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MICROPROPAGATION OF *Commiphora wightii* (ARNOTT) BHANDARI — A THREATENED MEDICINAL PLANT OF SEMI-ARID REGION

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A simple and efficient micropropagation method is developed for *Commiphora wightii* from the cotyledonary nodal segments of twenty one-day-old *in vitro* grown seedlings. Multiple shoots were initiated on MS medium containing IAA 0.1 mgl^{-1}, BA 1.5 mgl^{-1} and additives consisting of 50 mgl^{-1} ascorbic acid, 25 mgl^{-1} each of citric acid, arginine and adenine sulphate. The isolated shoots were multiplied on MS medium containing IAA 0.1 mgl^{-1}, BA 1.0 mgl^{-1} and additives as in the initiation medium. Shoots were rooted on 1/2 strength MS medium containing 0.25 mgl^{-1} IBA. The rooted plantlets were hardened and transferred to soil.

Key words : *Commiphora wightii*, micropropagation, rooting, hardening

Increasing human activities and wide range exploitation of plants for their useful components caused disturbances in the habitats of plants. This resulted in complete loss of gradual reduction of important germplasm of economically and medicinally important plant species. Emphasis has been given on the conservation of these plant species either by conventional or unconventional methods (Mohamed *et al.*, 1999). Multiplication and conservation of threatened and rare plant species by using biotechnological methods is being done for various species (Arora and Bhojwani, 1989; Anand *et al.*, 1999) and is being utilized for conservation of important plant germplasm for posterity.

Commiphora wightii (Arnott) Bhandari of Burseraceae, is a balsamiferous shrub, which is apomictic in nature (Gupta *et al.*, 1996). Crude and wide range tapping for oleogum resins resulted in the decline of natural population of this plant and is threatened in nature (Jain and Sastry, 1980). Medicinal plants are of great interest to the researchers of plant biotechnology, as most of the drug industries depend, in part on plants for the production of pharmaceutical compounds (Chand *et al.*, 1997). *C. wightii* is exploited for its products used in indigenous ayurvedic medicine. Its antiasthmatic as well as hypocholosterolaemic properties have been reported (Satyavadi, 1990) and so many commercial products have been marketed. It yields guggul, an important oleogum resin used as incense, fixative and perfumery. Natural propagation by conventional methods is very slow and extremely poor under semi-arid conditions and is not adequate to meet with the increasing demands of this plant. There is an important need to conserve its germplasm and also to ensure sustainable supply of this raw material. Establishment of *in vitro* cultures of *C. wightii* and plantlet regeneration is described in this communication.

MATERIALS AND METHODS

1. Collection and inoculation of seeds

Ripe fruits of *C. wightii*, collected from Kaylana, Rajasthan during the month of April-May

were depulped by keeping the seeds in running tap water and then removing the remaining pulp by rubbing the seeds in a muslin cloth. Seeds were dried and the hard seed coat was removed by treating the seeds with concentrated sulphuric acid for 3-4 minutes. The softened seed coat was removed with a scalpel and needle and the seeds were recovered. Elaborate seed treatment methods were not required for the recovery of viable seeds from the fruits as described by Barve and Mehta (1993). The seeds were surface sterilized with 0.075% (w/v) mercuric chloride solution for four minutes, washed with sterilized distilled water 6-7 times and kept in sterilized distilled water for 20-25 minutes for the seeds to absorb water and then inoculated on to hormone free MS medium (Murashige and Skoog, 1962) for germination.

2. Initiation and multiplication of cultures

From 20-25 days old seedlings, cotyledonary nodal portion, cotyledon, hypocotyl and roots were excised and kept for shoot regeneration in MS and White's (Gamborg *et al.*, 1968) media containing IAA and NAA (0.05, 0.10 and 0.25 mgl^{-1}) along with BA (0.5, 1.0, 1.5, 2.0 and 2.5 mgl^{-1}) and TDZ (0.05, 0.10, 0.15, 0.20 and 0.25 mgl^{-1}). Both the media were adjusted with 50 mgl^{-1} ascorbic acid, 25 mgl^{-1} each of citric acid, arginine and adenine sulphate. The pH of the media were adjusted to 5.7 prior to the addition of agar (0.7% w/v) and sterilized at 120°C for 20 minutes. Cultures were incubated at 27 ± 2°C and light intensity of 40 μ E m^{-2} s^{-1} and photoperiod of 16h/d. The shoots induced from the cotyledonary nodal portion were excised after 14 days and kept horizontally in MS medium containing IAA (0.05, 0.1, 0.25 and 0.50 mgl^{1}) and BA (0.5, 1.0, 2.5 and 5.0 mgl^{-1}) along with the same additives used for the initiation of shoots.

3. Rooting of shoots, acclimatization and pot transfer of plantlets

The shoots of 3-4 cm length were isolated from the shoot clump and kept in varying concentrations of MS salts (full MS, 3/4 MS, 1/2 MS and 1/4 MS) along with auxins IAA, NOA and IBA (0.05, 0.10, 0.25 and 0.50 mgl^{-1}). The rooted plantlets were removed from the culture and adhered agar was removed from the plantlets and kept in culture bottles containing autoclaved soilrite moistened with 1/4 MS nutrient salts and was kept in green house at 80 per cent relative humidity. The caps of the bottles were gradually removed after seven days and the plantlets were exposed to outer environment. After one month of hardening, the plantlets were transferred to sandy soil in pots.

All the experiments were repeated at least thrice with ten replicates each and observations were recorded during an interval of 21 days for all the experiments. Data were analyzed statistically by using ANOVA.

RESULTS AND DISCUSSION

1. Initiation and multiplication of cultures

Of the two media tested, maximum growth was observed in MS medium containing IAA 0.1 mgl^{-1}, BA 1.5 mgl^{-1} along with 50 mgl^{-1} of ascorbic acid, 25 mgl^{-1} each of citric acid, arginine and adenine sulphate. Effects of these additives on shoot initiation of various species from the arid region have been reported (Arya and Shekhawat, 1986). Two to three shoots were initiated from cotyledonary nodes during the culture period of 21-25 days (Fig. 1). The data

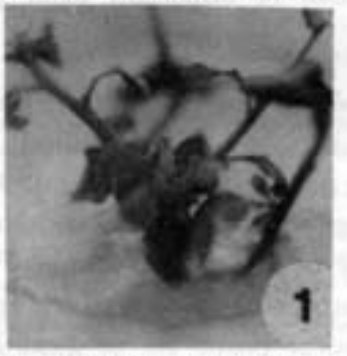

Fig. 1. Initiation of shoots from the cotyledonary nodal portion of *C. wightii* on MS medium containing IAA 0.1 mgl^{-1}, BA 1.0 mgl^{-1} and additives.

shows IAA, an unstable auxin under light, is more efficient for shoot regeneration than NAA, which is stable under light conditions. The results also shows that cotyledonary nodal explants needed an unstable auxin for a short period of time in order to regenerate shoots, because at low cytokinin levels, the rate of regeneration was more frequent as IAA concentration was increased, but a stable auxin like NAA could suppress shoot regeneration. George (1993) made similar observations for the synergetic effects of auxin and cytokinins. Higher concentrations of BA produced large number of shoots, which were small in size. TDZ at low concentrations induced large number of fasciated and vitrified shoots which senesced during the subculturing period (Table 1). Similar observations for TDZ are reported in the case of *Malus pumila* (Huetteman and Preece, 1993). White's medium along with the auxins and cytokinins, did not induce shoots from any of the explants used. Cotyledon and hypocotyl induced white callus initially, from the whole surface of the explant which eventually turned brown in all the media concentrations tried. From the root segments, white globular bodies were produced, which were attached to the explant and later turned brown and did not show any regeneration potential in any of the media used.

Table 1. Effect of various auxins, cytokinins and TDZ on shoot induction from the cotyledonary nodal segments of *C. wightii* in MS medium containing additives. (Data collected after 21 days)

Medium	Auxin(s) mgl^{-1}	Cytokinin (s) mgl^{-1}	Mean shoot number/ explant ± SE	Mean shoot length (cm) ± SE
MS (control)	-	-	1.1 ± 0.31^{a}	1.04 ± 0.12^{d}
	IAA	BA		
	0.1	0.5	1.3 ± 0.48^{a}	1.80 ± 0.13^{h}
	0.1	1.0	1.9 ± 0.56^{bc}	1.59 ± 0.12^{g}
	0.1	1.5	2.6 ± 0.67^{cde}	1.04 ± 0.19^{cd}
	0.1	2.0	2.5 ± 0.53^{de}	0.88 ± 0.13^{b}
	0.1	2.5	3.1 ± 0.74^{f}	0.61 ± 0.18^{a}
	NAA			
	0.1	0.5	1.3 ± 0.48^{a}	1.59 ± 0.51^{f}
	0.1	1.0	1.5 ± 0.52^{ab}	1.21 ± 0.18^{e}
	0.1	1.5	2.0 ± 0.60^{bcd}	0.91 ± 0.15^{bc}
	0.1	2.0	2.4 ± 0.69^{cde}	0.83 ± 0.12^{b}
	0.1	2.5	2.7 ± 0.82^{e}	0.59 ± 0.11^{a}
		TDZ		
		0.05	2.2 ± 0.69^{a}	1.1 ± 0.22^{e}
		0.10	2.8 ± 0.80^{ab}	1.2 ± 0.14^{d}
		0.15	3.6 ± 0.70^{ab}	1.1 ± 0.14^{c}
		0.20	3.8 ± 0.40^{b}	1.0 ± 0.11^{b}
		0.25	4.1 ± 0.60^{b}	0.6 ± 0.21^{b}

Any having the same letter is not significant at the 5% level

Three to four shoots were induced from single nodes of the isolated shoots on MS medium containing IAA 0.1 mgl^{-1}, BA 1.0 mgl^{-1} and additives within a period of 16-20 days (Fig. 2).

Fig. 2. Multiple shoot induction from the isolated shoots of *C. wightii* on IAA 0.1 mgl^{-1}, BA 1.0 mgl^{-1} and additives.

At higher concentration of BA, the shoots induced were small and during subculture to fresh media dried off (Table 2). Increasing the level of auxins resulted in the formation of white friable callus from the entire surface of the explant.

2. Rooting and acclimatization of plantlets

Half strength MS medium containing 0.25 mgl^{-1} IBA was found to be the best for strong and viable root induction from the isolated shoots within 10-12 days. Two or three roots (*ca.* 3-4 cm in length) induced from the shoots during the culture period (Fig. 3). The root inducing capacity of IBA along with half strength MS

Table 2. Effect of different concentrations of IAA and BA on shoot multiplication of *C. wightii* in MS medium containing additives. (Data collected after 21 days)

IAA + BA (mgl^{-1})	No. of shoots ± SE	No. of shoots ± SE	Callus intensity
0.05 + 0.5	1.2 ± 0.8^{a}	1.1 ± 0.2^{d}	-
0.05 + 1.0	1.4 ± 0.6^{b}	1.0 ± 0.2^{c}	-
0.05 + 2.5	2.1 ± 0.4^{bcd}	1.0 ± 0.1^{c}	+
0.05 + 5.0	2.8 ± 0.6^{ab}	1.0 ± 0.1^{c}	+
0.10 + 0.5	1.8 ± 0.5^{bc}	2.5 ± 0.7^{d}	-
0.10 + 1.0	3.3 ± 0.6^{cde}	3.1 ± 0.5^{e}	-
0.10 + 2.5	3.5 ± 0.4^{f}	1.8 ± 0.6^{a}	+
0.10 + 5.0	3.8 ± 0.6^{cde}	1.5 ± 0.2^{b}	+
0.25 + 0.5	1.6 ± 0.4^{ab}	1.2 ± 0.6^{c}	-
0.25 + 1.0	1.8 ± 0.2^{bcd}	1.1 ± 0.4^{bc}	-
0.25 + 2.5	2.2 ± 0.1^{cd}	1.0 ± 0.2^{ab}	+
0.25 + 5.0	2.6 ± 0.2^{ef}	1.0 ± 0.4^{bc}	+
0.50 + 0.5	1.2 ± 0.2^{ab}	1.0 ± 0.2^{d}	-
0.50 + 1.0	1.4 ± 0.4^{a}	1.1 ± 0.4^{d}	-
0.50 + 2.5	2.1 ± 0.2^{b}	1.0 ± 0.6^{b}	+
0.50 + 5.0		2.8 ± 0.4^{b}	+

- no callus, + moderate callus
Any having the same letter is not significant at the 5% level

Fig. 3. Root induction from the isolated shoots of *C. wightii* on half strength MS medium containing 0.25 mgl^{-1} IBA.

medium is reported to be better than IBA along with full strength MS medium (Rout *et al.*, 1999). Higher concentrations of IBA induced white friable callus from the basal portion of the shoots and tip burning of shoots. All the media along with IAA and NOA were less effective for root induction as they induced small fibrous roots along with watery callus from the basal portion of the shoots. Concentrations of NOA used, produced weak roots with brownish callus (Table 3). Full strength

Table 3. Effect of different auxins on root induction from the isolated shoots of *C. wightii* on half strength MS medium. (Data collected after 21 days)

Auxins used (mgl^{-1})	No. of roots/shoot ± SE	Length of roots ± SE	Callus intensity
Control	1.4 ± 0.5^{a}	0.5 ± 0.1^{a}	-
IAA			
0.05	1.0 ± 0.2^{a}	0.8 ± 0.1^{a}	-
0.10	1.2 ± 0.8^{ab}	0.9 ± 0.2^{ab}	+
0.25	1.0 ± 0.6^{a}	0.6 ± 0.3^{a}	+
0.50	-	-	+
IBA			
0.05	1.0 ± 0.1^{ab}	0.5 ± 0.06^{a}	-
0.10	2.0 ± 0.6^{cd}	0.8 ± 0.08^{ab}	+
0.25	3.3 ± 0.5^{cde}	1.7 ± 0.30^{bcd}	-
0.50	2.0 ± 0.6^{de}	1.4 ± 0.4^{cde}	+
NOA			
0.05	1.0 ± 0.1^{ab}	0.3 ± 0.1^{a}	-
0.10	1.4 ± 0.1^{abc}	0.5 ± 0.1^{a}	+
0.25	1.6 ± 0.2^{bcd}	0.4 ± 0.1^{a}	+
0.50	1.8 ± 0.2^{cde}	0.2 ± 0.1^{ab}	+

- no callus, + moderate callus, ++ high callus
Any having the same letter is not significant at the 5% level

MS, 3/4 MS and 1/4 MS nutrient media were found to be inefficient and induced only small fibrous roots with good amount of callus from the basal portion of the shoots.

Actively growing plantlets were transferred to autoclaved soilrite in glass bottles (Fig. 4)

Fig. 4. Hardening of rooted plantlets of *C. wightii* in glass bottles containing autoclaved soilrite enriched with one fourth MS nutrient salts.

moistened with 1/4 liquid MS nutrient salts and were transferred to pots containing sandy soil (Fig. 5). All the plantlets transferred to the soil survived.

Fig. 5. Forty five days old pot transferred hardened plantlets of *C. wightii*.

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