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DIRECT SHOOT MULTIPLICATION IN *OCHRADENUS ARABICUS*, AN ENDEMIC MEDICINAL PLANT OF SAUDI ARABIA

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Abstract

In vitro studies were carried out with *Ochradenus arabicus* for rapid micropropagation from embryo explants. Direct regeneration of shoot buds of *O. arabicus* was observed in MS basal medium supplemented with varying levels of either benzyladenine (BA) or kinetin (Kn). Embryos produced multiple shoots on Murashige and Skoog's medium (MS) containing 1.0 µM BA after 25 days of inoculation. Number of shoots depended on type of cytokinin and its concentrations. Maximum number of shoots (56) was derived from MS medium supplemented with 1.0µM BA, which declined with a further increase in BA concentration. Kinetin also induced multiple shoots at higher concentration (2.0µM) but the number of shoots produced was very low (15). The shoot buds elongated well on MS medium with 1.0µM BA. The shoots thus obtained were rooted on MS medium supplied with 0.5µM NAA after 20 days of culture. The plantlets so developed were transferred to soil after proper acclimatization.

Introduction

The genus *Ochradenus* Del. belongs to family Resedaceae, which comprises about eight species of shrubs mainly found growing in desert and arid regions of North-East Africa, and South-West and central regions of Saudi Arabia. Plants of *Ochradenus* have complex branching system with deciduous leaves. They bear occasional polygamous flowers with or without ephemeral corolla. All these characteristics are considered as important adaptations against severe aridity (Martin-Bravo *et al.*, 2007). Only one species *O. baccatus* Del. is widespread while most of the other species are restricted as endemics in the South Arabian Peninsula and the horn of Africa (Miller 1984).

The medicinal value of herbal plants depends on various types of phyto-constituents, which accumulate in the aerial and non-aerial parts of a plant. Undoubtedly, the contents of these phyto-constituents are greatly affected by a variety of biotic and abiotic factors. *Ochradenus baccatus* is known for its numerous metabolites such as flavonoids derived from areal parts (Shabana *et al.*, 1990).

Several native desert plants are being threatened and are near to extinction mainly due to anthropogenic factors (Sudhersan *et al*., 2003; Al-Abbasi *et al.*, 2010). Thus, such endangered desert plants need to be preserved and propagated using potential biotechnological approaches (Khan *et al*., 2012). Unfortunately, the propagation and establishment techniques being employed these days for the conservation of valuable native plants are not very effective (AboEl-Nil, 1997; Al-Abbasi *et al.*, 2010). Keeping in view this problem, an *in vitro* protocol was developed to propagate plantlets of *O. arabicus* which could be able to tolerate extreme adverse conditions in addition to their possible potential use in urban landscaping. Perhaps, this is the first report on *In vitro* multiplication of *O. arabicus*.

Materials and Method

Plant material: Some mature plants of *Ochradenus arabicus* bearing seeds were collected from the central region of Saudi Arabia. Plants were got identified by a plant taxonomist and a specimen was deposited in the herbarium of Department of Botany and Microbiology, College of Science, King Saud University, Riyadh (Fig. 1). The mature seeds were used for germination as well as for shoot and root development.

Fig. 1. *Ochradenus arabicus*.

Shoot and root regeneration: The seeds were first washed well with tap water for 30 min and then treated with liquid detergent Labolene (Qualigens, India; 5% solution) and 0.1% (w/v) Bavistin (Bayer, India) for 10 min and rinsed well with distilled water. The seeds so treated were then surface sterilized with sodium hypochlorite containing 4% available chlorine for 10 min and rinsed well with sterilized double distilled water. The sterilized seeds were then inoculated in glass Petri plates at a rate of 25 seeds per plate. When the embryos grew to two cotyledonary stage they were used for *in vitro* shoot induction and multiplication. The MS media (Murashige & Skoog, 1962) supplemented with 2 percent sucrose solution and BA and kinetin ranging from 0.5-5.0µM was used for shoot induction. Primary shoots produced in the culture media were further multiplied by culturing nodal

explants in MS media supplemented with optimum concentration of BA, i.e., 1.0µM. For *in vitro* root formation IBA, NAA or 2,4-D (0.5-10.0 µM) was added to the agar gelled MS basal medium. The pH of media was maintained at 5.8 before autoclaving for 15 min at 121°C and 104 kPa. Based on requirement, media were poured into the Petri plates (30 ml/plate) or in 250ml Erlenmeyer flasks (50 ml/flask). For shoot regeneration, each treatment comprised 3 replicates (3 explants per/plate) and for rooting, 3 replicates per treatment (5 shoots per flask). Each experiment was repeated three times. All cultures were placed in a culture room maintained at 55–60% RH and 25-28°C under a 16 h photoperiod of 40 µmol m^{-2} s^{-1} PPFD by cool white fluorescent tubes. Tukey's range test (Gomez & Gomez, 1984) was employed to test the significant differences among the mean values.

Results and Discussion

In our study, the adventitious bud producing capacity was found to be significantly dependent on the addition of a growth regulator and its concentration to the growth medium. For example, seedlings grown on MS basal medium containing no any growth regulator failed to show regeneration. Furthermore, addition of cytokinins (BA or Kn) to the medium was found to be essential for adventitious shoot organogenesis. The first noticeable changes in the cultured embryos appeared during the third week of culture (Fig. 2a). Although the embryos grew in size, they became deformed so that the adventitious shoot buds arose directly from the surface (Fig. 2b). Shoot buds continued to emerge from the explants till the end of the month of culture; thereafter there was a substantial decrease in shoot bud differentiation (Fig. 2c). The cytokinin BA alone particularly at 1.0µM, showed the highest response in terms of shoot bud formation. However, a further increase in BA concentration from 2-5 µM resulted in the frequency of shoot bud production. Embryos at two cotyledonary stage produced a maximum of 56.0 shoots per explant (Table 1).

Table 1. Effect of cytokinin on shoot regeneration from mature zygotic embryos cultured on MS medium.

S. No.	Treatment µM	Number of shoots (Mean± S.E.)	
		BA	Kn
1.	0.5	6.0 ± 1.53 bc	3.66 ± 1.20 ab
2.	1.0	56.0 ± 5.86 a	6.66 ± 0.88 b
3.	2.0	15.67 ± 0.88 b	15.00 ± 0.57 a
4.	3.0	8.67 ± 0.67 bc	4.00 ± 1.00 ab
5.	4.0	3.67 ± 1.86 bc	4.66 ± 0.88 ab
6.	5.0	1.33 ± 1.33c	3.00 ± 0.57 ab

Mean of three replicates. Tukey's range test significance level at p=<0.05

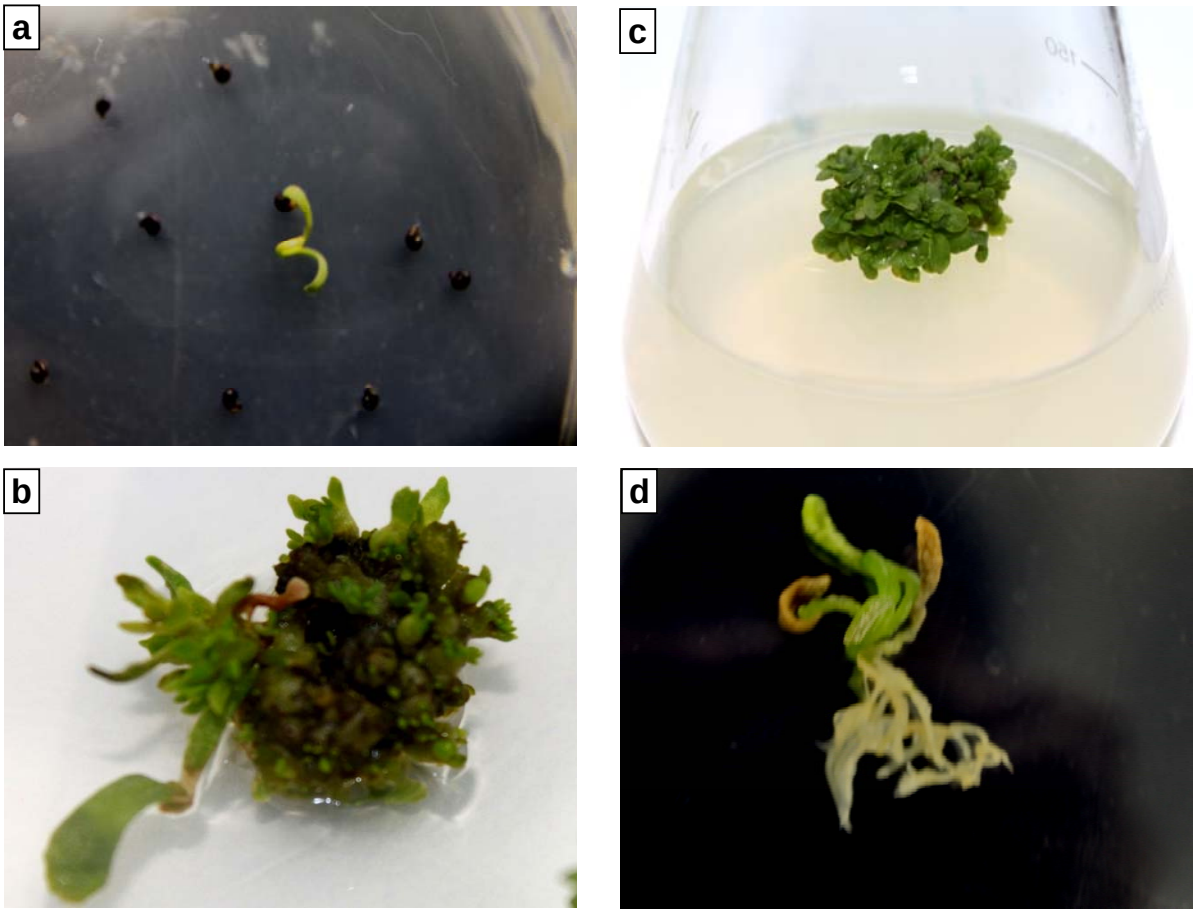

Fig. 2. a: Embryo at two cotyledonary stage; b: Appearance of shoot buds on MS media containing BA; C: Shoot elongation and growth; d: Induction of root in individual shoot on MS medium containing 0.5 µM NAA.

The other PGR tested for shoot induction was kinetin (Kn) which was able to cause shoot induction at higher concentration (Kn 2.0µM). However, the number of shoots produced (15 shoots) was much lower than that obtained by BA. Elongation of induced shoots and expansion of leaves was achieved by transferring the cultures to MS basal medium containing no growth regulator. For shoot multiplication, the primary shoots regenerated from embryos were excised and multiplied using nodal explants in MS medium containing the optimum concentration of BA (1.0µM), and these regenerated shoots were used for the rooting experiment. None of the shoots cultured in a medium without auxin produced roots. Roots initiated between 20 and 30 days of culturing (Fig. 2d).

Shoots (2.62 cm length after rooting) cultured in medium supplemented with NAA showed a very slow rooting response. At 0.5 µM of NAA, root initiation took place after 20-30 days of culture and a maximum of 80% rooting with an average number (2.75) of roots (1.3cm) per shoot was achieved. The shoots inoculated for rooting were replaced by callus development in media with NAA, 2,4-D and IBA at high concentration, i.e., 2.0µM or higher within two weeks. The plantlets produced through *in vitro* propagation protocol were planted in pots containing sterile soilrite. About 65 percent of the acclimated plants were transferred to full sun where they grew prosperously without any detectable phenotypic variation.

As is evident from the results presented here application of BA was found to be very effective in inducing multiple shoots. BA-induced adventitious shoot regeneration from embryos, was also reported in crops such as *Vigna unguiculata* L. (Aasim *et al*., 2010) and *Cajanus cajan* (Prasad *et al.*, 2011). BA ranging in concentration from 0.25-1.0 mg/l was found to be also effective to induce organogenesis in callus derived from hypocotyls of *Embellica officinalis* (Hai-Tao *et al.*, 2006), from mature embryo in *Vigna unguiculata* L. (Odutayo *et al.*, 2005). In the present study, the induction of multiple shoots decreased considerably with increase in concentration of BA beyond the optimal level (1.0µM). Reduction in the number of shoots regenerated per explant at BA concentration higher than the optimal level (1.0µM) was also earlier reported in *Annona squamosa* (Nagori & Purohit 2004). Considerable rooting was achieved in a medium containing NAA compared to that with 2,4-D or IBA, which in fact could not induce any rooting. Among various concentrations tested, NAA at 0.5µM concentration produced highest response. Similar rooting response was also observed in *Dendrocalamus asper* (Arya *et al*., 1999). The protocol employed in the present study for *In vitro* multiplication of *Ochradenus arabicus*, an important plant species from Saudi Arabia can be useful for conservation of the endemic plants.

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