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EFFICIENT REGENERATION OF A POTENTIAL MEDICINAL PLANT *OCHRADENUS BACCATUS* DELILE FROM COTYLEDON AND SHOOT AXIS

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Abstract

Ochradenus baccatus (Del.) is one of the high value medicinal plant of Saudi Arabia. The plant is used particularly in lowering blood cholesterol level and counteracting malaria. The propagation of the species in Saudi Arabia and other allied areas is scarce. Therefore, we have developed an effective regeneration method of mass propagation of this potential plant. *In vitro* shoot multiplication was obtained from cotyledon and shoot axis on the MS medium containing 2.0 µM (BA) and 4.0 µM (NAA). The shoots were further multiplied on the MS medium containing (0.5-5.0 µM) BA and 2ip individually. BA was better in enhancing the multiplication of shoots. These shoots were routinely cultured on the MS medium with BA at 1.0 µM concentration which resulted in a fair amount of shoots per subculture at one month interval. The multiplied shoots were exposed to the MS medium supplemented with IBA and NAA. This resulted in reduced percent rooting. Rooting was enhanced when the shoots pulse treated with IBA (25-200 µM) at 5, 10 and 15 days. When the shoots were transferred to the MS medium without any PGR maximum (96.6%) rooting was achieved on treatment with 100.0 µM IBA for 10 days. Plants were acclimatized on different soils. Highest (70%) survival was obtained on sterilized soil with FYM. Efforts are being made to transplant the i*n vitro* developed plants to the natural habitat.

Introduction

Ochradenus baccatus is found growing on sandy and stony places in the Kingdom of Saudi Arabia (Al-Fredan, 2010). It has also been reported from almost all the deserts of Egypt (Tackholm, 1974; Abd El-Wahab *et al.*, 2008). This species is medicinally very important as it has already been shown that it can effectively lower blood cholesterol level in rats and showed a strong growth inhibition of the malaria parasite *Plasmodium falciparum* (Sathiyamoorthy *et al.*, 1991). This plant being of high medicinal value has been characterized for its composition. Barakat *et al.*, (1991) have isolated a number of compounds including some novel flavonoids from the leaves of this plant.

The demand of *O. baccatus* has increased tremendously during the last few decades. Therefore, it is imperative to adopt different ways of propagation and multiplication of the plant. In this regard the plant tissue culture is a rapid multiplication technique which can be successfully used to generate a large number of plantlets using small amounts of explants (Edson *et al*., 1997). However, plant regeneration is essential for genetic transformation to improve their yield and quality traits such as drought resistance, heat registance, longer self life, nutritional quality and acidic soil adaptation (Lal & Lal, 1993). Thus, this technique after proper optimization can facilitate large scale multiplication and conservation of germplasm of threatened, endangered, rare and important medicinal plants (Arora & Bhojwani, 1989).

The objective of this study was to develop an appropriate, fast and efficient method of propagation for *O. baccatus*. We describe here micropropagation of *O. baccatus* from two sources of explants i.e., i*n vitro* grown cotyledons and shoots.

Materials and Method

The seeds were first washed thoroughly with tap water and then treated with liquid detergent Labolene and 0.1% (w/v) Bavistin for 10 min, and rinsed thoroughly with distilled water. The seeds so treated were surface sterilized with sodium hypochlorite solution containing 4% available chlorine for 7-10 min and washed (X3) with sterilized double distilled water. The sterilized seeds were then inoculated in glass Petri plates. The MS media (Murashige & Skoog 1962) supplemented with 2 percent sucrose, BA (benzyl adenine) and NAA (naphthalene acetic acid) ranging from 1.0-6.0 µM was used for shoot induction. Primary shoots produced in the culture media were further multiplied by culturing nodal explants in the MS media supplemented with optimum concentration of 0.5-5 µM BA and 2ip (2, isopentynyl adenine). For i*n vitro* root formation IBA (Indole butyric acid) and NAA (0.5-10.0 µM) were added to the agar gelled MS basal medium. In a second experiment IBA at higher concentration 25-200 µM was applied for 5, 10, and 15 days. For root induction the shoots were transferred to the MS medium alone. The pH of the media was maintained at 5.8 and then autoclaved them for 15 min at 121°C and 104 kPa. Based on requirement, the media were poured into the Petri plates (30 ml/plate) or in Erlenmeyer flasks (50 ml/flask). For shoot and root regeneration, each treatment comprised 3 replicates, i.e., 3 explants per/plate for shoot and and 10 shoots per flask for root. All cultures were placed in a culture room set at 55–60% RH and 25-28°C under PPFD of 40 µmol m^{-2} s^{-1} for 16 h photoperiod. One month after rooting, acclimatization of the plants was carried out in a controlled green-house at 27-30 ºC and 80% RH. Plants were watered daily for the first five days, then as and when required to achieve sufficient soil moisture in pots. Data were analyzed with the SPSS program using one-way ANOVA. Post-hoc tests were performed using the Tukey's Multiple Range test at $p< 0.05$.

Results and Discussion

The seeds cultured on the MS medium containing different concentrations of NAA and BA (1.0-6.0 µM, Table 1) showed germination (emergence of radicle

considered germination) after 7 days of inoculation. The culture exhibited expansion in cotyledon as well as in the embryonic axis. After 10 days, some protuberance started appearing from the surface of cotyledonary leaves on the MS medium supplemented with NAA (4.0 µM) and BA (2.0 µM, Table 1). Simultaneously some shoot multiplication was observed on the same medium but the number of shoots (3.3 shoot/explant, Table 1 and Fig. 1A) was much lower than the shoot buds produced from the cotyledon (19.0 shoots/explant, Table 1 and Figs. 2 A, B). After one month of culture, these shoots were detached from mother plant and cultured on the MS medium supplied with BA and 2ip in the range of 0.5-5.0 µM to optimize shoot multiplication. It was noted that number (12.0 from shoots and 16.3 from cotyledons, Table 2) of shoots increased on the MS medium supplied with BA (1.0 µM). In case of 2ip a significant multiplication was not achieved. Therefore, a further sub-culturing was carried out the medium with 1.0 µM BA for the maintenance of the culture. The source of shoot multiplication was significant on the first subculture whereas it normalized on a subsequent subculture (data not presented). This report describes the adventitious shoot formation and plant regeneration from cotyledon explants in *Ochradenus*. Plant regeneration has been achieved by using cotyledons as explants in a number of plant species. In most cases, cotyledonary tissues were obtained from young seedlings such as in squash *Cucurbita pepo* (Ananthakrishnan *et al*., 2003), bottle gourd (*Lagenaria siceraria* Standl) (Han *et al.*, 2003), oilseed crop, niger (*Guizotia abyssinica* (Murthy *et al.*, 2003) and *Althia rosea* (Munir *et al.*, 2012). Similarly, shoot regeneration was also reported (Ghareb *et al*., 2007 and Nadeem *et al.*, 2012) from nodal explant treated with BA on the MS medium.

Table 1. Multiplication of shoots from cotyledon and embryonal axis on MS medium containing different concentrations of BA and NAA.

S.No.	PGRs concentration (µM)		Number of shoots ± SD	
	BA	NAA	Cotyledon	Shoot
1.	1.0	0	-	2.3 ± 0.57
2.	2.0	0	-	1.6 ± 0.57
3.	4.0	0	-	3.0 ± 1.00
4.	6.0	0	-	1.6 ± 1.15
5.	0	1.0	-	-
6.	0	2.0	-	-
7.	0	4.0	-	-
8.	0	6.0	-	-
9.	1.0	1.0	4.6 ± 1.15	2.3 ± .57
10.	1.0	2.0	2.3 ± 0.57	2.6 ± 1.15
11.	1.0	4.0	2.6 ± 0.57	2.0 ± 0.00
12.	1.0	6.0	1.3 ± 0.57	2.0 ± 1.00
13.	2.0	1.0	2.0 ± 1.0	3.0 ± 1.00
14.	2.0	2.0	2.6 ± 1.15	1.6 ± 1.15
15.	2.0	4.0	19.0 ± 3.60*	3.3 ± 1.15*
16.	2.0	6.0	6.6 ± 1.15	2.0 ± 1.00
17.	4.0	1.0	5.3 ± 1.15	1.6 ± 0.57
18.	4.0	2.0	8.3 ± 1.52	1.6 ± 1.15
19.	4.0	4.0	3.3 ± 1.15	2.6 ± 1.15
20.	4.0	6.0	6.3 ± 1.52	3.0 ± 1.73
21.	6.0	1.0	3.6 ± 1.15	1.6 ± 1.15
22.	6.0	2.0	4.6 ± 2.30	1.3 ± 0.57
23.	6.0	4.0	4.3 ± 1.52	1.6 ± 1.15
24.	6.0	6.0	4.0 ± 0.00	2.0 ± 0.00

Mean of three replicates. Tukey's range test significance level at $p< 0.05$. Highly significant results within a treatment are marked

Table 2. Shoot multiplication from shoots of two origins of *O. baccatus.*

S.No.	PGRs concentration (µM)	Number of shoots/shoot ± SD	Number of shoots /cotyledon ± SD
1.	BA (0.5)	5.0±1.0	9.3 ± 1.1*
2.	BA (1.0)	12.0±4.0*	16.3 ± 3.2*
3.	BA (2.5)	5.3±0.5	11.3 ± 1.1*
4.	BA(5.0)	3.6±0.5	8.0 ± 1.0
5.	2ip (0.5)	3.0±1.0	8.0 ± 1.0
6.	2ip (1.0)	3.6±1.1	5.0 ± 1.0
7.	2ip (2.5)	3.0±1.0	3.3 ± 1.5
8.	2ip (5.0)	2.6±0.5	6.0 ± 2.0

Mean of three replicates. Tukey's range test significance level at $p=<0.05$. Highly significant results within a treatment are marked

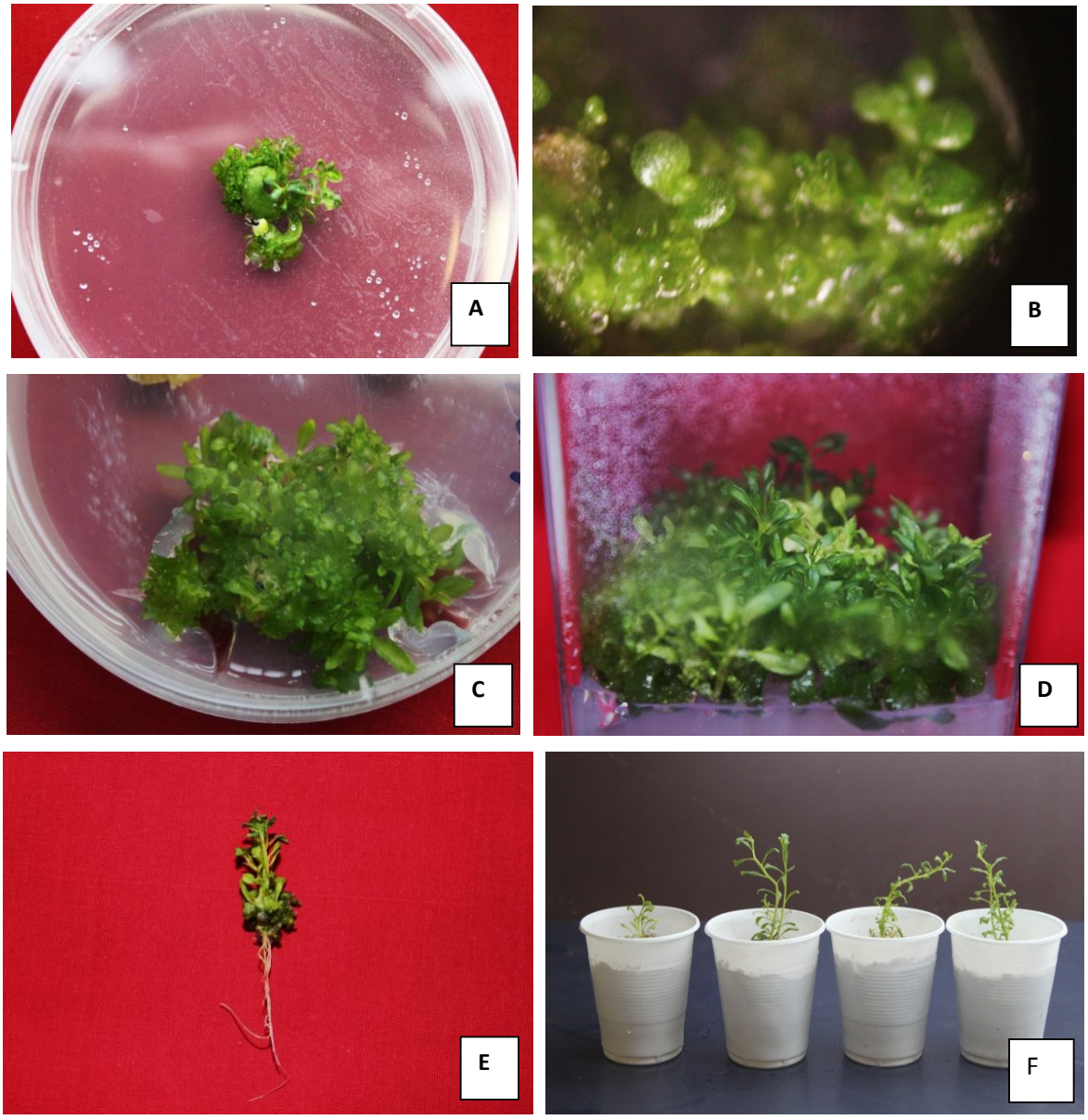

Fig. 1. A: Shoot regeneration from cotyledons of *Ochradenus baccatus* in MS medium containing BA and NAA (2.0+4.0 µM), B & C: shoot growth in MS medium containing BA (1.0 µM), D: Shoot multiplication from stem segments on MS medium supplemented with BA (1.0 µM), E: Rooting in individual shoot on MS medium supplemented with IBA (100.0 µM) pulse treatment, F: Acclimatized plants of *O. baccatus.*

Shoots of about 1.5 cm length were subjected to two sets of experimentation for root initiation. In the first experiment, the shoots were cultured on the MS medium containing IBA and NAA (0.5-10.0 µM). Root initiation occurred after 20 d of culture on both PGRs. Low rooting percentage was observed with both the PGRs on the MS medium, i.e., about 20% (maximum) in NAA and 16% (maximum) in IBA, respectively. Keeping this in mind, another strategy was adopted to optimize the rooting by treating the shoots with higher concentration of IBA (25-200 µM). This is the pulse treatment for 5,10 and 15 days on the MS medium supplemented with higher concentration of the PGR for a short period of time and then transferring the shoots to PGR free medium for further growth and development. Maximum (96.6%) rooting was noted in 100.0 µM IBA (Table 3) treatments for 10 days incubation period, which took place after 5 days of transfer to the PGR free MS medium. After one month of culturing, average shoot length from all treatments ranged from 2.20-2.54 cm and root length from 1.38-1.74 cm (Table 4). There were no significant differences found among the different treatments on the growth of shoot and

root . In the present study, IBA was found to be more effective than NAA for root induction and development in *O. baccatus*. These results are in good agreement with those of Ahad *et al.,* (1994) in watermelon, Rani *et al.,* (2006) in *Coleus blumei*, Kaliamoorthy *et al.,* (2008) in *Harpagophytum procumbens*, Hasan *et al.,* (2008) in *Cassia alata* and in *Sinapis alba* (Abbasi *et al.,* 2011) .

The plantlets thus obtained from the previous experiment were subjected to acclimatization and hardening under greenhouse environment on different types of soils. These soils were sterilized and non-sterilized i.e., garden soil, garden soil with FYM, perlite, and peat moss. Maximum survival about 70% (Fig. 2) was recorded in the sterilized garden soil with FYM after 45 days of acclimatization under greenhouse conditions. The survival of plants decreased in non-sterilized soils. It was minimal (46.6 %) in garden soil following by peat moss (46.6 %), perlite (50%) and garden soil with FYM (60%). There was no sign of phenotypic variation among the plants when grew naturally.

The propagation protocol presented was found to be highly efficient and simple. This method can be beneficial for developing a large scale multiplication systems for any propagation program of *O. baccatus*. This will also fulfil the demand of plant material required for extraction of active ingredients from this potential medicinal plant.

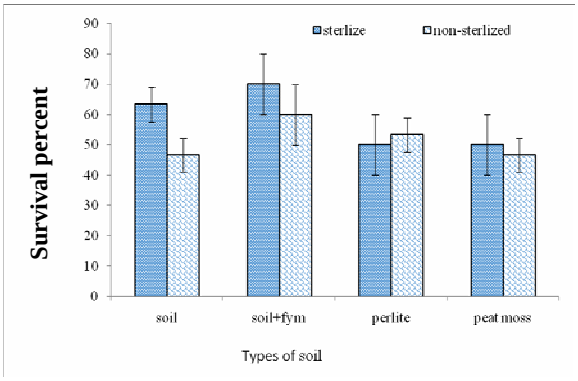

Fig. 2. Percent survival of plants in different soil types under greenhouse conditions.

Table 3. Rooting without pulse treatment in *O. baccatus* on MS medium containing NAA or IBA.

S.No.	PGRs concentration (µM)	Rooting (%) ± SD	Shoot length (cm) ± SD	Root length (cm) ± SD
1.	NAA (0.5)	13.3 ± 5.7	2.5 ± 0.20	1.5 ± 0.13
2.	NAA (2.5)	20.0 ± 10.0*	2.5 ± 0.20	1.5 ± 0.19
3.	NAA (5.0)	16.6 ± 5.7	2.6 ± 0.18	1.4 ± 0.16
4.	NAA (10.0)	10.0 ± 0.00	2.6 ± 0.21	1.4 ± 0.14
5.	IBA (0.5)	13.3 ± 5.7	2.5 ± 0.15	1.6 ± 0.20
6.	IBA (2.5)	16.6 ± 11.5*	2.6 ± 0.17	1.4 ± 0.13
7.	IBA (5.0)	13.3 ± 5.7	2.6 ± 0.16	1.4 ± 0.20
8.	IBA (10.0)	10.0 ± 0.00	2.7 ± 0.13	1.4 ± 0.11

Mean of three replicates. Tukey's range test significance level at p=<0.05. Highly significant results within a treatment are marked.

Table 4. Rooting with pulse treatment of IBA after 5, 10 and 15 days for induction.

S.No.	Time interval (days)	IBA Treatment (µM)	Mean rooting (%) ± SD	Mean shoot length (cm) ± SD	Mean root length (cm) ± SD
1.		25	26.6 ± 5.7	2.3 ± 0.19	1.7 ± 0.25
2.		50	33.3 ± 5.7	2.3 ± 0.20	1.5 ± 0.19
3.	5	100	93.3 ± 5.7*	2.3 ± 0.14	1.5 ± 0.18
4.		150	73.3 ± 5.7*	2.4 ± 0.11	1.5 ± 0.21
5.		200	63.3 ± 5.7	2.4 ± 0.17	1.4 ± 0.17
6.		25	23.3 ± 5.7	2.4 ± 0.12	1.6 ± 0.16
7.		50	26.6 ± 5.7	2.4 ± 0.11	1.7 ± 0.22
8.	10	100	96.6 ± 5.7*	2.5 ± 0.18	1.7 ± 0.24
9.		150	66.6 ± 5.7*	2.5 ± 0.15	1.6 ± 0.20
10.		200	53.3 ± 5.7	2.3 ± 0.15	1.6 ± 0.15
11.		25	16.6 ± 5.7	2.4 ± 0.11	1.7 ± 0.26
12.		50	23.3 ± 5.7	2.5 ± 0.15	1.7 ± 0.32
13.	15	100	70.0 ± 10.0*	2.4 ± 0.18	1.7 ± 0.29
14.		150	53.3 ± 5.7	2.2 ± 0.09	1.7 ± 0.22
15.		200	46.6 ± 5.7	2.2 ± 0.10	1.7 ± 0.25

Mean of three replicates. Tukey's range test significance level at p=<0.05. Highly significant results within a treatment are marked

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