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DIRECT PLANTLET REGENERATION FROM SEGMENTS OF ROOT OF *BALANITES AEGYPTIACA* DEL. (L.)- A BIOFUEL ARID TREE

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ABSTRACT

Shoot organogenesis and plant establishment has been achieved for *Balanites aegyptiaca* (L.) Del. from excised root explants. Young root explants obtained from 4 weeks old aseptic seedlings were used for the induction of direct shoot regeneration by incorporating various cytokinins either singly or in combination with auxins in Murashige and Skoog (MS) basal medium. Root explants cultured on MS medium enriched with 5.0 μM Benzyladenine (BA) produced organogenic nodular meristemoids which developed into shoots at the cut ends as well as at the middle surface of the explant without formation of callus within 2 weeks of incubation. The highest frequency (68%) of shoot regeneration and maximum number (7.20 ± 0.15) of shoots per explant was obtained on MS medium containing a combination of 5 μM BA and 1.0 μM α-naphthalene acetic acid (NAA). The microshoots were rooted best on half strength MS medium supplemented with 1.0 μM indole-3-butyric acid (IBA). Plantlets derived via shoot organogenesis from root explants were successfully hardened and acclimatized under greenhouse and fared well with 75% survival rate in field conditions. The protocol could be harvested in obtaining biodiesel from the plants and genetic transformation for the benefit of mankind.

KEYWORDS: **Direct regeneration, Cytokinins, Auxins, Biodiesel, Balanitaceae, Desert date**

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INTRODUCTION

Balanites aegyptiaca (L.) Del. (Balanitaceae) is a spiny, evergreen, semi arid tree distributed throughout India on the plains and in hilly regions [1]. Indiscriminate collection resulted in the disappearance of this plant from wild sources and the species is found to be confined in the Amravati district region of Maharashtra, western region of Rajasthan in India. Extracts from several parts of the plant have been intensively used for various medicinal purposes. The fruits and roots contain diosgenin, a sapogenin compound which is very useful in pharmaceutical industries as a natural source of steroidal hormones. Also, this tree species has been used as a model for the utilization of esters of Balanites oil as a biodiesel for compression ignition engines in India [2]. The Root being the medicinally useful part, destructive harvesting poses a serious threat to the sustenance of the tree. This plant is conventionally propagated through seeds which have short viability and poor germination rate. Also, vegetative propagation through root suckers is slow, difficult and cumbersome [3]. So, propagation through tissue culture methods appears to be a viable alternative. Among the possible initial explants, roots have proven to be vastly regenerative explants for *in vitro* regeneration in different species, including forest ones [4]. According to Morton and Browse [5], root explants are advantageous over other explants in terms of their easy manipulation, higher regeneration and excellent susceptibility for *Agrobacterium* transformation. Roots have also received considerable attention as a potential production system for stable metabolite production [6]. Besides of being useful for micropropagation, the root culture could be successfully applied for germplasm preservation [7]. There are few reports available on micropropagation of *Balanites aegyptiaca* using nodal segments [3,8,9] and cotyledons derived calli [10]. However, to date, only one report on direct regeneration from root explant [11] is available and that cannot be used for micropropagation of the species because the regeneration frequency reported is very low. Attempts to induce adventitious shoot regeneration from root explants of *Balanites aegyptiaca* have not met with success so far. In the present communication, results from pilot study on the effect of both cytokinins and auxins for inducing high frequency adventitious shoot regeneration and multiplication in *in vitro* grown root segments of *Balanites aegyptiaca* are presented.

MATERIALS AND METHODS

Plant Material

Mature seeds of *Balanites aegyptiaca* obtained from a 10 year- old candidate plus tree growing at Arid Forest Research Institute, Jodhpur, India, were thoroughly washed in running tap water for 30 min to remove adherent particles, immersed in 1% (w/v) solution of Bavistin, a fungicide, for 30 min then treated with 5% (v/v) Teepol solution for 20 min and rinsed thrice with sterile distilled water followed by surface sterlization in 0.1% (w/v) mercuric chloride solution under sterile conditions for 10 min and rinsed thrice with sterile distilled water. The disinfected seeds were germinated aseptically on MS basal medium [12]. Root segments (1-2 cm) excised from the sub-apical portion to basal region of roots of 4 weeks old aseptic seedlings were used as explants.

Culture media and culture conditions

MS medium supplemented with 3% (w/v) sucrose (Qualigens, Mumbai, India) and 0.8% (w/v) agar (Qualigens, Mumbai, India) was used during the study. The medium was adjusted to pH 5.8 using 1N NaOH or HCl and sterilized by autoclaving at 121°C and 105 kPa pressure for 20 min. Cultures were incubated at 25 ± 2°C under 16:8h light: dark photoperiod maintained through cool white fluorescent tubes (Phillips, India) with a photon flux density of 50 μmol $m^{-2}s^{-1}$ (PPFD) at 55-60% relative humidity.

Adventitious shoot induction
Root segments were inoculated on MS medium supplemented with three cytokinins (BA, Kn, 2-iP and TDZ) at different concentrations (1.0, 2.5, 5.0, 10.0, 12.5 and 15.0 µM) (Table 1 and 3) singly. The MS basal medium without plant growth regulators was used as a control. The data on percentage explants responding, number of shoot induced per explant produced and shoot length was recorded after 4 weeks of culture.

Shoot multiplication and elongation
Multiple shoots obtained in the induction media were transferred on shoot multiplication and elongation media in which an optimal concentration (5.0 µM) of cytokinin (BA) was added separately with different concentrations of auxins NAA, IAA and IBA (0.5, 1.0, 2.0 and 2.5 µM) (Table 2) to evaluate the combined effect of cytokinin and auxin on shoot multiplication and elongation. Also, TDZ induced cultures were transferred to hormone free MS medium for obtaining shoot proliferation. Subculturing was done after every 2 weeks at regular intervals. The data on percentage explants producing shoots, number of shoots per explant and shoot length was recorded after 8 weeks of culture and similarly parameters of five passages of TDZ induced culture onto MS basal medium were recorded after each subculture.

In vitro rooting
Isolated shoots (4- 5 cm) were transferred on half strength MS medium supplemented with IBA, NAA and IAA at different concentrations (0.1, 0.5, 1.0, 2.0 and 5.0 µM) single for rooting. The data on percent rooting, number of roots per shoot and root length was recorded after 4 weeks.

Hardening and transfer of plantlets to soil
Shoots with well developed roots were removed from the culture tubes and rinsed with running tap water to remove agar media, placed in the plastic cups filled with sterilized soilrite (Keltech Energies Ltd. Bangalore, India) and irrigated with tap water. The plants were covered with polythene bags to maintain high humidity. These plants were maintained in the culture room for 4 weeks under the following atmospheric conditions: temperature, 25 ± 2°C; light, 16h photoperiod with light intensity of 50 µmol m^{-2} s^{-1} PPFD; relative humidity 65 ± 5 %. The plants were then removed from the cups and transferred in the earthen pots containing garden soil and moved to greenhouse after 4 weeks.

Histology
Regenerative tissues were fixed in FAA (formaldehyde/ glacial acetic acid/ 70% ethanol, 5:5:90, v/v/v) for 24 h, dehydrated through graded TBA (tertiary butyl alcohol) series, each for 24 h, and embedded in saturated paraffin wax. Sections (10 µM thickness) were then cut using the Spencer 820 rotatory microtome (American Optical Corp., Buffalo, NY, USA) and mounted on glass slides. Tissues were dewaxed in xylol series, rehydrated in a graded ethanol series, and then stained with 1.0 % (w/v) safranin. Excess stain was removed by washing briefly with water and then tissues were dehydrated in a graded ethanol series. The sections were observed under an optical microscope (CH 20i, Olympus, Tokyo, Japan) and photographed using a micrographic attachment (Canon Powershoot SX120, IS, Beijing, China).

Statistical Analysis
All experiments were set up in completely randomised design under factorial arrangement. Each treatment consisted of ten replicates having one explant per culture tube and all experiments were repeated thrice. Analysis of variance (ANOVA) was used to test the statistical significance, and the significance of differences among means was carried out using Duncan's [13] multiple range test at P = 0.05.

RESULTS

Effect of cytokinins singly and in combination with auxins
Adventitious buds were formed from root explants in all the treatments except higher

concentrations of each cytokinins (BA, Kn and 2-iP) and statistically significant differences in the adventitious shoot regeneration capacity of root explants were noted in almost all the shoot induction media (Table 1). The adventitious bud formation was induced as a direct process without formation of callus. Enlarged and developed protuberances (or nodular meristemoids) were initially observed in the middle as well as at the cut ends of the root explants within 2 weeks of culture (Fig. 1A-B). Subsequently, the protuberances differentiated into dark green adventitious buds which underwent normal growth and development (Fig. 1C-D). Furthermore, histology confirmed the direct organogenesis pathway showing shoot differentiation from root explants (Fig. 1E). Root explants inoculated on plant growth regulator free medium yielded no shoot buds while the frequency of bud formation on a medium containing cytokinins ranged from 18 to 54%. Cytokinins at higher concentrations produced callus and did not show any morphogenesis even after 4 weeks of culture. Among the three cytokinins tested, 5.0 μM BA was found to be most effective in induction of maximum number (4.50 ± 0.32) of shoots per explant (Fig. 2A) and shoot length (5.86 ± 0.44 cm) whereas at the same concentration, Kn and 2-iP induced 3.46 ± 0.26 and 2.00 ± 0.05 shoots per explant respectively after 4 weeks of induction (Table 1). Moreover, the growth of the shoots remained arrested after 4 weeks of culture onto their respective media. The multiple shoots were then transferred on the media containing cytokinin-auxin combinations after 4 weeks for further growth.

A synergistic influence of auxin and cytokinin was evident when combinations of optimal concentration of BA with different concentrations of NAA, IAA and IBA were tested during the study. The results are summarized in table 2. Upon transfer of the buds on the shoot multiplication and elongation media, new adventitious buds started to multiply while the pre-existing adventitious shoots proliferated rapidly within 2 weeks followed by the formation of leaves and elongation of shoots in another 2 weeks of incubation. Moreover, the addition of NAA in the BA containing medium enhanced the multiplication and elongation of shoots induced from the root explants and found to be most effective than the media containing IAA or IBA. Among various cytokinin-auxin combinations used, the highest shoot regeneration frequency (68%), mean number of shoots per explant (7.20 ± 0.15) and mean shoot length (4.93 ± 0.03 cm) was recorded in MS medium containing a combination of 5.0 μM BA and 1.0 μM NAA after 8 weeks of culture (Table 2; Fig. 2B). However, a consistent decline in the percent regeneration, the number of shoots per explants and shoot length was observed with an increase in the concentrations of auxins (2.0 μM, 2.5 μM) as it resulted in the formation of stunted shoots.

Effect of TDZ and subculturing

The responding root explants swelled and turned light green, whereas the non-responding explants turned to brown. Regeneration was evident after 2 weeks, with multiple clusters of dark green protuberances appearing from the surface and the cut ends of the explants cultured in TDZ media. Data generated from the experiment conducted with root explants to optimize the concentration of TDZ demonstrated that there was no bud formation in the absence of TDZ in the media whereas statistical difference was observed among the different concentrations of TDZ on root explants in terms of overall average results (Table 3). The range of percentage of shoot regenerating explants was 10-50% and the average number of shoot buds per explant varied significantly at different concentrations of TDZ (Table 3). The frequency of adventitious shoot induction and the number of shoots per explant increased with the increase in concentration of TDZ up to an optimum level. The greatest multiple shoot formation rates was obtained on MS medium supplemented with 2.5 μM TDZ. This enabled a 50% regeneration rate and produced an average of 3.03 ± 0.08 shoots more than 2.63 cm long per regenerating explant (Table 3). Increasing the TDZ concentration to 15.0 μM resulted in

callus formation and no shoot morphogenesis was observed even after 4 weeks of incubation.

The multiple shoot buds obtained with various concentrations of TDZ showed vitrification and failed to elongate even after 4 weeks of culture. So, MS medium lacking TDZ was used in an effort to stimulate the shoot proliferation and elongation. After 4 weeks on TDZ-induced medium, adventitious buds were transferred to MS basal medium without plant growth regulators and subcultured for five passages. Repeated transfers in MS medium eliminated the deleterious effects of TDZ from the explants, thereby supporting differentiation of the buds to healthy shoots. Although TDZ induced a good number of adventitious buds but all did not differentiated simultaneously. Excision of elongated shoots from the clusters possibly reduced the dominance exerted by the elongated shoots and hastened the elongation of remaining shoots. Furthermore, during the first to fourth passages, the number of shoots increased and thereafter it got stabilized at fifth passage (Fig.3). The regenerated shoots were healthy with well developed leaves.

Root initiation and acclimatization of the plantlets

The microshoots from regenerated cultures failed to produce roots on full strength MS basal medium. Only one root was formed on half strength MS basal medium after 4 weeks. The addition of an auxin in half strength MS medium was essential for root induction. So, to promote the development of the root system, 4-5 cm long microshoots were transferred on MS medium augmented with IBA, NAA and IAA alone at different concentrations (0.1, 0.5, 1.0, 2.0 and 5.0 µM) (Table 4). The development of roots was observed without an intervening callus phase on all the auxin containing rooting media after two weeks of incubation.The addition of IBA to half strength MS medium enhanced rooting significantly with the formation of healthy and longer roots. On the medium containing NAA and IAA singly, roots were thick and underdeveloped. The maximum rooting percentage was obtained in half strength MS medium with 1.0 µM IBA (75%) at 1.0 µM. The addition of IAA either at lower or higher concentrations than 1.0 µM resulted in lower percentage of rooting as compared to those obtained with NAA and IBA.

Statistics expressed as the number of roots per shoot and root length supported the trend established for the rooting experiment. On lower concentration of IBA (0.1 µM), the number of roots per shoot was considerably less i.e. 2.00 ± 0.00 roots per shoot was recorded after 4 weeks of culture. Also, at higher (5.0 µM) concentration of IBA, the number (1.90 ± 0.10) of roots and length (2.03 ± 0.20 cm) got reduced. Similar response was also recorded for lower and higher concentrations of NAA and IAA (Table 4). Nevertheless, the best rooting response was recorded at 1.0 µM IBA where an average of 6.86 ± 0.54 roots of root length 4.30 ± 0.36 cm was recorded after 4 weeks of culture (Table 4; Fig. 2C). On this treatment, upper shoot growth was appeared with new leaf expansion and stem elongation. The roots were moderately thin which helped in establishing the plantlets (Fig. 2D) firmly in the soil with 75% survival rate after 4 weeks in the field. All the plantlets were morphologically homogeneous and exhibited normal characteristics similar to those of the source plant.

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Table 1
Effect of cytokinins on multiple shoot induction from root explant excised from aseptic seedlings after 4 weeks of culture

Cytokinins (µM)			% Response	Mean no. of shoots/ explant	Mean shoot length (cm)
BA	Kn	2-iP			
1.0	-	-	22	2.20 ± 0.43[bcd]	2.93 ± 1.03[bc]
2.5	-	-	31	2.80 ± 0.54[bc]	3.86 ± 0.08[b]
5.0	-	-	54	4.50 ± 0.32[a]	5.86 ± 0.44[a]
10.0	-	-	-	+	-
12.5	-	-	-	+	-
15.0	-	-	-	+	-
-	1.0	-	23	2.00 ± 0.60[cd]	1.56 ± 0.72[c]
-	2.5	-	28	2.03 ± 0.60[cd]	3.16 ± 0.44[bc]
-	5.0	-	40	3.46 ± 0.26[ab]	3.63 ± 0.84[b]
-	10.0	-	-	+	-
-	12.5	-	-	+	-
-	15.0	-	-	+	-
-	-	1.0	18	1.30 ± 0.43[d]	1.33 ± 0.44[c]
-	-	2.5	20	1.43 ± 0.23[cd]	2.30 ± 0.35[bc]
-	-	5.0	37	2.00 ± 0.05[cd]	2.70 ± 0.61[bc]
-	-	10.0	-	+	-
-	-	12.5	-	+	-
-	-	15.0	-	+	-

(+) represent no response. Values represent means ± SE. Means followed by the same letter within columns are not significantly different (P=0.05) using Duncan's multiple range test.(Source: Varshney, 2012)[37]

Table 2
Effect of auxins at different concentrations with an optimal concentration of BA (5.0 µM) in MS medium on shoot multiplication from root explants excised from aseptic seedlings after 8 weeks of culture

Auxins (µM)			% Response	Mean no. of shoots/explant	Mean shoot length (cm)
NAA	IAA	IBA			
0.5	-	-	60	3.90 ± 0.05[b]	4.30 ± 0.26[b]
1.0	-	-	68	7.20 ± 0.15[a]	4.93 ± 0.03[a]
2.0	-	-	59	3.16 ± 0.21[c]	4.00 ± 0.05[bc]
2.5	-	-	56	3.03 ± 0.13[c]	3.80 ± 0.26[bcd]
-	0.5	-	61	2.86 ± 0.12[cd]	3.20 ± 0.05[def]
-	1.0	-	63	3.13 ± 0.06[c]	3.60 ± 0.15[cde]
-	2.0	-	50	2.20 ± 0.23[e]	3.33 ± 0.14[def]
-	2.5	-	49	2.10 ± 0.20[e]	3.00 ± 0.05[efg]
-	-	0.5	50	2.36 ± 0.27[de]	2.53 ± 0.38[ghi]
-	-	1.0	56	2.53 ± 0.41[cde]	2.80 ± 0.05[fgh]
-	-	2.0	45	2.00 ± 0.05[e]	2.33 ± 0.08[hi]
-	-	2.5	40	2.00 ± 0.15[e]	2.10 ± 0.28[i]

Values represent means ± SE. Means followed by the same letter within columns are not significantly different (P=0.05) using Duncan's multiple range test. (Source: Varshney, 2012)[37]

Table 3
Effect of TDZ on multiple shoot induction from root explants excised from aseptic seedlings after 4 weeks of culture

TDZ (μM)	% Response	Mean no. of shoots/explant	Mean shoot length (cm)
1.0	30	1.90 ± 0.05^{b}	1.53 ± 0.08^{bc}
2.5	50	3.03 ± 0.08^{a}	2.63 ± 0.31^{a}
5.0	40	2.23 ± 0.33^{b}	2.03 ± 0.08^{b}
10.0	10	1.00 ± 0.05^{c}	1.00 ± 0.00
12.5	-	+	-
15.0	-	+	-

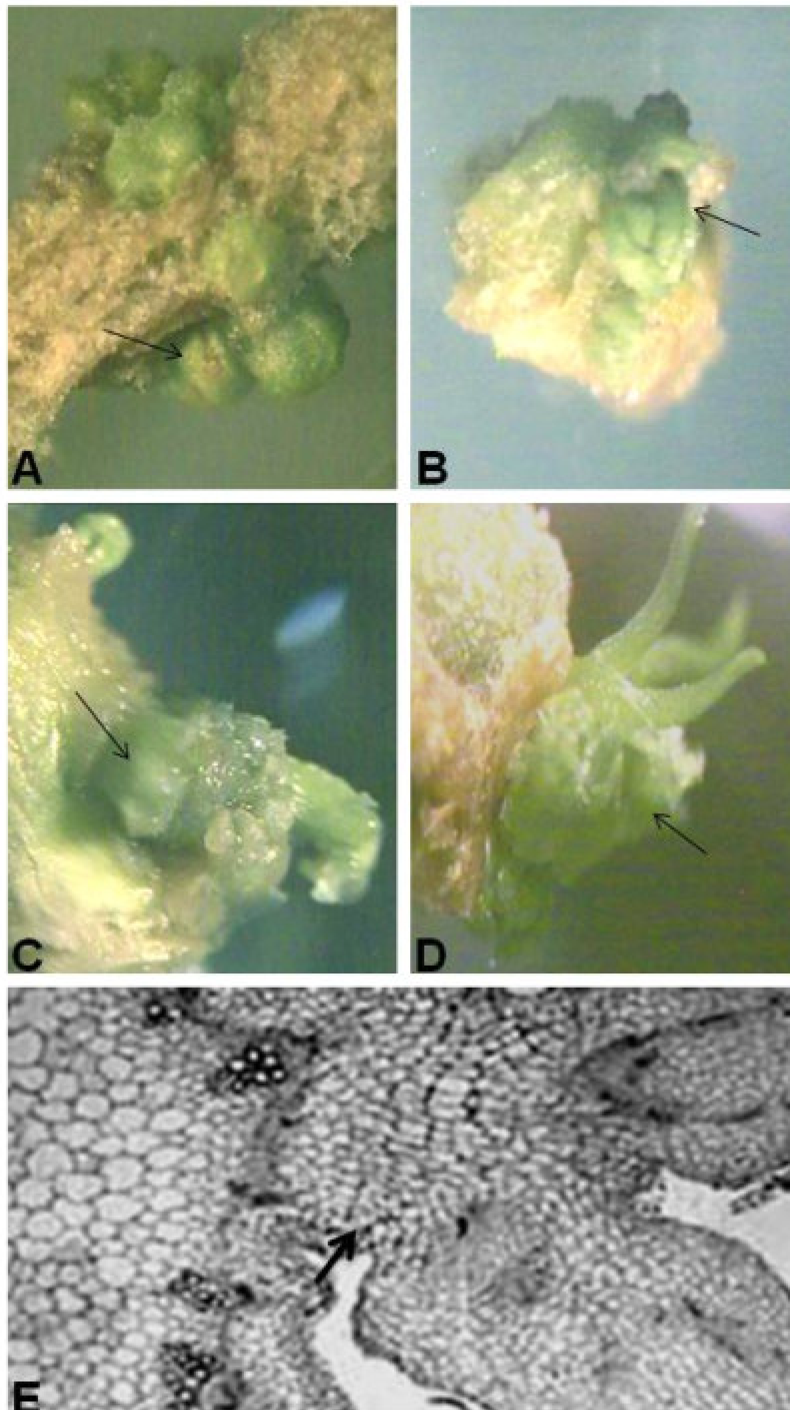
(+) represent no response. Values represent means ± SE. Means followed by the same letter within columns are not significantly different (P=0.05) using Duncan's multiple range test. *(Source: Varshney, 2012)[37]*

Table 4
Effect of different auxins in half strength MS medium on in vitro Rooting of the microshoots after 4 weeks of culture

Auxins (μM)			% Rooting	Mean no. of roots /shoot	Mean root length (cm)
IBA	NAA	IAA			
0.1	-	-	51	2.00 ± 0.00^{def}	1.53 ± 0.20^{ef}
0.5	-	-	60	2.40 ± 0.11^{cd}	1.90 ± 0.05^{de}
1.0	-	-	75	6.86 ± 0.54^{a}	4.31 ± 0.36^{a}
2.0	-	-	62	3.23 ± 0.46^{b}	2.66 ± 0.20^{c}
5.0	-	-	48	1.90 ± 0.10^{ef}	2.03 ± 0.20^{cde}
-	0.1	-	30	1.43 ± 0.12^{efg}	1.10 ± 0.05^{f}
-	0.5	-	36	2.03 ± 0.08^{de}	1.93 ± 0.03^{de}
-	1.0	-	55	3.16 ± 0.33^{d}	3.53 ± 0.37^{b}
-	2.0	-	45	2.53 ± 0.26^{bcd}	1.90 ± 0.15^{de}
-	5.0	-	20	1.93 ± 0.12^{def}	1.66 ± 0.24^{ef}
-	-	0.1	25	1.50 ± 0.00^{efg}	1.06 ± 0.08^{f}
-	-	0.5	22	1.83 ± 0.18^{fgh}	1.43 ± 0.12^{ef}
-	-	1.0	40	2.80 ± 0.15^{bc}	2.56 ± 0.29^{cd}
-	-	2.0	30	1.23 ± 0.88^{fg}	1.46 ± 0.27^{ef}
-	-	5.0	23	1.00 ± 0.00^{g}	1.00 ± 0.00^{f}

Values represent means ± SE. Values followed by the different letter within columns are significantly different (P=0.05) using Duncan's multiple range test.

Figure 1
(A-E) Direct organogenesis from root explant of Balanites aegyptiaca



A-B. Emergence of direct shoot buds from root explant on MS medium augmented with 5.0 µM BA within 2 weeks of culture (from Varshney, 2012)[37].
C-D. Advanced stages of culture showing differentiation of shoots (from Varshney, 2012)[37].
E. Histological section showing the direct induction of adventitious shoot in root explant.

Figure 2
(A-D) Adventitious shoot multiplication and plantlet formation from root explants of Balanites aegyptiaca

- *A. Adventitious shoots produced directly from root explants after 4 weeks of in vitro culture on MS medium supplemented with 5.0 µM BA (from Varshney, 2012)[37].*
- *B. Cultures showing an elongation and proliferation of healthy adventitious shoots induced from root explants after 8 weeks of incubation on MS medium containing 5.0 µM BA + 1.0 µM NAA (from Varshney, 2012)[37].*
- *C. In vitro rooted shoots cultured on half strength MS medium with 1.0 µM IBA after 4 weeks.*
- *D. An acclimatized plant in soilrite*

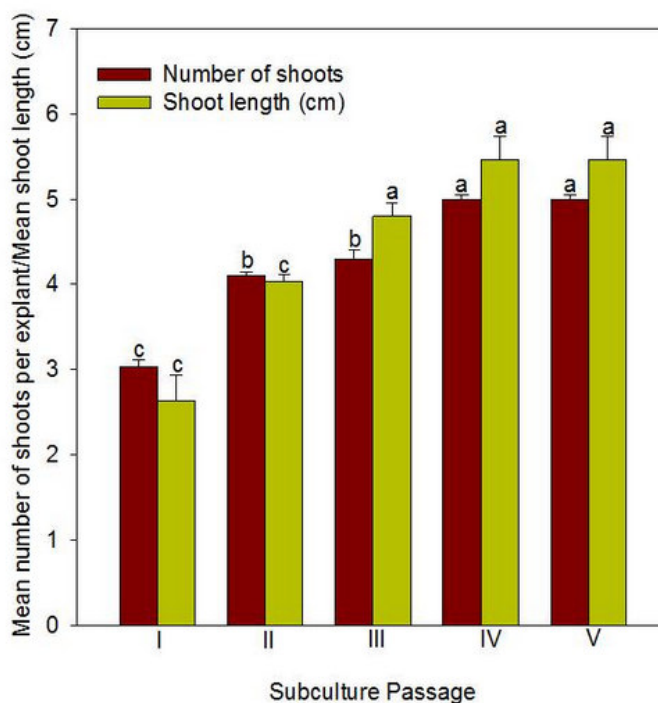


Figure 3

Growth and development of shoots obtained from root explant excised from aseptic seedlings subcultured for different number of passages on MS medium without TDZ. Bars represent means ± SE. Bars denoted by the same letter within the response variables are not significantly different (P=0.05) using Duncan's multiple range test (from Varshney, 2012)[37].

DISCUSSION

An efficient adventitious organogenesis was recognized from root explants cultured in a medium supplemented with different plant growth regulators. The effects on shoot proliferation observed in the explants probably reflect differences in the internal organization and hormonal balance. The higher shoot proliferation rates observed in root segments (middle and proximal sections) could be related to the existence of higher endogenous hormones or growth factors that are required for bud regeneration. The results are in corroboration with reports on other species where only the proximal and middle root sections exhibited direct organogenesis in *Holostemma annulare* [14], *Hypericum perforatum* [6], *Tylophora indica* [15], *Swertia chirata* [16]. Application of BA was proved to be useful in inducing multiple shoots. Like our results, BA-induced adventitious shoot regeneration has been reported in *Citrus aurantifolia* [17], *Populus tremula* [18], *Melia azedarach* [19], *Swertia chirata* [16]. The percentage response and the induction of multiple shoots declined with increase in concentration of BA beyond the optimal level (5.0 µM). Reduction in the number of shoots regenerated per explant at BA concentration higher than the optimal level was also reported in *Albizia lebbeck* [20].

TDZ was found to be least effective of all the cytokinins tested in the present study. Similar to our observations, the formation of stunted shoots or the fasciation of the shoots on TDZ supplemented medium has been reported in several plant species such as *Pyrus malus* [21], *Rhododendron* [22], *Dalbergia sissoo* [23]. The inhibition of shoot elongation may be due to the high cytokinin activity of TDZ whereas the presence of a phenyl group in TDZ may be possible cause of shoot bud fasciation [24].Formation of

multiple shoots induced from root segments in TDZ containing medium in *Balanites aegyptiaca* are in corroboration with the results in *Albizia julibrissin* [25], *Populus deltoides* [7], *Populus alba* [26]. The pattern of regeneration was also observed through histology and inveterated the origin of morphogenesis. Nodular proliferation and subsequent shoot bud formation from root explant has also been observed in *Acacia albida* [27], *Brassica napus* [28] and *Cassia angustifolia* [29].

A synergism of BA (5.0 µM) and NAA (1.0 µM) was considered to be the optimal growth regulator combination for maximum adventitious shoot regeneration in *Balanites aegyptiaca* among all the treatments. The addition of NAA in the cytokinin containing medium enhanced the multiplication and elongation of shoots induced from the root explants. However, a consistent decline in the percent regeneration, the number of shoots and shoot length was recorded with an increase in the concentrations of NAA (5.0 µM) as it resulted in the formation of stunted shoots. BA and Kn, each 5.0 µM with NAA (1.0 µM) were less effective than BA (5.0 µM) and NAA (1.0 µM) but significantly better than other treatments. Our results are in sharp contrast with the previous findings in *Balanites aegyptiaca* [11] where root segments cultured on B5 [30] medium containing 0.02mg/l NAA alone showed the morphogenetic potential of shoot regeneration in only 15.3 % of root segments after 4 weeks of culture. In previous studies, a combination of BA and NAA has been found to be ideal treatment for direct shoot multiplication of *Balanites aegyptiaca* using nodal segments [8,3,9]. Similarly, the combination of BA and NAA showed better results for direct adventitious shoot regeneration from root explants. This was probably due to variation in endogenous growth regulator contents and physiological status of the plant species and genotype. In the combination treatment, it appears that cytokinins acted specifically on shoot multiplication, while auxins on shoot elongation. Like our results, initiation of shoots from root explants has been described in several plant species, indicating a possibility of developing regenerative excised root culture for mass multiplication, their germplasm preservation and extraction of valuable secondary metabolites viz., *Acacia albida* [27], *Albizzia julibrissin* [25], *Shorea robusta* [7], *Melia azedarach* [19], *Populus alba* [26], *Azadirachta indica* [31], *Albizia lebbeck* [20] and *Cassia angustifolia* [29]. In these aforesaid plant species, it has been observed that addition of auxins and cytokinins in the regeneration medium has augmented the shoot bud induction and multiplication from root segments. It is apparent that addition of two different plant growth regulators is essential for increased adventitious shoot multiplication and elongation from root segments of *Balanites aegyptiaca*. Greater rooting was observed in IBA incorporated media compared to media containing NAA and IAA. Stimulation of rooting by IBA agrees with similar findings in other medicinal plants which are difficult-to-root both under *in vitro* and *in vivo* conditions such as *Syzygium alternifolium* [32], *Ziziphus spina- christi* [33], *Melia azedarach* [19], *Cotinus coggygria* [34], *Vitex negundo* [35], *Murraya koenigii* [36].

CONCLUSION

In conclusion, the present study has revealed the morphogenic potential of root segments of *Balanites aegyptiaca* grown *in vitro* as an alternative source for high-frequency regeneration and micropropagation by organogenesis pathway. The culture procedure for mass plant regeneration could be helpful in the study of secondary metabolite production (diosgenin, saponins for pharmaceuticals), production of transesters for diesel engine and genetic transformation.

ABBREVIATIONS

2- iP 2- Isopentenyl adenine
B5 Gamborg medium
BA 6-Benzyladenine
IAA Indole-3-acetic acid
IBA Indole-3-butyric acid
Kn Kinetin
MS Murashige and Skoog medium
NAA α- Naphthalene acetic acid

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REFERENCES

1. Anonymous (2001) *The Wealth of India: A dictionary of Indian Raw Materials and Industrial Products. Publication and Information Directorate, CSIR, New Delhi*, *2*,3-5.
2. Deshmukh, S.J. & Bhuyar, L.B. (2009) *Biomass and Bioenergy, 33,* 108-112.
3. Siddique, I. & Anis, M. (2009) *New Forests, 37,* 53-62.
4. George, E.F. (1993) *Plant Regeneration by Tissue Culture, 2nd Ed., Exegetics Ltd*., Part I, pp 574.
5. Morton, L. & Browse, J. (1991) *Plant Cell Reports, 10,* 235- 239.
6. Zobayed, S.M.A. & Saxena, P.K. (2003) *Plant Sciences, 165,* 463-470.
7. Chaturvedi, H.C., Sharma, M., Sharma, A.K., Jain, M., Agha, B.Q. & Gupta, P. (2004) *Indian Journal of Biotechnology, 3,* 305-315.
8. Ndoye, M., Diallo, I. & Gassama/Dia, Y.K. (2003) *African Journal of Biotechnology, 2,* 421-424.
9. Anis, M., Varshney, A. & Siddique, I. (2010) *Agroforestry Systems, 78,* 151-158.
10. Gour, V.S., Sharma, S.K., Emmanuel, C.J.S.K. & Kant, T. (2007) *Journal of Plant Biochemistry and Biotechnology, 16,* 151-153.
11. Gour, V.S., Emmanuel, C.J.S.K. & Kant, T. (2005) *Multipurpose Trees in the Tropics: Management and Improvement Strategies,* pp 701-704.
12. Murashige, T. & Skoog, F. (1962) *Physiologiae Plantarum, 15,* 473-497.
13. Duncan, D.B. (1955) *Biometrics,11,* 1-42.
14. Sudha, C.G., Krishnan, P.N., Seeni, S. & Pushpangadan, P. (2000) *Current Science, 78,* 503-508.
15. Chaudhari, K.N., Ghosh, B. & Jha, S. (2004) *Plant Cell Reports, 22,* 731-740.
16. Pant, M., Bisht, P. & Gusain, M.P. (2010) *Nature and Science, 8,* 244-252.
17. Bhat, S.R., Chitralekha, P. & Chandel, K.P.S. (1992) *Plant Cell Tissue and Organ Culture, 29,* 19-25.
18. Vinocur, B., Tzfira, T., Ziv, M., Vainstein, A. & Altman, A. (1997) *In: Altman A, Waisel Y; eds. Biology of root formation and development. New York: Plenum Press,* pp 217-219.
19. Vila, S., Gonzalez, A., Rey, H. & Mroginski, L. (2005) *In Vitro Cellular and Developmental Biology- Plant, 41,* 746-751.

20. Perveen, S., Varshney, A., Anis, M. & Aref, I.M. (2011) *Journal of Forestry Research, 22,* 47-52.
21. Van Nieuwkerk, J.P., Zimmerman, R.H. & Fordham, I. (1986) *HortScience, 21,* 516-518.
22. Preece, J.E. & Imel, M.R. (1991) *Science Horticulturae, 48,* 159-170.
23. Pradhan, C., Kar, S., Pattnaik, S. & Chand, P.K. (1998) *Plant Cell Reports, 18,* 122-126.
24. Huetteman, C.A. & Preece, J.E. (1993) *Plant Cell Tissue and Organ Culture, 33,* 105-119.
25. Sankhla, D., Davis, T.D. & Sankhla, N. (1996) *Plant Growth Regulators, 14,* 267-272.
26. Tsvetkov, I., Huasman, J.F. & Jouve, L. (2007) *Bulgarian Journal of Agricultural Sciences, 13,* 623-626.
27. Ahee, J. & Duhoux, E. (1994) *Plant Cell Tissue and Organ Culture, 36,* 219-225.
28. Sharma, K.K., Yeung, E.C. & Thorpe T.A. (1993) *Annals of Botany, 71,* 461–466.
29. Parveen, S. & Shahzad, A. (2011) *Acta Physiologiae Plantarum, 33,* 789-796.
30. Gamborg, O.L., Miller, R.A. & Ojima, K. (1968) *Experimental Cell Resesearch, 50,* 151-158.
31. Arora, K., Sharma, M., Srivastava, J., Ranade, S.A. & Sharma, A.K. (2011) *In vitro* cloning of *Azadirachta indica* from root explants. *Biologia Plantarum, 55*,164-168.
32. Sha Valli Khan, P.S., Prakash, E. & Rao, K.R. (1997) *Plant Cell Reports, 16,* 325-328.
33. Sudhersan, C. & Hussain, J. (2003) *Turkish Journal of Botany,* 27, 167-171.
34. Metivier, P.S.R., Yeung, E.C., Patel, K.R. & Thorpe, A.T. (2007) *In Vitro Cellular and Developmental Biology- Plant, 43,* 119-123.
35. Ahmad, N. & Anis, M. (2011) *European Journal of Forest Research, 130,* 135-144.
36. Rani, U., Ismail, N., Zibbu, G., & Batra A. (2010) *International Journal of Pharma and Bio Sciences*,1-7.
37. Varshney, A (2012) Ph.D Thesis, *Aligarh Muslim University, Aligarh, India.*