Plant Biotechnology and Molecular Markers
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15. Development of High Efficiency Micropropagation Protocol of an Adult Tree—*Wrightia tomentosa*

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Abstract: Highly efficient and reproducible micropropagation protocol for *Wrightia tomentosa* using sexually adult material has been developed. Multiple shoots were induced from nodal shoot segments through forced axillary branching *in vitro*. Nature and management of the donor tree, season of collecting explant and their orientation on the medium strongly influenced the initial establishment of cultures. Explants collected in April-June period and placed vertically on the MS medium containing 2 mgl^{-1} BAP produced shoots from axillary nodes *in vitro*. Management of donor tree by serial harvesting of explants every fortnight was necessary to obtain vigorous growth of shoots *in vitro*. Explants of the fifth flush (F_5) were found most suitable to obtain more than 7 shoots per node on the above medium. The rate of multiplication in subsequent subcultures was a little more than two and half-fold. Incorporation of phloroglucinol (100 mgl^{-1}) into the multiplication medium containing BAP (2 mgl^{-1}) accelerated the rate of multiplication to 3-fold per subculture. Similar response could be obtained by using 10 mM thidiazuron (TDZ) alone in the multiplication medium. Nodal segments from *in vitro* raised shoots were also used to initiate a new culture cycle. The shoots could be multiplied for at least 24 months without loss of vigor. More than sixty per cent shoots obtained after sixth subculture developed roots when treated with pre-autoclaved indole-3-butyric acid solution (100 mgl^{-1}) for 10 min and implanted on modified MS medium (major salts reduced to $^1/_4$ strength and 400 mgl^{-1} activated charcoal). Successfully rooted plants were hardened *in vitro* in glass bottles containing Soilrite™ irrigated with $^1/_4$ strength MS salt solution (pH 5.0). More than 5,000 plantlets were successfully hardened *in vitro* and transferred to greenhouse for acclimatization. The survival rate of the plants during hardening was more than 95 per cent.

1. Introduction

Wrightia tomentosa (Roxb.) Roem et Schult (Apocynaceae), once a common tree species of Aravallis in Rajasthan (India), has traditionally been exploited for its ivory-white wood in making toys and as fuel. High rate of seedling mortality, lack of suitable method for natural regeneration and overexploitation has reduced its population drastically and the plant has been listed as an endangered species [1]. There is, therefore, a strong need for an alternative method to produce large number of plants of superior types for conservation and regeneration. Micropropagation methods have been widely applied for clonal propagation of tree species for afforestation, woody biomass production and conservation of elite and rare germplasm [2, 3]. These methods have been successfully integrated with modern forest tree management programs for rapid restoration of the degraded lands [4]. A lab-scale protocol for micropropagation of *W. tomentosa* using adult material was reported by Purohit et al. [5]. Only a limited number of plants could be produced by that

method with major constraint being in hardening of *in vitro* developed plants. The present article describes a highly efficient and reproducible micropropagation protocol that is being taken up for scaling-up production by this laboratory for large-scale plantation by the foresters.

2. Materials and Methods

Trees of *W. tomentosa* selected and marked for quality of wood (age of tree more than 30 years) were used as a source of explants. Shoots were harvested from these plants round the year, divided into four distinct periods viz. April-June, July-September, October-December and January-March. Management of donor tree was done by lopping one major fork and juvenile shoots produced near cut ends were collected for explantation. Such newly flushed shoots were serially harvested fortnightly and successive flushes were termed as first (F_1), second (F_2), third (F_3), fourth (F_4) and fifth (F_5). Nodal shoot segments (1.5, 3.0 and 4.5 cm long and 0.2–0.5 cm in diameter) were prepared as explants. Two different orientations of explant on medium was tested. Horizontal placement was done in two ways: (i) explant lying horizontally on medium (H_1) and (ii) one side of node, having axillary bud, inserted in medium while other side exposed to air (H_2). Vertical placement of explant was done in three different ways: (a) node completely immersed in medium (V_1), (b) node on the surface of medium (V_2) and (c) node one centimeter above the medium (V_3).

Explants were washed thoroughly with sterilized distilled water containing few drops of Tween-20 and then surface-sterilized with 0.1% (w/v) mercuric chloride for 5 min followed by thorough washing with sterile distilled water. Surface sterilized explants were inoculated on standard multiplication medium containing MS salts [6] with 2 mg l^{-1} BAP. Explants were also inoculated on MS medium containing different concentrations of Kn (0.5–5.0 mg l^{-1}), TDZ (50–10,000 nM) and GA_3 (1.0–2.0 mg l^{-1}). Proliferated shoots from nodes of F_5 flush were further subcultured on MS medium with various concentrations of TDZ (0.1, 1.0 and 10.0 µM) or BAP (2 mgl^{-1}). Phloroglucinol (50, 100 and 250 mg l^{-1}) was also added to standard shoot multiplication medium. Cultures in conical flasks (100, 150 ml) covered with non-absorbent cotton plugs were kept under controlled conditions of temperature (28 ± 2°C), light (45 µmol m^{-2} s^{-1} for 16 h/day provided by fluorescent tubes) and 60–70% relative humidity. Once culture conditions for optimum shoot induction from explants were established, the shoots produced *in vitro* were subcultured on fresh medium every 3 weeks.

Shoots having passed through three, six and nine passages in multiplication medium were used for rooting. Shoots (2.0–3.0 cm) were excised and their cut ends were dipped in different concentrations of IBA solution (50, 100, 200 and 500 mg l^{-1}) for different duration (5–15 min) followed by their implantation on standard rooting medium containing quarter strength MS salts, sucrose (1%) and agar (0.6%). Activated charcoal (50, 100, 200 and 400 mg l^{-1}) was also tested in standard rooting medium. Initially the culture vessels were kept wrapped with black paper or in darkness for 5–7 days at 30 ± 2°C temperature at 60–70% relative humidity.

Rooted shoots from 3-week-old cultures were hardened prior to *ex vitro* exposure. Hardening was attempted by three different methods. In first method, individual plantlets were planted carefully in Soilrite™ (Karnataka Explosives, Bangalore, India) filled netted pots (3 cm high) and placed in horizontally kept pickle bottles (30 cm long) which accommodated 25 such pots (W_1). In second method, individual plantlets, transferred to netted pots were placed in glass troughs (30 cm diameter) covered with polythene sheets (W_2). Thirdly, autoclaved 400 ml screw

cap glass bottles one-fourth filled with soilrite™ irrigated with 40 ml inorganic salt solution (major salts of MS medium reduced to $^1/_4$ strength, pH 5.0) were used (W_3). Each bottle containing 4 plantlets were kept in culture room for 30 days.

After 30 days, plantlets hardened by methods described as W_1 and W_2 were shifted individually to plastic pots (10 cm high) and covered with polythene bags. Gradually, humidity was lowered by perforating polythene cover, then opening it for 1 h/day and finally completely removing it.

Plantlets hardened *in vitro* (W_3) were kept in closed bottles till they touched the caps of bottles (nearly after 30 days). The caps were loosened and finally opened in misthouse (with 70–85% RH). After one month, plants were transferred to pots and kept under greenhouse conditions where a gradient of humidity (80–40%) was maintained by a Fan-Pad evaporative cooling system.

2.1 Statistical Analyses

Standard analysis procedures [7, 8] were followed for CRD analysis. Abnormality, non-additivity and heterogeneity of variance in raw data of different experiments were minimized using square root ($\sqrt{\chi}$ and $\sqrt{\chi} + 0.5$) transformation [9]. In ANOVA, test for significance (F test), standard error of mean and critical difference at 5 and 1 per cent probability was calculated on transformed data which were tabulated along with retransformed values in each experiment. In case of discrimination amongst two treatments '*t*' test was used [8].

3. Results

3.1 Initiation of Shoot Cultures

Bud break frequency was strongly influenced by the nature and management of donor tree, season of explant collection and their orientation on the medium. April-June was found to be the best period for collection of explant to obtain maximum (98%) bud break response with minimum (5.0%) loss due to contamination (Table 1). The explants collected during the months of July-September developed fungal growth associated with shoot bud proliferation. Least bud break response was observed in the winter months of October-December. Explants prepared from one-year-old mature branches responded poorly in cultures as compared to explants taken from freshly flushed branches (Table 2).

Table 1. Effect of season of harvest on shoot initiation from mature node explants of *W. tomentosa* on standard multiplication medium

Period	Per cent contamination*	Per cent response*	Callus intensity
April–June	5 ± 1.08	98 ± 13.85	++
July–September	90 ± 8.78	78 ± 12.68	++++
October–December	81 ± 7.02	20 ± 4.12	+++++
January–March	72 ± 8.60	45 ± 8.02	+++

*Mean ± SE.

Explants of different size showed varied response in terms of bud break and amount of associated callus during shoot initiation. Maximum bud break was found in explant measuring

Table 2. Effect of source of explant on shoot initiation in *W. tomentosa* on standard multiplication (SM) medium

Source of explant	Per cent explant sprouted	Mean number of shoots per node	Mean shoot length (cm)
Mature branches	52	2.17	0.85
Juvenile branches	98	3.83*	2.92*

*Significant at 1% level using *t* test.

3.0 and 4.5 cm in length (Table 3). However, the size of explant did not make significant difference in shoot bud proliferation both in terms of their number and length. In very small explants (1.5 cm) the basal callus developed upto node, posing difficulty in further subculturing.

Table 3. Effect of length of explant on shoot initiation in *W. tomentosa* on standard multiplication medium

Size of explant	Per cent explant sprouted	Mean number of shoots per node	Mean shoot length (cm)
1.5 cm	82	4.32 (2.08)	1.92
3.0 cm	98	4.64 (2.15)	2.33
4.5 cm	97	4.47 (2.11)	2.00
SEm±	0.07	0.18	
$CD_{.05}$		NS	NS′

*Figures in parentheses are $\sqrt{\chi}$ transformed values.

Orientation of explant on medium was a significant factor in shoot proliferation from nodal segments. Between horizontal and vertical orientation of explants on medium, the latter was found significantly superior (Table 4). Maximum number of shoots were produced in explants oriented in vertical position V_3 followed by V_2 and horizontal position H_2, both statistically at par in terms of number and length of shoots. When positioned vertically, callusing was associated with lower internodal region of explant only while it extended to whole surface in horizontally placed explants. Nodal explants placed 1.0 cm above the medium provided callus-free shoot proliferation.

Management of donor tree from which the explants were collected was a very important step in accelerating the number of shoots per node during initial phases of cultures establishment. Explants obtained from serially lopped branches producing different flushes of juvenile shoots exhibited graded increase in shoot bud proliferation. An increase in per cent bud break response and number of shoots per explant from first flush (F_1) to fifth flush (F_5) was noted with a concomitant decrease in per cent contamination (Table 5). Explants from F_5 flush exhibited initiation of ca 7.33 axillary shoots per node as compared to 3.98 shoots induced in explants from F_1 flush. However, effect of flushes on length of proliferated shoots was non-significant.

Explants from F_5 flushes responded differently as compared to that of F_1 flush (Table 6). About 90 per cent bud break response was observed when explants from F_5 flush were inoculated on the MS medium containing any of the four growth regulators tested. In explants from F_1

Table 4. Effect of node orientation on shoot initiation in *W. tomentosa* on standard multiplication medium

Orientation of nodal explant	Per cent explant sprouted	Mean number of shoots per node*	Mean shoot length (cm)
Horizontal placement			
Both sides of node on the medium (H_1)	98	2.45 (1.57) c	1.42 c
One side inserted into the medium (H_2)	94	3.29 (1.81) b	2.50 b
Mean		2.85 (1.69)	1.96
Vertical Placement			
Node immersed in the medium (V_1)	10	1.30 (1.14) d	1.00 d
Node on the surface of the medium (V_2)	95	3.98 (1.99) b	3.83 b
Node nearly 1 cm above the medium (V_3)	98	5.31 (2.30) a	3.33 a
Mean		3.28 (1.81)*	2.38*
SEm±		0.09	0.18
$CD_{.05}$		0.25	0.52
$CD_{.01}$		0.35	0.71

*Figures in parentheses are $\sqrt{\chi}$ transformed values.

Means followed by different letters in the same column differ significantly.

*Placement of explant (horizontal or vertical) differ significantly.

Table 5. Effect of serial harvesting on *in vitro* response by MN of *W. tomentosa* on standard multiplication medium

Flush number	Per cent contamination	Per cent explant sprouted	Mean number of shoots per node*	Mean shoot length (cm)
I flush (F_1)	10	88	3.98 (1.99) d	2.54
II flush (F_2)	10	90	5.14 (2.27) c	2.18
III flush (F_3)	8	91	5.49 (2.34) bc	2.43
IV flush (F_4)	5	95	6.51 (2.48) b	2.17
V flush (F_5)	1	98	7.33 (2.71) a	2.31
SEm±			0.06	0.17
$CD_{.05}$			0.17	NS
$CD_{.01}$			0.23	NS

*Figure in parentheses are $\sqrt{\chi}$ transformed values.

Means followed by different letters in the same column differ significantly.

flush, mean number of axillary shoots initiated were statistically insignificant on tested concentrations of any of the four growth regulators except GA_3 with least shoot formation. However, influence of PGRs on shoot initiation was marked in explants from F_5 flush, maximum being on BAP (2 mg l^{-1}). None of the other three growth regulators (Kn, TDZ and GA_3) at any of the concentrations showed better response in terms of number of axillary shoots induced per node. Numerically, 2 mg l^{-1} BAP produced maximum number of shoots, followed by 250 nM TDZ.

Table 6. Effect of different PGRs on shoot initiation in *W. tomentosa*

MS + PGR		F_1 flush		F_5 flush	
		Per cent explant sprouted	Mean number of shoots/node*	Per cent explant sprouted	Mean number of shoots/node
BAP	2 mg l^{-1}	84	3.74 (1.93)a	97	6.52 (2.55)a
Kn	0.5 mg l^{-1}	48	2.91 (1.71)ab	89	3.73 (1.93)b
	2.0 mg l^{-1}	58	2.69 (1.64)ab	90	3.19 (1.79)bc
	2.5 mg l^{-1}	52	3.19 (1.79)ab	88	3.11 (1.79)bcd
	5.0 mg l^{-1}	65	2.44 (1.56)abc	95	2.91 (1.71)bcd
TDZ	50 nM	61	2.44 (1.56)abc	98	2.91 (1.71)bc
	250 nM	60	3.44 (1.85)a	92	3.48 (1.87)bc
	500 nM	52	2.69 (1.64)ab	98	2.91 (1.71)bcd
	1,000 nM	61	2.91 (1.76)ab	97	3.48 (1.87)bc
	10,000 nM	53	2.69 (1.64)ab	92	3.19 (1.79)bc
GA_3	mg l^{-1}	35	1.72 (1.31)bc	95	2.23 (1.49)cd
	2 mg l^{-1}	24	1.45 (1.21)c	92	1.93 (1.39)d
SEm±		0.14			0.13
CD.05		0.40			0.38
$CD_{.01}$		NS			0.52

*Figure in parentheses are $\sqrt{\chi}$ + 0.5 transformed values.

Means followed by different letters in the same column differ significantly.

3.2 Shoot Multiplication

Shoots after their initial proliferation from F_5 explants on medium containing 2.0 mgl^{-1} BAP along with the mother explant were further subcultured onto standard multiplication medium after every 3 weeks. Substitution of BAP with TDZ in subcultures increased shoot multiplication rate, highest being on medium containing 10 µM TDZ (2.92 fold) which was significantly superior to the rate obtained on standard multiplication medium containing 2.0 mg l^{-1} BAP (Table 7). Incorporation of phloroglucinol (PG) in standard multiplication medium increased the rate of shoot multiplication above 3-fold. PG also induced healthy cultures with dark green and lustrous leaves (Table 8).

Table 7. Effect of different concentrations of TDZ on shoot multiplication in *W. tomentosa* cultures

MS + TDZ (µM)	Multiplication fold*	Callus intensity
Control (SM medium)	2.42b	+
0.1	1.92c	+
1.0	2.67ab	+++
10.0	2.92a	+++
SEm±	0.14	
$CD_{.05}$	0.42	
$CD_{.01}$	0.58	

*Means followed by different alphabets in the same column differ significantly.

Table 8. Effect of different concentrations of phloroglucinol on shoot multiplication in *W. tomentosa*

MS + BAP (2 mgl^{-1}) +PG (mgl^{-1})	Multiplication fold*	Callus intensity
Control	2.34 b	+++
50	1.08 c	+++
100	3.33 a	+++
250	1.92 b	++++
SEm±	0.20	
$CD_{.05}$	0.61	
$CD_{.01}$	0.83	

*Means followed by different letters differ significantly.

3.3 Rooting in Shoots

Those shoots having passed through six multiplication cycles, responded to rooting treatments (Table 9). With the increasing number of shoot multiplication cycles, the rooting response was more favorable, showing early root initiation and reduced callusing.

Table 9. Effect of number of subcultures on rooting in IBA pulse treated shoots of *W. tomentosa* on standard rooting medium

Shoots harvested after subculture	Per cent rooting response	Mean number of days to rooting	Callus intensity
III subculture	00.0	00.0	+++
VI subculture	15.4	35.6	+++
IX subculture	40.3	18.1	+

* IBA pulse treatment (100 mgl^{-1} for 10 min).

Concentration of IBA and duration of treatment affected the root induction process considerably. Among various IBA concentrations tested for pulse treatment, rooting response was maximum in shoots treated with 100 mg l^{-1} IBA solution for 10 min (Table 10). Such shoots exhibited ca 2.92 roots with 2 cm mean root length. Higher or lower concentrations of IBA did not improve rooting response. Rooting percentage was found to be positively related with concen-tration of activated charcoal (AC) added to rooting medium (Table 11). Maximum rooting response (69.7%) was observed when the IBA-treated shoots were placed in medium containing 400 mgl^{-1} AC. Addition of AC helped in early root initiation, increased root number and reduced callusing at the root-shoot junction.

3.4 Hardening and Acclimatization

Plantlets raised *in vitro* initially posed problems in hardening and acclimatization. Rooted plants when transferred directly to pots without prior hardening started wilting, no sooner they were transferred, and desiccated completely within 24 h. Seedlings were also used in experimentation to understand their requirements for hardening. Even the seedlings were prone to transplantation shock similar to *in vitro* developed plantlets.

Table 10. Effect of IBA pulse treatment on rooting in shoots of *W. tomentosa* on standard rooting medium

Strength of IBA solution (mgl^{-1})	Duration (min)	Per cent rooting response	Mean number of roots*	Mean root length (cm)	Callus intensity
50	5	11	1.30 (1.14) d	0.78 d	–
	10	15	1.49 (1.22) cd	1.25 bc	+
	15	18	1.84 (1.36) bcd	1.50 b	+
100	5	40	2.00 (1.41) bc	1.50 b	++
	10	59	2.92 (1.71) a	2.00 a	++
	15	48	1.90 (1.38) bc	1.85 a	+++
200	5	18	2.37 (1.54) ab	1.85 a	+++
	10	21	1.96 (1.40) bc	1.00 cd	+++
	15	19	1.79 (1.34) bcd	1.00 cd	++++
500	5	0	–	–	
	10	0	–	–	
	15	0	–	–	
$SEm_{\pm}$			0.08	0.11	
$CD_{.05}$	0.23	0.31			
$CD_{.01}$	0.31	0.42			

*Means followed by different alphabet in same column differ significantly.

Table 11. Effect of activated charcoal (AC) on rooting in pulse treated shoots of *W. tomentosa* on standard rooting medium

Medium + AC (mgl^{-1})	Per cent rooting response	Mean number of roots*	Mean root length (cm)	Mean shoot length (cm)	Callusing intensity
Control	59	2.89 (1.70) b	1.87 b	3.74	+
AC 50	45	2.93 (1.71) b	1.92 b	3.67	–
100	50	2.76 (1.66) b	2.11 ab	3.56	–
200	65	3.06 (1.75) b	2.41 a	3.89	–
400	69.7	3.78 (1.94) a	2.06 b	3.60	–
$SEm_{\pm}$		0.07	0.11	0.18	
$CD_{.05}$		0.21	0.34	NS	
$CD_{.01}$		0.30	0.46	NS	

*Values in parentheses are $\sqrt{\chi}$ transformed values.

Means followed by different letters in the same column differ significantly. IBA pulse treatment (100 mgl^{-1} for 10 min).

Owing to fast desiccation of rooted plants on direct pot transfer, other methods of hardening were employed. Rooted plants with nearly 4 cm long shoot, 1 to 2 cm long root and 4 to 6 leaves in number were hardened by three different methods as described in materials and methods. Apical growth was visible in more than 95 per cent of plantlets reared through any of the three methods. After 30 days, plantlets attained an average height of 5.4 cm with 2.1 cm long root (root shoot ratio being 0.37) and 6-8 broad leaves. Such plantlets were transferred to individual

plastic pots and covered with polythene bags in case of W_1 and W_2 while the caps of glass bottles were loosened in W_3 plantlets. Plantlets exhibited wide variation in growth during this period. Most of the plantlets in all the three methods exhibited good shoot growth while root growth was better only in case of plantlets hardened through W_3 method. Generally, the plants were ca 9.08 cm long with 3.67 cm long roots and 8 to 10 leaves. These plants on an average accumulated 20.22 mg dry matter in shoots (without leaves), 7.95 mg in roots and 13.54 mg in each leaf. During gradual exposure to *ex vitro* conditions in greenhouse, all plants remained green and healthy for initial 15 days. Plants hardened through W_1 and W_2 modes exhibited yellowing of leaves and leaf fall in next 15 days. The rate of survival was 26.8% after 30 days of transfer to pots which declined to 2.4% after 60 days. Plantlets hardened through W_3 mode grew vigorously having rigid and thick stem, highly branched root system and green, lustrous, healthy and broad leaves. Survival rate of such plants was more than 95 per cent. More than 5,000 plantlets have been successfully hardened and acclimatized and are ready for transplantation into field (Fig. 1).

Fig. 1. Hardened plants of *W. tomentosa* kept in a nursery.

4. Discussion

An adult superior tree can be micropropagated for desired attributes by enhanced axillary proliferation. The buds residing in the axil of twigs are induced to proliferate and generate multiple shoot buds *in vitro*. Proliferation of these axillary buds may be difficult due to micro-organism contamination [10], phenolic oxidation [11] and tissue maturity [12]. Maturity of tissue is accompanied with reduced growth rate, reduced/lack of rooting ability and sometimes plagiotrophy [13]. By reverting a part of tree to complete/partial juvenility by *in vivo* and *in vitro* methods problems associated with maturity can be minimized. In *W. tomentosa* the explants collected from previously lopped trees showed better proliferation when shoots were harvested serially. Severe pruning has been found to be an efficient method for rejuvenation [14]. In *Quercus robur* forced flushing method, related to severe pruning, was adopted [15].

The season of explant collection greatly influenced establishment of *W. tomentosa* cultures *in vitro*. Effect of season on bud sprouting was also noted in many tree species viz. *Tectona grandis*

[16], guava [17], *Tecomella undulata* [18], *Prosopis cineraria* [19] and *W. tinctoria* [20]. Vertical orientation of *W. tomentosa* explants was found better than horizontal orientation in terms of number of proliferated shoots. On the contrary, horizontal orientation of explants was found better in *Fraxinus angustifolia* [21] and *Quercus robur* [15].

Cytokinins promote cell division in plant tissues under specific conditions and are found obligatory for shoot differentiation [22]. Hu and Wang [23] reported superiority of BAP among cytokinins in differentiation of shoots from explants of trees. Buising et al. [24] suggested that transient exposure of soybean embryonic axes to BAP interrupted chromosomal DNA replication and reprogrammed the developmental fate of a large number of cells in shoot apex. In our case, maximum number of shoots were obtained in medium containing BAP in comparison to other PGRs. Recently, TDZ has been found to be one of the most active cytokinin-like substances used for woody plant tissue culture [25]. In present study, TDZ did not supercede the response obtained with BAP in shoot induction, but it did enhance multiplication of shoots in subcultures. Incorporation of phloroglucinol in the multiplication medium improved shoot multiplication rate. Similar results have been reported in apple root stock M.7 by Jones [26]. The effect could be related to hastening of rejuvenation process *in vitro* by phloroglucinol.

Rooting by dip treatment of auxin has been recommended by Harry and Thorpe [27]. It is supposed to eliminate the inhibitory effect on root growth when IBA is incorporated in the media [28]. Purohit et al. [5] have recommended IBA pulse treatment for rooting in *W. tomentosa* shoots. This method of root induction has been successfully employed in the present studies also.

Hardening is most critical factor for achieving success in pot transfer of regenerated plantlets. We have observed that a gradual shifting of plants from medium to culture bottles containing low salt concentration without sucrose allowed stress, compelling plants to become partially autotrophic. This step proved useful in achieving more success in hardening.

The results have demonstrated the feasibility of application of this protocol for raising large number of *W. tomentosa* which would greatly help in afforestation programmes in Aravallis in Rajasthan (India). Large numbers of plants are ready for field transfer that can be used for field evaluation studies.

Acknowledgements

Authors thank Dr. N.S. Shekhawat, Incharge, Plant Biotechnology Laboratory, J.N. Vyas University, Jodhpur, India for providing hardening facilities. Thanks are also due to the Department of Biotechnology, Govt. of India, New Delhi, for financial support.

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