stem cuttings in an aeroponic growth chamber

Auxin concentration ( $\text{g l}^{-1}$ )	Percent rooting	Number of roots (mean $\pm$ SD)	Root length (cm) (mean $\pm$ SD)
Control	$41.6 \pm 3.21^c$	$4.10 \pm 0.92^{efg}$	$4.43 \pm 0.63^{ef}$
<b>IAA</b>			
1.0	$15.33 \pm 2.51^{ij}$	$4.79 \pm 0.93^{ef}$	$4.17 \pm 0.60^{ef}$
2.0	$23.00 \pm 1.73^g$	$6.66 \pm 0.83^{cd}$	$5.08 \pm 0.55^{def}$
3.0	$47.33 \pm 2.51^d$	$7.95 \pm 1.08^{bc}$	$6.69 \pm 0.60^{bcd}$
5.0	$29.30 \pm 1.15^f$	$7.16 \pm 0.60^{cd}$	$5.42 \pm 0.85^{cde}$
10.0	$14.00 \pm 1.00^{ijk}$	$4.24 \pm 0.68^{efg}$	$3.61 \pm 0.45^{fgh}$
<b>IBA</b>			
1.0	$42.00 \pm 2.64^e$	$5.83 \pm 0.86^{de}$	$7.07 \pm 0.87^{abc}$
2.0	$79.00 \pm 1.00^b$	$9.41 \pm 0.96^b$	$8.24 \pm 0.66^{ab}$
3.0	$60.66 \pm 1.52^c$	$7.79 \pm 0.74^{bc}$	$7.36 \pm 0.49^{ab}$
5.0	$49.33 \pm 0.57^d$	$7.62 \pm 0.60^c$	$6.55 \pm 0.53^{bcd}$
10.0	$18.00 \pm 3.46^{hi}$	$3.87 \pm 0.73^{fg}$	$4.67 \pm 0.64^{ef}$
<b>NAA</b>			
1.0	$7.66 \pm 2.08^l$	$2.54 \pm 0.45^g$	$2.10 \pm 0.09^h$
2.0	$10.60 \pm 1.15^{kl}$	$3.24 \pm 0.17^{fg}$	$2.44 \pm 0.20^{gh}$
3.0	$13.30 \pm 1.52^{jk}$	$3.91 \pm 0.57^{fg}$	$3.83 \pm 0.53^{efg}$
5.0	$20.30 \pm 4.50^{gh}$	$4.33 \pm 0.60^{efg}$	$4.08 \pm 0.44^{efg}$
10.0	$0.00 \pm 0.00^m$	$0.00 \pm 0.00^h$	$0.00 \pm 0.00^i$
<b>IBA + IAA</b>			
2.0 + 1.0	$87.00 \pm 2.64^a$	$11.58 \pm 0.98^a$	$8.54 \pm 0.88^a$
<b>IBA + NAA</b>			
2.0 + 1.0	$61.00 \pm 3.60^c$	$7.29 \pm 0.81^{cd}$	$6.86 \pm 0.41^{abc}$

Data was recorded after 15 days of insertion. Values are the mean of three independent experiments. Mean values within a column followed by different letters are significantly different from each other at  $P < 0.5$  (according to DMRT test)

**Table 2** Effect of auxin concentrations on AR formation in *T. aphylla* stem cuttings in soil condition

Auxin concentration (g l <sup>-1</sup> )	Percent rooting	Number of roots (mean ± SD)	Root length (cm) (mean ± SD)
Control	0.00 ± 0.00 <sup>g</sup>	0.00 <sup>g</sup>	0.00 ± 0.00 <sup>f</sup>
<i>IBA</i>			
1.0	15.00 ± 3.00 <sup>e</sup>	4.63 ± 1.00 <sup>de</sup>	2.63 ± 0.10 <sup>d</sup>
2.0	22.00 ± 2.00 <sup>d</sup>	5.40 ± 0.55 <sup>c</sup>	2.95 ± 0.44 <sup>c</sup>
3.0	39.66 ± 1.50 <sup>b</sup>	6.43 ± 0.52 <sup>b</sup>	3.62 ± 0.52 <sup>b</sup>
5.0	34.00 ± 1.00 <sup>c</sup>	5.20 ± 0.33 <sup>cd</sup>	3.14 ± 0.64 <sup>c</sup>
10.0	11.66 ± 1.52 <sup>f</sup>	3.66 ± 0.71 <sup>f</sup>	2.31 ± 0.18 <sup>e</sup>
<i>IBA + IAA</i>			
2.0 + 1.0	48.00 ± 2.00 <sup>a</sup>	7.32 ± 0.74 <sup>a</sup>	3.91 ± 0.47 <sup>a</sup>
<i>IBA + NAA</i>			
2.0 + 1.0	39.66 ± 2.51 <sup>b</sup>	4.10 ± 0.92 <sup>ef</sup>	3.06 ± 0.30 <sup>c</sup>

Data was recorded after 15 days of insertion. Values are the mean of three independent experiments. Mean values within a column followed by different letters are significantly different from each other at  $P < 0.5$  (according to DMRT test)

### Adventitious root formation in soil condition

Stem cuttings responded poorly in soil conditions when independently treated with IAA or NAA. Maximum rooting percentage (40.0%) was recorded with IBA 3.0 g l<sup>-1</sup>, when applied independently. However, a combination of IBA (2.0 g l<sup>-1</sup>) and IAA (1.0 g l<sup>-1</sup>) induced maximum rooting (48.0%) in soil conditions with average  $7.32 \pm 0.74$  number of roots (Table 2; Fig. 1e).

Stem cuttings used as control did not show any rooting response. AR in aeroponics chamber was found to yield higher number of rooted cuttings and in shorter time as compared to soil conditions. Slow rate of rhizogenesis might be due to lesser aeration in soil and hindrance in root growth by soil particles.

Successfully rooted plants were transferred to the polybags with 95% survival rate and shifted in nursery conditions (Fig. 1f). The average duration of AR formation and clonal propagation was comparatively lesser through aeroponics than conventional methods of propagation. Roots generated through aeroponics are clean and profuse with optimal lateral roots and root hair (Mehandru et al. 2014). The technique has also been utilized for production of quality potato seeds tubers (Muthoni et al. 2011). Aeroponics could also be utilized to produce the pharmaceutical crops which are highly diverse and challenging to cultivate (SANBI 2014). Moreover, this technique allows easy access to the root system (Peterson and Krueger 1988) therefore, could be useful for the procurement of root biomass having medicinal properties.

It is concluded that *T. aphylla*, an ecologically valuable haloxeric tree, was successfully propagated in controlled conditions through aeroponics. Aeroponics propagation was proved superior over conventional propagation method. The technique could be successfully employed for

large scale multiplication of *T. aphylla* which is a potential tree for afforestation of marginal lands and agroforestry practices in drought and salinity prone areas.

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### Compliance with ethical standards

**Conflict of interest** Authors declare that they have no conflict of interest.

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