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Research Paper

REGENERATION FROM CALLUS WHICH IS PRODUCED FROM COTYLEDON OF *ANTIRRHINUM MAJUS*

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Antirrhinum majus L. (Snapdragon) is a herbaceous ornamental plant which is cultivated for its attractive view. In this study, three experiments included callus induction, regeneration from callus and *in vitro* rooting were carried out as a complete randomized design in 3 replications (10 samples). The maximum callus induction frequency (90%) from cotyledon explant was observed on MS medium supplemented with 1.0 mg/L 2,4-D along with 0.1 mg/L BAP. The highest regeneration frequency (93.33%) followed by maximum number of multiple shoots (2.96) as well as length (2.43 cm) were obtained on MS medium supplemented with 1.0 mg/l BAP along with 0.1 mg/L IBA. One hundred percent of the microshoots were rooted on MS medium supplemented with 2.0 mg/L IBA or NAA. A total of 85% survival was achieved when rooted explants were acclimatized *ex vitro* using 1 perlite: 1 cocopeat mixture.

Keywords: Acclimatization, Callus induction, Regeneration, Rooting, Snapdragon

INTRODUCTION

Antirrhinum majus (snapdragon) is a species of flowering plant belonging to the Scrophulariaceae family. It is native to the Mediterranean region, from Morocco and Portugal north to southern France, and east to Turkey and Syria (Oyama and Baum, 2004). The common name "snapdragon", originates from the flowers reaction to having their throats squeezed, which causes the "mouth" of the flower to snap open like a dragon's mouth (Tank *et al.*, 2006).

Snapdragon if propagated by seeds cannot ensure genetic uniformity, may produce undesired phenotypes and subsequently influence the quality and regeneration potential of the plants, as the plants may be randomly selected without taking necessary care. This will result in negative economic implications on mass production of this plant (Sheyab *et al.,* 2010). Thus, the development of *in vitro* culture techniques would increase the knowledge about this germplasm with potential relevance as an ornamental crop.

So far, a few studies has been done on *in vitro* propagation of *Antirrhinum majus* L. (Sangwan and Harada 1975; Newbury 1986; Atkinson et al. 1988; Okubo *et al.,* 1991; Sheyab *et al.,* 2010). Therefore, the aim of this work was to establish

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a reliable regeneration system by indirect organogenesis in *Antirrhinum majus,* which may be further applied for genetic transformation.

MATERIALS AND METHODS

The experiment was conducted at tissue culture laboratory, Department of Horticulture, Ramin University of Agriculture and Natural Resources, Khoozestan, Iran. The seeds were washed under running water for 30 min and five to six times rinse with tap water and then with liquid soap solution followed by washing with tap water. Further surface sterilization treatment was conducted in a laminar air flow chamber. The seeds were surface sterilized with 70% aqueous ethanol for 4 seconds, and dipped into 10 min in 10% (v/v) NaOCl with 5.25% active chlorine solution, and then washed with 3 times in sterilized distilled water. The sterilized seeds were inoculated on one-tenth strength MS (Murashige and Skoog, 1962) medium. Medium were supplemented with 3% w/v sucrose, adjust 5.8 pH and solidified with 0.6% agar than autoclaved 121°C for 15-20 min, poured into culture bottles (4 cm in diameter and 15 cm in height). All cultures were incubated in culture room at 26 ± 2°C in light of 1000 to 1200 lux.

Explants with 0.5–1.0 cm length, from cotyledon of 1-week-old seedlings (Figure 1a) were cultured on a basal MS medium containing different concentrations of plant growth regulators for callus induction (Table 1). All of the cultures were incubated at 25±2°C in the dark.

Figure 1: Indirect Organogenesis of *Antirrhinum majus* L. via Cotyledon Explant; (a) *In vitro* Seed Germination of *Antirrhinum majus* L., (b) Friable Callus Formed from cotyledon cultured on MS medium supplemented with 2.0 mg/l 2,4-D + 0.2 mg/l BAP, after 4 weeks in darkness, (c) Indirect organogenesis from cotyledon on MS medium supplemented with 1.0 mg/l BAP along with 0.1 mg/l IBA after 1 and (d) 4 weeks of culture in the presence of light, (e) *In vitro* rooting of micro-shoots on MS medium supplemented with 1.0 mg/l IBA, (f) An acclimatized plant after 20 days

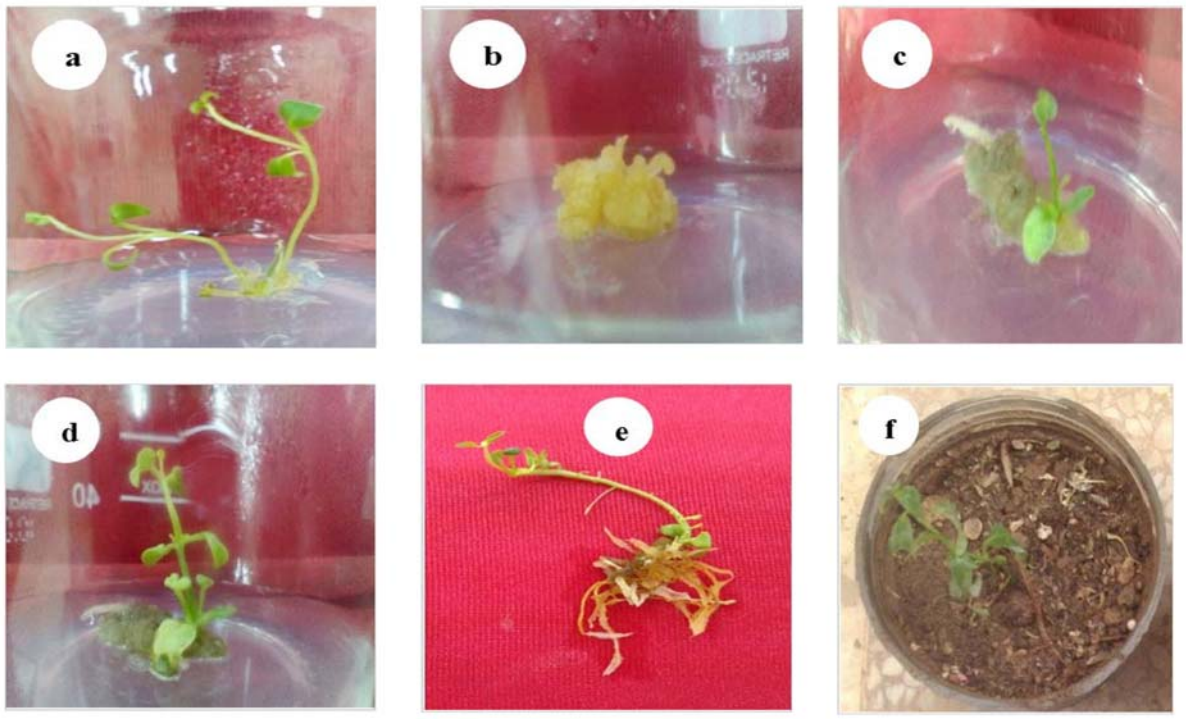

Table 1: Effect Of Different Plant Growth Regulators In Ms Medium On Callus Formation Of *A. Majus* From Cotyledon Explant.

Growth Regulator	Callus Induction (%)	Callus Weight (G)
Free Of Pgrs	0.00 C	0.00 D
0.5 Mg/L 2,4-d + 0.05 Mg/L Bap	73.33 B	1.10 B
1.0 Mg/L 2,4-d + 0.1 Mg/L Bap	90.00 A	1.56 A
1.5 Mg/L 2,4-d + 0.15 Mg/L Bap	76.66 B	1.20 B
2.0 Mg/L 2,4-d + 0.2 Mg/L Bap	70.0 B	0.89 C

Mean Comparison To The 5% Level According To Duncan's Multiple Range Test. Columns With Common Characters, No Significant Difference.

In the regeneration experiment, the callus with the greatest weight was chosen. Then the callus were cultured on a basal MS medium containing different concentrations of plant growth regulators for regeneration (Table 2). Glass culture at 16 h light and 8 h dark photoperiod condition and from 1000 to 1200 lux light intensity and temperature of +25±2°C were maintained in a growth chamber. After 4 weeks, organogenesis produced shoots.

The micro-shoots (2-3 cm high) were transferred to rooting medium consisted of MS medium and 3% sucrose with different concentrations of plant growth regulators and a MS basal medium control treatment (Table 3).

In vitro-grown plants were removed from the culture vessels 4 weeks after transfer to MS medium, and washed several times with distilled water to remove traces of medium on root surfaces. Then, the plants were transferred to perlite and cocopeat (1:1). The trays were placed in glass chambers (80 cm × 40 cm × 40 cm) at 26 ± 2°C under a 16-h photoperiod, for 4 weeks. In order to reduce the relative humidity inside the chambers, the covers were gradually opened after the first week and completely removed 4 weeks after transplanting. Survival rates of plants were recorded after this period.

The experiments were set up in Completely Randomized Design (CRD) and there were 10 replicates per treatment and each treatment was repeated in three sets. The data were analyzed by Analysis of Variance (ANOVA) followed by Duncan's multiple range test. Data analysis was carried out by using SAS version 9.3.

RESULTS AND DISCUSSION

First experiment was set up to find out the most appropriate combination between plant growth regulators for calli induction. The results showed that the maximum callus weight (1.56 g) and percent of callus induction (90.00%) after 4 weeks was obtained on MS medium supplement with 1.0 mg/l 2,4-D along with 0.1 mg/l BAP (Table 1; Figure 1b) which is somewhat in agreement with Sangwan and Harada (1975) who have reported that acceptable callus formation from stem explants of *A. majus* was observed in MS basal medium supplemented with 1.0 mg/L 2,4-D. Studies have shown that, 2,4-D is one of the most effective auxins for the induction and growth of callus (Burbulis *et al.,* 2007).

Indirect organogenesis was achieved from cotyledon explant cultured in the presence of all PGRs. The best regenerating response percentage of explant forming shoots (93.33%), for number of shoots per explant (2.96) and shoot length (2.43 cm) after 4 weeks were observed in MS medium supplement with 1.0 mg/l BAP plus 0.1 mg/L IBA. (Table 2; Figure 1c,d). Sangwan

Table 2: Effect of Different Plant Growth Regulators in MS Medium on Indirect Organogenesis of *A. majus*.

Growth Regulator	% Of Explant Formingshoots	Number Of Shoots Perexplant	Length Of The Shoots (Cm)
Free of PGRs	0.000 e	0.00 f	0.00 e
0.5 mg/l BAP + 0.05 mg/l IBA	63.33 d	2.30 c	1.56 c
1.0 mg/l BAP + 0.1 mg/l IBA	93.33 a	2.96 a	2.43 a
2.0 mg/l BAP + 0.2 mg/l IBA	83.33 b	2.56 b	1.93 b
1.0 mg/l KIN + 0.1 mg/l IBA	73.33 c	1.36 e	1.36 d
2.0 mg/l KIN + 0.2 mg/l IBA	76.66 bc	1.83 d	1.43 d
3.0 mg/l KIN + 0.3 mg/l IBA	56.66 d	1.56 e	1.63 c

Mean comparison to the 5% level according to Duncan's multiple range test. Columns with common characters, no significant difference.

Table 3: Effect IBA and NAA On Root Induction in Regenerated Shoots Of *A. Majus* In Ms Medium.

Growth regulator	Rooting response (%)	Root number	Root length (cm)
Free of PGRs	0.00 e	0.00 g	0.00 g
0.5 mg/l IBA	76.66 d	8.03 d	3.36 d
1.0 mg/l IBA	83.33 cd	14.86 a	3.16 e
2.0 mg/l IBA	100 a	9.73 b	2.93 f
0.5 mg/l NAA	86.66 bc	9.33 c	4.53 c
1.0 mg/l NAA	93.33 ab	6.93 e	5.86 a
2.0 mg/l NAA	100 a	3.06 f	5.16 b

Mean comparison to the 5% level according to Duncan's multiple range test. Columns with common characters, no significant difference.

and Harada (1975) reported that the interaction of IAA and kinetin resulted in the regeneration of the whole plant of *A. majus* from stem explants. Also, Atkinson *et al.* (1989) reported that the regeneration was achieved in *A. majus* when callus was produced from segments of hypocotyl and was most effective using MS medium containing 0.25 mg/L NAA plus 1.0 mg/L BAP.

The BAP was found as more suitable cytokinin for shoot proliferation in the present study. The edge of BAP over other cytokinins is well documented in other studies also (Newbury, 1986; Okubo *et al.,* 1991; Sheyab *et al.*, 2010).

Rooting of the *in vitro* regenerated shoots was not observed on PGRs free medium. Rooting was induced with different frequencies in different medium. Maximum frequency of root induction (100%) after 4 weeks was observed on both of MS medium supplemented with 2.0 mg/L IBA and MS medium supplemented with 2.0 mg/L NAA. Maximum number of roots (14.86) was observed on MS medium supplemented with 1.0

mg/L IBA. However, maximum length of roots (5.86 cm) was observed on MS medium supplemented with 1.0 mg/L NAA (Table 3, Figure 1e). Root induction was also achieved, from the bases of excised shoots, in the presence of IBA and NAA, which is similar to previous findings by Sheyab *et al.* (2010) in *A. majus*.

Rooted plants, when moved to acclimatization conditions, showed 85% survival. Plantlets resumed normal growth in the greenhouse, developing new leaves within 20 days (Figure 1f). Sheyab *et al.* (2010) reported that 90% survival was achieved when rooted explants of Snapdragon were acclimatized *ex vitro* using 1 soil: 1 perlite: 1 peat mixture.

CONCLUSION

The overall result of this preliminary study revealed that mass scale micropropagation of *Antirrhinum majus* is possible through induction of indirect organogenesis in cotyledon tissue. It was further proved that induction of tissue growth and its differentiation was dependent on PGR sup-plements in the medium. Though the percentage survival of the transferred plantlets to the outside environment was very high, this technique could still be adopted profitably by our herbal industries. Future experiments are in progress to pinpoint the factors related to improvement of this culture technique and the establishment procedure.

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