Physiol Mol Biol Plants (July–September 2014) 20(3):365–373
DOI 10.1007/s12298-014-0240-8

RESEARCH ARTICLE

Evaluation of aeroponics for clonal propagation of *Caralluma edulis*, *Leptadenia reticulata* and *Tylophora indica* – three threatened medicinal Asclepiads

Pooja Mehandru · N. S. Shekhawat · Manoj K. Rai · Vinod Kataria · H. S. Gehlot

Received: 11 February 2014 / Revised: 19 May 2014 / Accepted: 2 June 2014 / Published online: 22 June 2014

Abstract The present study explores the potential of aeroponic system for clonal propagation of *Caralluma edulis* (Paimpa) a rare, threatened and endemic edible species, *Leptadenia reticulata* (Jeewanti), a threatened liana used as promoter of health and *Tylophora indica* (Burm.f.) Merill, a valuable medicinal climber. Experiments were conducted to asses the effect of exogenous auxin (naphthalene acetic acid, indole-3-butyric acid, indole-3-acetic acid) and auxin concentrations (0.0, 0.5, 1, 2, 3, 4 or 5gl^{-1}) on various root morphological traits of cuttings in the aeroponic chamber. Amongst all the auxins tested, significant effects on the length, number and percentage of rooting was observed in IBA treated nodal cuttings. Cent per cent of the stem cuttings of *C. edulis* rooted if pre-treated with 2.0 gl^{-1} of IBA for 5 min while 97.7 % of the stem cuttings of *L. reticulata* and 93.33 % of stem cuttings of *Tylophora indica* rooted with pre-treatment of 3.0 gl^{-1} of IBA for 5 min. Presence of at least two leaves on the nodal cuttings of *L. reticulata* and *T. indica* was found to be a prerequisite for root induction. In all the species, the number of adventitious roots per cutting and the percentage of cuttings rooted aeroponically were significantly higher than the soil grown stem cuttings. Shoot growth measured in terms of shoot length was significantly higher in cuttings rooted aeroponically as compared to the cuttings rooted under soil conditions. All the plants sprouted and rooted aeroponically survived on transfer to soil. This is the first report of clonal propagation in an aeroponic system for these plants. This study suggests aeroponics as an economic method for rapid root induction and clonal propagation of these three endangered and medicinally important plants which require focused efforts on conservation and sustainable utilization.

Keywords Aeroponics · Adventitious roots · *Caralluma edulis* · *Leptadenia reticulata* · *Tylophora indica*

Abbreviations

IAA	Indole −3-acetic acid
IBA	Indole-3-butyric acid
NAA	Naphthalene acetic acid
ARF	Adventitious root formation

Introduction

Mass vegetative multiplication of elite genotypes can offer quick productive gains (Kesari et al. 2009). Adventitious root formation (ARF) is a key step in vegetative propagation of woody or economically important species and the problems associated with rooting of cuttings frequently cause economic losses (de Klerk et al. 1999). ARF is a complex physiological process that is influenced by different endogenous and environmental factors (Ahkami et al. 2013). Poor adventitious root formation is a major obstacle in vegetative propagation via cuttings (de Klerk 2002).

Aeroponically grown plants show good root hair development due to highly aerated environment surrounding the

P. Mehandru · N. S. Shekhawat (✉) · M. K. Rai · V. Kataria
Biotechnology Centre, Department of Botany, Jai NarainVyas University, Jodhpur 342033, Rajasthan, India
e-mail: biotechunit@gmail.com

P. Mehandru
e-mail: lalitpooja@hotmail.com

H. S. Gehlot
BNF & Stress Biology laboratory, Department of Botany, Jai Narain Vyas University, Jodhpur, Rajasthan 342001, India

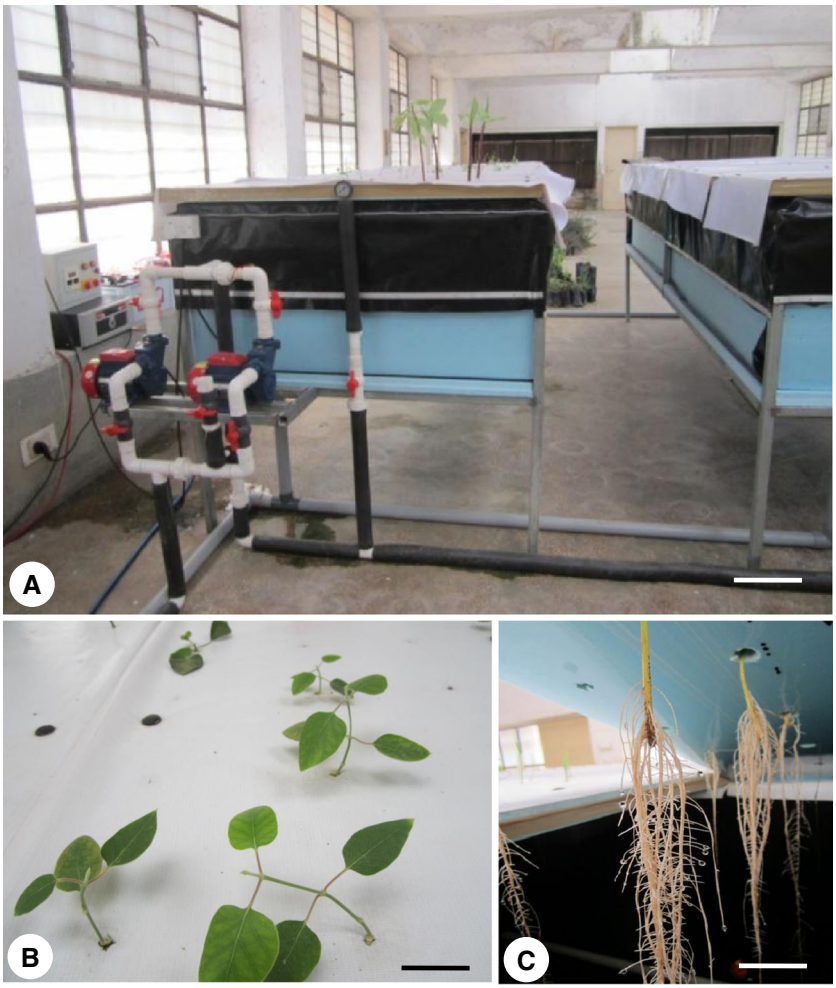

Fig. 1 Aeroponic unit. a Aeroponic chamber in a greenhouse (*scale bar* 15 cm), b Stem cutting inserted in an aeroponic chamber c (*scale bar* 2 cm) Profuse adventitious roots within the aeroponic chamber (*scale bar* 4.8 cm)

root (Laurent et al. 1997). This technology has been used to study various biological phenomena such as drought stress, symbiotic relationships, disease effect, mineral nutrition, canopy – root interrelationships, overall plant morphology and physiology (Weathers and Zobel 1992; Waisel 2002; Eshel and Grunzweig 2012). This system has been utilized for crop production such as potato seed tuber production (Muthoni et al. 2011) and by high tech greenhouse operations throughout the world (Despommier 2013). We have designed an aeroponic unit suited for adventitious root formation of threatened and medicinally important plants. Such an aeroponic system allows easy observations and phenotyping of adventitious root formation from stem cuttings and minimise risk of disease as root tissues are free from any rooting medium (Hayden et al. 2004).

Leptadenia reticulata (Retz.) Wight. & Arn. (Asclepiadaceae) is commonly known as Jeevanti, Kharkhodi/Dodi/Dudhi, Doodeeshak, Jhumka and Madhusrava. This plant is known to be medicinally important because of the active constituent 'stigmasterol' which has lactogenic/galactagogue effect. It has also been mentioned as stimulant, eye tonic, astringent, used in controlling habitual abortion and maintaining pregnancy (Arya et al. 2003). It is an important ingredient of poultry feed for increasing the egg laying capacity and to maintain the egg size. This plant propagates naturally through seeds. However, very low seed setting and low germination rate of seeds reduces its propagation through seeds. Besides this, increasing demand and overexploitation of this plant in pharmaceutical industries has caused wide spread habitat destruction, which is one of the reasons for poor reproduction and propagation. Hence, this plant is threatened in its natural habitat (Shetty and Singh 1993). There is a need for developing alternative methods for rapid propagation and conservation of this plant (Arya et al. 2003; Rathore et al. 2013). The genus *Caralluma* (Asclepiadaceae) comprises about 260 species of which some are used as dietary ingredient for suppressing appetite (Dutt et al. 2012) while some are used as traditional medicine for the treatment of rheumatism, diabetes, leprosy, paralysis, inflammation and have antimalarial, antitrypanosomal,

Table 1 Effect of different auxin concentrations on rooting of *Caralluma edulis*, *Leptadenia reticulata* and *Tylophora indica* in an aeroponic growth Chamber

	% of rooted Cuttings (mean±SE)			Root number (mean±SE)			Root Length (cm) (mean±SE)		
Auxin concentration (g l^{-1})	*C. edulis*	*L. reticulata*	*T. indica*	*C. edulis*	*L. reticulata*	*T. indica*	*C. edulis*	*L. reticulata*	*T. indica*
0.0	62.1±2.22^{g}	37.7±4.43^{h}	6.65±3.83^{kl}	1.4±0.17^{i}	2.88±0.30^{i}	2.16±0.03^{f}	1.06±0.05^{i}	1.71±0.06^{g}	0.12±0.03^{h}
IAA (g l^{-1})									
0.5	75.5±2.23^{ef}	48.8±2.20^{g}	8.88±2.22^{jkl}	1.6±0.16^{hi}	11.1±0.54^{h}	2.20±0.15^{f}	1.28±0.02^{hi}	2.38±0.21^{cdef}	0.15±0.02^{hgf}
1	77.7±3.86^{de}	62.2±5.87^{ef}	15.55±2.22^{ik}	2.8±0.13^{ef}	17.9±0.23^{e}	2.33±0.17^{f}	1.43±0.10^{gh}	2.76±0.10^{c}	0.18±0.01^{hgf}
2	82.2±2.20^{cd}	68.8±4.43^{de}	22.22±2.22^{gij}	3.6±0.16^{de}	19.7±0.33^{d}	2.86±0.08^{f}	2.01±0.09^{e}	3.73±0.16^{b}	0.24±0.02^{hg}
3	71.0±2.23^{efg}	55.5±2.23^{f}	37.77±4.44^{eh}	2.9±0.18^{ef}	16.4±0.26^{f}	9.06±0.50^{d}	1.61±0.03^{fg}	3.40±0.11^{b}	0.45±0.03^{fe}
4	68.8±4.43^{fg}	46.6±2.83^{gh}	51.10±2.22^{d}	2.2±0.13^{fgh}	14.2±0.24^{g}	13.8±0.17^{c}	1.47±0.10^{gh}	2.64±0.10^{cde}	0.79±0.05^{ba}
5	66.6±3.84^{fg}	42.2±2.20^{h}	35.55±5.87^{ef}	1.6±0.2^{hi}	12.7±0.43^{h}	9.30±0.40^{d}	1.40±0.4^{gh}	2.43±0.23^{cdef}	0.62±0.06^{dc}
IBA (gl^{-1})									
0.5	75.5±2.23^{de}	71.0±3.86^{cd}	17.7±3.86^{hij}	2.8±0.13^{ef}	19.5±0.3^{d}	2.3±0.30^{f}	2.29±0.07^{d}	2.7±0.15^{cd}	0.18±0.02^{hgf}
1	88.8±2.23^{bc}	84.4±2.20^{b}	26.6±6.66^{fgh}	4.2±0.20^{d}	27.2±0.32^{c}	2.93±0.20^{f}	2.67±0.15^{c}	3.4±0.16^{b}	0.26±0.01^{gf}
2	100±0.00^{a}	88.8±2.20^{b}	53.3±6.66^{d}	8.0±0.59^{a}	31.4±1.08^{b}	12.83±1.48^{c}	3.57±0.11^{a}	3.7±0.15^{b}	0.41±0.06^{e}
3	93.3±3.86^{ab}	97.7±2.23^{a}	93.3±6.66^{a}	6.0±0.21^{b}	46.6±0.93^{a}	23.7±1.24^{a}	2.94±0.03^{b}	5.1±0.18^{a}	0.89±0.06^{a}
4	88.8±2.23^{bc}	77.6±2.23^{c}	84.4±3.84^{b}	5.1±0..18^{c}	30.2±0.44^{b}	17.1±0.86^{b}	2.02±0.04^{e}	4.0±0.21^{b}	0.63±0.05^{dc}
5	75.55±2.22^{de}	73.33±3.85^{cd}	75.5±2.22^{c}	4.3±0.36^{d}	26.1±0.69^{c}	14.10±0.95^{c}	1.96±0.08^{e}	3.46±0.31^{b}	0.60±0.04 ^{dc}
NAA (gl^{-1})									
0.5	64.4±4.43 g	53.3±3.86^{fg}	4.44±2.22^{l}	2.3±0.15^{fg}	13.2±0.41^{g}	2.13±0.03^{f}	1.22±0.11^{hi}	1.8±0.16^{fg}	0.14±0.02^{hg}
1	66.6±3.83^{fg}	55.5±2.23^{efg}	8.88±2.22^{jkl}	3 .4±0.16^{e}	18.3±0.30^{e}	2.16±0.02^{f}	1.34±0.04^{h}	2.1±0.17^{ef}	0.16±0.01^{hgf}
2	71.0±2.23^{efg}	66.6±2.20^{e}	13.33±0.00^{ijkl}	2.5±0.167^{fg}	16.1±0.33^{f}	2.4±0.30^{f}	1.78±0.06^{f}	2.7±0.18^{cd}	0.18±0.02^{hgf}
3	66.6±0.00^{fg}	53.3±3.86^{fg}	22.22±2.22^{ghi}	2.2±0.13^{fgh}	13.3±0.26^{g}	4.73±0.93^{e}	1.44±0.04^{gh}	2.3±0.12^{cdef}	0.28±0.02^{f}
4	64.4±2.20^{fg}	42.1±2.24^{h}	48.88±2.22^{d}	1.9±0.04^{ghi}	11.2±0.21^{h}	8.36±0.32^{d}	1.30±0.042^{hi}	2.1±0.17^{def}	0.68±0.05^{cb}
5	62.2±2.22^{g}	39.9±3.84^{h}	28.88±2.22^{efg}	1.4±0.26^{i}	10.5±0.31^{h}	7.73±0.14^{d}	1.23±0.28^{hi}	1.8±0.17^{fg}	0.52±0.06^{ed}

Each mean is based on three replicates, each of which consisted of 15 cuttings. Values are the means of three independent experiments. Mean values followed by the different letter under different treatments within a column are significantly different from each other at $P<0.05$ (according to DMRT test)

Fig. 2 Aeroponically rooted plantlets after 14 days. **a** Rooting of stem cuttings of *C. edulis* through aeroponics on pre-treatment with 2.0 gl^{-1} IBA for 5 min (*scale bar* 1.6 cm) **b** Effect of IBA (3.0 gl^{-1}) on the rooting of cuttings of *L. reticulata* through aeroponics (*scale bar* 2.5 cm) **c** Rooting of stem cuttings of *T. indica* through aeroponics on pre-treatment with 3.0 gl^{-1} IBA for 5 min (*scale bar* 3.5 cm)

anti-ulcer, antioxidant, antinociceptive, and antiproliferative activities. Presence of pregnane glycosides in this genus is indicative for the appetite suppressing property (Astell et al. 2013). A strong antioxidant activity has also been reported in aerial parts of *Caralluma edulis* (Ansari et al. 2005). These findings have generated great interest in extracts of *C. edulis* for possible phytotherapeutic uses in prevention of ageing related disease and Alzheimer disease. The young shoots of *C. edulis* are edible which are cooked as vegetables and preserved as pickle. This plant is already disappearing from this region (Kaur et al. 1992; Rathore et al. 2008). The genus *Tylophora indica* (Burm.f.) Merill (Asclepiadaceae) is a medicinal climber. The pharmacologically active component of *Tylophora* extract is tylophorine used to treat bronchial asthama and other immunological ailments (Ganguly and Sainis 2001). Overexploitation, uncontrolled harvesting and lack of cultivation have led to a rapid decline in the wild population of this plant. Moreover it is difficult to propagate via seed or by vegetative propagation (Faisal et al. 2007). Therefore large scale vegetative propagation of these plants via aeroponics offers a viable alternative to meet pharmaceutical demands and for conservation of these plants.

Table 2 Comparison of effect of IBA on rooting of *Caralluma edulis* in soil and aeroponic chamber

	% of rooted cuttings		Mean no. of roots per cutting		Mean length of roots per cutting (cm)	
IBA (g l^{-1})	Soil	Aeroponic Chamber	Soil	Aeroponic Chamber	Soil	Aeroponic Unit
0.00	57.77 ± 4.44^{d}	64.4 ± 4.43^{d}	1.2 ± 0.13^{e}	1.4 ± 0.17^{f}	0.51 ± 0.02^{b}	1.06 ± 0.05^{f}
0.5	62.21 ± 4.44^{cd}	75.5 ± 2.23^{c}	1.6 ± 0.16^{d}	2.8 ± 0.13^{e}	0.54 ± 0.03^{b}	2.29 ± 0.07^{d}
1	66.66 ± 5.87^{bc}	88.8 ± 2.23^{b}	1.9 ± 0.10^{cd}	4.2 ± 0.20^{d}	0.67 ± 0.02^{b}	2.67 ± 0.15^{c}
2	71.10 ± 2.22^{ab}	100 ± 0.00^{a}	2.9 ± 0.10^{b}	8.0 ± 0.59^{a}	2.14 ± 0.25^{a}	3.57 ± 0.11^{a}
3	75.55 ± 2.23^{a}	93.3 ± 3.86^{b}	4.1 ± 0.18^{a}	6.0 ± 0.21^{b}	2.31 ± 0.20^{a}	2.94 ± 0.03^{b}
4	73.33 ± 3.85^{a}	88.8 ± 2.23^{b}	2.2 ± 0.13^{c}	$5.1\pm0..18^{c}$	2.20 ± 0.14^{a}	2.02 ± 0.04^{e}

Data were recorded after 10 days of planting. Values are the means of three independent experiments. Mean values followed by the different letter under different treatments within a column are significantly different from each other at $P<0.05$ (according to DMRT test)

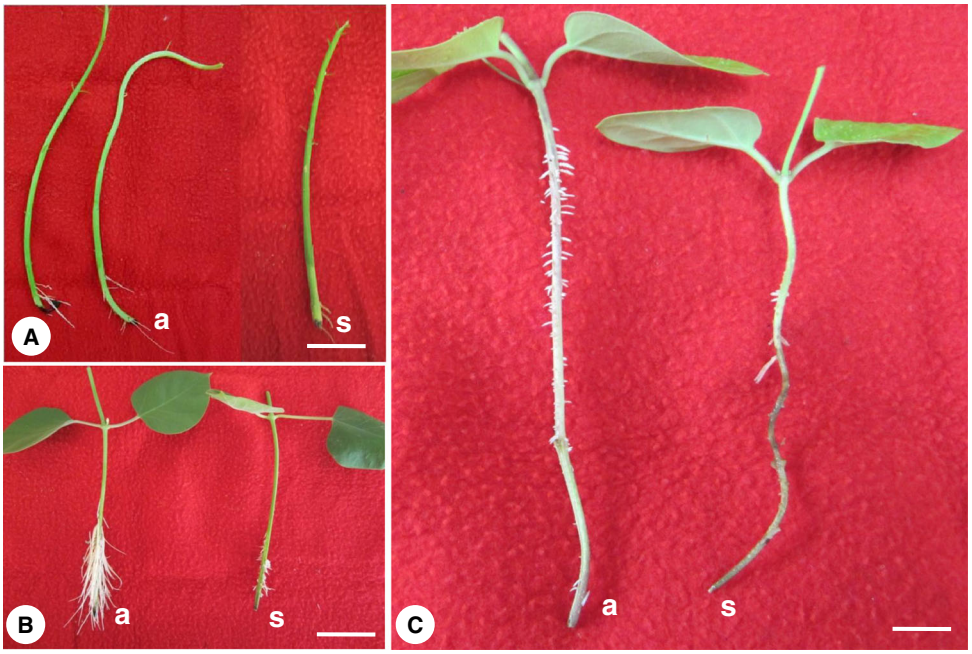

Fig. 3 Comparison of stem cuttings rooted aeroponically (a) and under soil (s) condition **a** *C. edulis* after 10 days (*scale bar* 2 cm) **b** *L. reticulata* after 21 days (*scale bar* 3 cm) and **c** *T. indica* after 10 days (*scale bar* 0.9 cm)

The objectives of the present study includes 1) evaluating rooting ability of mature stem cuttings in an aeroponic chamber in defined environmental conditions, 2) determining quality and quantity of suitable root-promoting auxin, 3) determining the time duration for rooting of cuttings, 4) analysing the morphological features of aeroponically induced roots in terms of root number, root length and presence of root hairs, 5) comparing aeroponically rooted cuttings to soil grown stem cuttings.

Materials and Methods

Plant material and preparation of cuttings

The stem cuttings of *Caralluma edulis* (Edgew.) Benth. & Hook., were harvested from the greenhouse maintained plants collected from remote area of Jaisalmer (Rajasthan) the Paimpathali (the land of Paimpa) region. The stem cuttings of *L. reticulata* and *Tylophora indica* were obtained from the field grown plants in the campus of J.N.V.U. Jodhpur. The multi nodal cuttings were harvested regularly from the greenhouse maintained and field grown plants. Average length of the cuttings used for the study was 18 cm with multiple nodes bearing at least two leaves in case of *L. reticulata* and *T. indica*. The cuttings were dipped in a solution of 0.1 % bavistin (fungicide, BASF Ltd, Mumbai) for 5 min, subsequently washed with distilled water and treated with root promoting auxins.

Effect of auxin on root induction

To induce root, the basal end of the cuttings were immersed in solutions of different concentrations (0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 gl^{-1}) of auxins i.e. IAA, IBA and NAA for 5 min.

Conditions of adventitious rooting

An aeroponic plant growth unit consisting of a styrofoam chamber (1.2 m×3.6 m×0.6 m) lined with black polysheet (Fig. 1a) was used for adventitious rooting in all the three plants selected in this study. To compare adventitious rooting in garden soil as well as aeroponic chamber, the cuttings were inserted in polybags containing soil and plant support structure of an aeroponic plant growth unit (Fig. 1b). In the aeroponic chamber, lower part of the cuttings was misted through 9 high pressure nozzels (50 μ), evenly spaced and fitted into 50 mm PVC pipes. The pipeline was connected to a 0.5 hp motor (Crompton Greaves) with attached filter to pump water at a pressure of 60 psi. Water was recirculated from the storage reservoir and was renewed every week. A digital timer was connected to the pump for controlling the spraying of mist in the chamber after set time intervals. Misting intervals lasted nearly 60 s

Table 3 Comparison of effect of IBA on rooting of *Leptadenia reticulata* in soil and aeroponic chamber

	% of rooted cuttings		Mean no. of roots per cutting		Mean length of roots per cutting (cm)	
IBA (g l^{-1})	Soil	Aeroponic Chamber	Soil	Aeroponic Chamber	Soil	Aeroponic chamber
0.00	0.00^{d}	37.7 ± 4.43^{e}	0.00^{e}	2.88 ± 0.30^{e}	0.00^{c}	1.7 ± 0.06^{d}
0.5	44.44 ± 2.22^{c}	71.0 ± 3.86^{d}	9.5 ± 0.16^{d}	19.5 ± 0.3^{d}	0.33 ± 0.08^{b}	2.7 ± 0.15^{c}
1	53.33 ± 3.85^{b}	84.4 ± 2.20^{bc}	11.1 ± 0.23^{c}	27.2 ± 0.32^{c}	0.40 ± 0.05^{b}	3.4 ± 0.16^{bc}
2	59.99 ± 3.84^{a}	88.8 ± 2.20^{b}	13.8 ± 0.32^{b}	31.4 ± 1.08^{b}	0.38 ± 0.06^{b}	3.7 ± 0.15^{b}
3	62.22 ± 3.85^{a}	97.7 ± 2.23^{a}	18.5 ± 0.4^{a}	46.6 ± 0.93^{a}	0.91 ± 0.10^{a}	5.1 ± 0.18^{a}
4	46.66 ± 3.84^{c}	77.6 ± 2.23^{cd}	13 ± 0.29^{b}	30.2 ± 0.44^{b}	0.41 ± 0.06^{b}	4.0 ± 0.21^{b}

Data were recorded after 20 days of planting. Values are the means of three independent experiments. Mean values followed by the different letter under different treatments within a column are significantly different from each other at $P<0.05$ (according to DMRT test)

with 800 s pause. The system was powered by electricity but a solar powered generator was used in case of electricity failure. The polybags containing soil and the aeroponic chamber were kept in a greenhouse which maintained an air temperature between 30–32 °C and an air relative humidity of 60 %. The temperature of the rhizospheric zone within the chamber was maintained between 28–32 °C while the relative humidity was approximately 80-90 %.

Statistical analysis

The experiment was conducted in a randomized block design with three replicates, each of which comprised 15 cuttings. The phenotypic observations were taken periodically according to the time period of root induction for all the three plants in an aeroponic chamber and in soil for various parameters such as rooting percentage, number and length of roots. A cutting having at least one root was considered as rooted. The data for shoot length were observed after 20 days of insertion of stem cuttings in the aeroponic chamber and soil. The data were analyzed statistically using SPSS v.17 (SPSS, Chicago, USA). The significance of differences among means was carried out using Duncan's multiple range test or paired sample *T* test at $P<0.05$. The results are expressed as means±SE of three experiments.

Results and discussion

Root development in plants mainly depends on endogenous auxin content, polar transport and auxin regulated signalling. Severance of cutting from the mother plant results in wound response leading to basal accumulation of auxins. Root growth and differentiation in plants is extensively linked to auxins. IAA being the major endogenous auxin is responsible for root system architecture and various stages of plant root development (Saini et al. 2013). There is substantial evidence that auxins contribute to adventitious root initiation in cuttings excised from plants (Osterc et al. 2009; Overvoorde et al. 2010). Auxins are known to increase the number of roots per cutting besides enhancing the rate of root development (Kesari et al. 2009). In the present study, the potential of aeroponic system for adventitious rooting (Fig. 1c) through application of auxins was evaluated in three asclepiads i.e. *C. edulis*, *L. reticulata* and *T. indica*. Amongst the three auxins (IBA, IAA & NAA) tested, IBA was most effective for root induction in all the three species (Table 1). In case of *C. edulis* 2.0 g l^{-1} of IBA induced cent percent rooting with maximum root number and root length (Fig. 2a; Table 1)

Table 4 Comparison of effect of IBA on rooting of *Tylophora indica* in soil and aeroponic chamber

	% of rooted cuttings		Mean no. of roots per cutting		Mean length of roots per cutting (cm)	
IBA (g l^{-1})	Soil	Aeroponic Chamber	Soil	Aeroponic Chamber	Soil	Aeroponic Unit
0.00	0.00^{d}	6.65±3.83^{g}	0.00^{f}	2.16±0.03^{d}	0.00^{d}	0.12±0.03^{e}
0.5	6.65±3.85^{d}	17.7±3.86d^{f}	0.90±0.7^{e}	2.30±0.30^{d}	0.08±0.04^{c}	0.18±0.02^{de}
1	15.5±3.86^{c}	26.6±6.66^{e}	1.70±0.30^{d}	2.93±0.20^{d}	0.13±0.03^{b}	0.26±0.01^{d}
2	17.7±3.86^{c}	53.3±6.66^{d}	2.23±0.18^{d}	12.83±1.48^{c}	0.15±0.02^{b}	0.41±0.06^{c}
3	28.8±3.86^{b}	93.3±6.66^{a}	4.73±0.72^{b}	23.70±1.24^{a}	0.16±0.03^{b}	0.89±0.06^{a}
4	57.7±3.85^{a}	84.4±3.84^{b}	7.0 0±0.43^{a}	17.10±0.86^{b}	0.24±0.02^{a}	0.63±0.05^{b}
5	53.3±6.66^{a}	75.5±2.22^{c}	3.26±0.46^{c}	14.10±0.95^{c}	0.14±0.02^{b}	0.48±0.04^{c}

Data were recorded after 10 days of planting. Values are the means of three independent experiments. Mean values followed by the different letter under different treatments within a column are significantly different from each other at $P<0.05$ (according to DMRT test)

while in *L.reticulata* and *T. indica* the cuttings treated with 3.0 gl^{-1} of IBA induced highest percent rooting with maximum number of roots and root length (Fig. 2b, Fig. 2c; Table 1). Appropriate relative humidity and high temperature i. e 30–32 °C of the rooting zone was a pre requisite for enhanced rooting of the cuttings in air. The nodal cuttings of the three plants selected responded poorly on treatment with NAA and IAA under soil conditions. Therefore, a comparative assessment of the rooting response of cuttings in soil and aeroponic chamber was carried through application of IBA. Highest percent rooting and maximum root number was observed under soil conditions with 3.0 gl^{-1} IBA in *C. edulis* (Table 2, Fig. 3a) and *L. reticulata* (Table 3, Fig. 3b) while 4.0 gl^{-1} IBA induced maximum rooting in *T. indica* (Table 4, Fig. 3c). It was observed that adventitious rhizogenesis in IBA treated nodal cuttings in all the three plants was more and rapid in an aeroponic chamber as compared to the traditional rooting in soil. Better rooting response on treatment with IBA may be due to the higher stability of IBA as compared to IAA or difference in the rate of uptake of the two auxins (De Klerk et al. 1997). Other studies also indicated that internal IBA levels rather than IAA levels, increase and stay elevated in IBA treated stem cutting (Nordstorm et al. 1991; Vander Krieken et al. 1992). Moreover, IBA is the best auxin for general use because it is non-toxic to plants over a wide concentration range than NAA or IAA (Hartmann et al. 2011) and is also effective in promoting rooting of a large number of plant species (Teklehaimanot et al. 1996; Ludwig- Müller 2003; Henrique et al. 2006).

Time required for root induction is associated with ease of propagation. More time taken for rooting may be associated with deterioration of tissues and limited development of these may impair the establishment of cuttings even when the roots form (Saranga and Cameron 2007). The cuttings of all the three plants rooted within 5–6 days of their insertion in an aeroponic chamber, contrarily the cuttings of *C. edulis* and *T. indica* rooted in 10 days and that of *L. reticulata* in 21 days under soil conditions. Leafless cuttings of *L. reticulata* and *T. indica* displayed low rooting percentage and delayed rooting. Presence of at least two leaves on the cuttings promoted faster root initiation and development suggesting that the leaves may act as the source of carbohydrates for a high energy requiring process of rooting. A study of ARF in *Petunia* shoot tip cutting suggests that early establishment of a carbohydrate sink at the site of root regeneration is a key

Table 5 Comparison of shoot length in *C. edulis*, *L. reticulata* and *T. indica* in soil and aeroponic chamber

Mode of Rooting	Shoot length of *C. edulis* (cm) (mean± SE)	Shoot length of *L. reticulata* (cm) (mean± SE)	Shoot length of *T. indica* (cm) (mean± SE)
Aeroponic Chamber	20.7±0.73^{a}	13.03±0.35^{a}	11.36±0.40^{a}
Soil	17.3±0.96^{b}	10.63±0.63^{b}	8.56±0.28^{b}

Data were recorded after 20 days of planting. Values are the means of three independent experiments. Mean values followed by the different letter under different treatments within a column are significantly different from each other at $P<0.05$ (according to paired sample *T* test)

Fig. 4 Aeroponically rooted plantlets of **a** *C. edulis* (*scale bar* 7 cm) **b** *L. reticulata* (*scale bar* 4 cm) and **c** *T. indica* (*scale bar* 1 cm) transferred to polybags containing soil

metabolic event (Ahkami et al. 2009). Many studies suggest that application of auxins for ARF stimulates mobilization of carbohydrates from leaves towards the rooting zone (Angeles et al. 2011). The leaf area influences the balance between assimilate gained by photosynthesis and water loss by transpiration and thereby affecting the rooting ability of cuttings (Tchoundjeu et al. 2002). Shoot growth measured in terms of shoot length was significantly ($P<0.05$) higher in cuttings rooted aeroponically as compared to the cuttings rooted under soil conditions (Table:5). This may indicate that a fast growing root system results in better shoot growth as it provides more water and nutrients to the shoot (Werner et al. 2010).

Aeroponically generated roots are clean and profuse with optimal lateral rooting and presence of root hair as compared to roots generated in soil. Moreover this approach provides the benefit of easy access to the root system (Peterson and Krueger 1988). This suggests that aeroponics can be successfully utilized for convenient procurement of root biomass of medicinally important plants. All the aeroponically rooted plantlets survived successfully on transfer to polybags containing garden soil (Fig. 4a, b & c). In all the three plants, the average duration for successful rooting and generation of clones was lesser through aeroponic propagation as compared to the conventional propagation method.

It is concluded that aeroponic propagation can be successfully and effectively employed for rapid commercial clonal propagation and ex situ conservation of *C. edulis*, *T.indica* and *L. reticulata*.

Acknowledgments Dr. Pooja Mehandru acknowledges DST for the WOS-A fellowship and for the funding of Aeroponic unit under the same. The authors also acknowledge Biotech Unit, Department of Botany, Jai Narain Vyas University where the aeroponic unit was installed and for all the technical support without which the study could not be conducted.

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