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Propagation of *Solenostemma arghel* (Del.) Hayne *In vitro*

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Abstract: A procedure for micropropagation of *Solenostemma arghel*, an endangered resulate endemic plant from South Sinai, was developed using shoot tips as explants for *in vitro* establishment. Hundred percent of explants survived, high proliferation of shoots (100%) and shoot length was obtained on Murashige and Skoog (MS) basal medium supplemented with a combination of 0.5 mg/l BA plus 0.5 mg/l NAA. The maximum number of proliferated shoots (6.6 shoots/explant) was obtained on MS medium supplemented with 3 mg/l BA + 1mg/l 2iP. Meanwhile, at 1.0 and 0.5 mg/l induced the highest shoot length. However, shoot elongation decreased with the increase of BA concentration. Eighty percent of the shoots rooted on half-strength MS medium supplemented with 2 or 3 mg/l indole butyric acid (IBA) to obtain complete plantlets. The maximum average root length was achieved using half-strength basal medium without IBA. High survival, over 80%, was obtained when the plantlets were transferred to green house conditions. The endangered *Solenostemma arghle* can be successfully micropropagated beginning with a shoot tip without significant damage to mother plant.

Key words: *Solenostemma arghel* · Micropropagation · Shoot tip

INTRODUCTION

Solenostemma arghel (Family Asclepiadaceae) is one of the most economic and important plants grown In South Sinai. *Solenostemma arghel* grows in a small area in Dahab and propagated naturally by seeds. However, due to overgrazing of this plant species, it failed to propagate naturally by seeds, so it became endangered and there is a need to keep this genetic resource. This plant contains medicinal derivatives, such as argelosides which was isolated by Perrone *et al.* [1] and flavonoids which were isolated by El Batran *et al.* [2]. They also isolated seven new keto pregnane glycosides (Stemmosides E-K). Also, Plaza *et al.* [3] isolated secopergnane glycosides from *Solenostemma arghel.* Conservation of the endemic or threatened plant species is carried out using different strategies. Micropropagation constitutes a powerful tool for *ex situ* conservation programs of this rich flora especially for species with much reduced populations or low seed production [4]. This technique facilitates the rapid establishment of a large number of stock plants forming minimum impact on the endangered wild population. With this technology, various endemic and endangered species have been successfully conserved [5].

This study was primarily initiated to develop a protocol for *in vitro* propagation of this endemic and rare plant, with the aim of *ex situ* conservation and commercial production. As it could be clonally propagated and grown as a medicinal plant.

MATERIALS AND METHODS

Plant Material: Plant material was collected from mature plants *Solenostemma arghel* grown naturally in South Sinai (Dahab), Egypt. Plant material was wrapped with wet paper and transferred in ice box to the Lab of the Tissue Culture Unit, Genetic Resources Department, Desert Research Centre. Shoot tip explants (2-3 cm) in length were excised and washed under running tap water. Prepared shoot tips were surface sterilized by immersing in an aseptic solution of 2% sodium hypochlorite with two drops of Tween 20 for 20 minutes in the culture cabinet "Laminar flow Hood". All traces of the used disinfectants were removed by soaking and rinsing the explants six times with sterile distilled water.

Nutrient Medium: The basal nutrient medium containing macro and micro elements was applied throughout the study according to Murashige and Skoog

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[6] plus 100 mg/l myo-inositol, 30 g/l sucrose and 0.4 mg/l thiamine HCl. The pH value of the nutrient media was adjusted at 5.7 to 5.8 with adding few drops of either 0.1 N of NaOH or HCl, prior the addition of 7 g/l agar. The media were dispensed into 25x150 mm tissue culture tubes, each contained 15 ml of culture medium. Sterilization of the medium was achieved by autoclaving the tubes containing media under pressure of 1.1 kg/cm^2 and at 120°C for 20 minutes. The tubes were transferred to the culture cabinet and left to cool in a slanted position till they were used.

Excising and Culturing Shoot Tips: Excising and culturing shoot tips were carried out in the culture hood which was previously disinfected. 0.3-0.5 cm long shoot tip containing four or more leaf primordia was excised and placed on the prepared media in the culture tubes. Each tube containing one shoot tip. Cultures were incubated at 25 ± 1°C and illuminated for 16 h photoperiod and light provided by cool white fluorescent tubes (2000 Lux).

Establishment and Shoot Multiplication: Shoot tips were placed on MS medium supplemented with different concentrations of 6 benzyl adenine (0.0, 1.0 2.0 and 3.0 mg BA/l) in combination with 6-naphthalene acetic acid (0.0, 0.1 and 0.5 mg NAA/l). Multiplication experiments were carried out using the most vigorous shoots from establishment stage. Nodal segments (2-3 cm long) containing two axillary buds were cut and cultured in MS medium containing BA (0.0, 1, 2 and 3 mg/l) in combination with 2-ispentenyle adenine (2iP) at (0.0, 0.5 or 1.0 mg/l) shoot proliferation was determined after six weeks of culture, The percentage of survival, shoots number/explant and average shoot length were recorded.

Rooting and Acclimatization of Plantlets: For rooting, individual shoots of 3-4 cm long were excised from the proliferated shoots and cultured on full, 75 and 50% strengths MS of basal medium supplemented with indole butyric acid (IBA) at different concentrations (0.0, 1.0, 2.0 and 3 mg/l). Shoots were cultured on these media for six weeks, thereafter; the following data were recorded as Percentage of rooting, Average number and length of roots formed per shoot. Plantlets were transplanted into plastic pots containing autoclaved mixture of peat and sand (1:1v/v) and covered with polyethylene bags, which were tightly closed to maintain high humidity. The plantlets were watered with 0.25 strength Hoagland's solution once a week for a period of 3 weeks. Thereafter, the polyethylene bags were removed and plantlets were maintained under nursery conditions.

Statistical Analysis: All obtained data were subjected to the analysis of variance and significant difference (L.S.D) according to Snedecor and Cochran [7].

RESULTS AND DISCUSSION

Culture Establishment: From data presented in Table 1, it could be noticed that survival of *Solenostemma arghel* cultures, using different combinations of BA and NAA including the control (without plant growth regulators), ranged from 80 to 100 percent. However, the combination of BA and NAA each at a concentration of 0.5 mg/L them was the best treatment since the survival attained 100%. The highest average shoot length (2.5cm) were obtained on the medium containing also 0.5 mg/l BA and 0.5 mg/l NAA and they decreased gradually with the decrease in BA and NAA concentration.

The general trend of responses of shoot growth to different auxin concentrations in the media may be interpreted by the fact that the auxin affects mainly the length of shoot tips and not through any other mechanism. On the other hand, the effect of some concentrations of auxin on morphogenetic responses of shoot tips varied relatively according to concentrations of cytokinin in the media. This might be due to low concentration of endogenous cytokinin that might play a role in the magnitude and effective level of exogenously added cytokinin concentration in the explant tissue. The difference in the interaction between the explants and different growth regulators may be due to the presence or absence of their mechanism action in the tissue or to the availability of such growth regulator basely in the explant, the matter which facilitates its function and did not need for further concentration [8]. The synergistic effect of BA in combination with an auxin was demonstrated in many medicinal plants of Asclepiadaceae, *Holostemma ada kodie* [9], *Ceropegia candelabrum* [11] and *Periploca angushfolia* [12].

Table 1: Establishment of *Solenostemma arghel* shoot tips using different combinations of BA and NAA after 6 weeks of culture

Treatments			
BA mg/l	NAA mg/l	Survival %	Average shoot length (cm)
0.0	0.00	80	1.27
0.5	0.10	90	1.50
1.0	0.10	90	2.36
0.5	0.50	100	2.52
1.0	0.50	90	1.90
LSD 0.05	0.16		

Table 2: Effect of various combinations of cytokinins on *in vitro* shoot multiplication of *Solenostemma arghel* after six weeks of culture

Treatments			
2iP mg/l	BA mg/l	Average no. of shoots per explant	Average shoot length (cm)
0.0	0.000	0.460	0.90
0.5	1.000	2.330	3.56
0.5	2.000	4.260	3.17
0.5	3.000	5.200	2.82
1.0	1.000	4.000	2.55
1.0	2.000	5.066	2.34
1.0	3.000	6.600	2.15
LSD 0.05	0.509	0.361	

Shoot Multiplication: Effect of various combinations of BA and 2iP on the multiplication of shoots is presented in Table 2. The cytokinin free medium gave the least average number of shoots indicating strong apical dominance. BA and 2iP stimulated the production of axillary shoots regardless of their concentration. BA and 2iP at concentration of 3.0 mg/l and 1.0 mg/l, respectively, induced significantly more axillary shoots (6.6 shoots per explant) than did the other treatments (Table 2). These results indicate that BA plays a key role in shoot proliferation of *Solenostemma arghel*.

Table 3: Effect of different concentrations of IBA and different MS strength on rooting of *Solenostemma arghel* after six weeks of culture

Treatments				
MS Strength%	IBA mg/l	Rooted shoots %	Average root no /shoot	Average root length (cm)
100	0.00	0.0	0.000	0.00
100	1.00	20	1.500	6.90
100	2.00	40	2.500	4.08
100	3.00	60	3.500	2.08
75	0.00	10	2.000	4.50
75	1.00	30	3.670	3.66
75	2.00	50	4.000	3.36
75	3.00	70	3.860	3.99
50	0.00	20	2.500	5.40
50	1.00	50	3.400	4.80
50	2.00	80	3.900	4.50
50	3.00	80	5.375	4.08
LSD 0.05	0.59	2.1		

The cytokinin (BA) is an efficient growth regulator for shoot multiplication in other endangered plants such as *Centaurea paui* [12] and *Centaurea juoniana* [13]. The present result suggests an inverse relationship between the elongation of shoots and increasing BA concentrations. It was also observed in the other previous micropropagation protocols [12]. The highest mean shoot length (3.56 cm) was found on MS medium supplemented with BA and

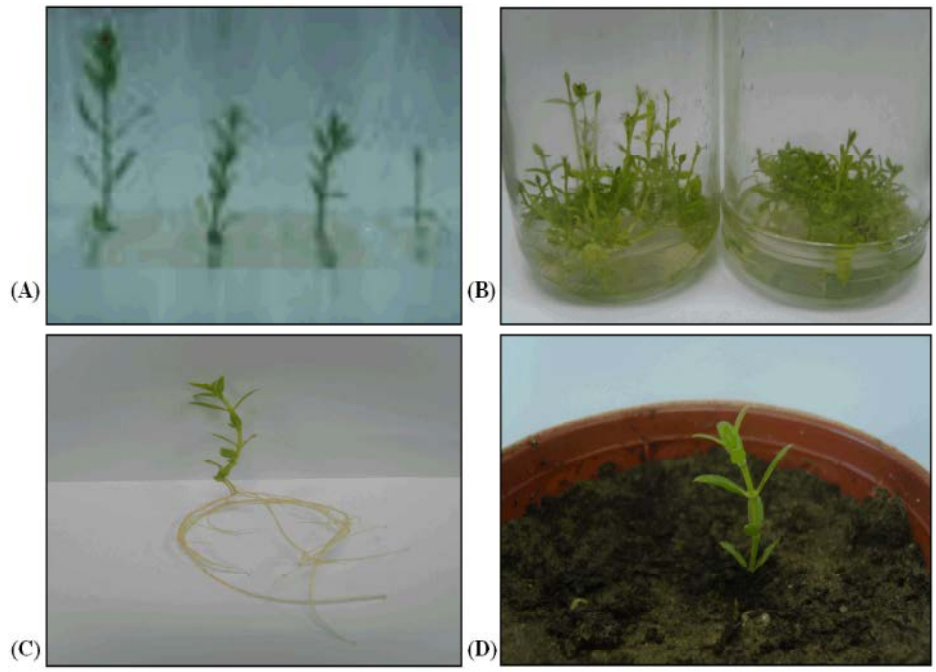

Fig. 1: Propagation of *Solenostemma arghel*.
(A) Establishment of *Solenostemma arghle.* (B) Multiplication of shoots.
(C*) In vitro* rooted plantlet after 6 weeks of culture. (D) A plantlet acclimatized in greenhouse.

2iP at their lowest (1.0 and 0.5 mg/l) concentrations. It seems that BA and 2iP could stimulate shoot growth if added to the media with low concentration while their high concentrations stimulate shoot multiplication.

Root Induction and Acclimatization: About 3-4 cm long shoots with two to three leaves, harvested from *in vitro* proliferated shoots, were used for rooting experiment. The rooting response differed according to concentration of IBA as well as the strength of the basal salt medium. As shown in Table 3, no rooting percentage was obtained in full strength MS medium without supplementing it with IBA. In half strength MS medium that was supplemented with 2.0 and 3.0 mg/l IBA; 80%.It could be concluded that, the proliferated shoots were rooted when the full strength 100% of MS media were supplemented with IBA. Rooted shoot percentage as well as number of roots/shoot were proportionally increased by the increase in concentration of supplemented IBA. The maximum rooting percentage as well as number of roots/shoot was obtained when supplemented IBA were added to MS media with concentration 3 mg/l. On the other hand, maximum root length (in cm) was attained when IBA was supplemented to full strength media with 1.0 mg/l IBA or without hormones. These results are in harmony with those obtained by Raghu *et al.* [14]. Best results in terms of average number and length of roots were obtained in half strength of the basal MS medium supplemented with 3.0 mg/l IBA. The results indicated that a relatively high auxin requirement is needed early in the rooting phase of *Solenostemma arghel*. The results in Table 3 are in contrast with those obtained for *Eriostemon myoporoides* where 42% rooting was obtained after 8 weeks on MS medium supplemented with 4.1M IBA [15]. Also the auxin IBA did not induce callus formation at shoots basis, which has a positive effect, since callus formation acts as a mechanical barrier to nutrient and water uptake [16].

Acclimatization: Plantlets regenerated *in vitro* with well developed root system were transferred to a glasshouse in plastic pots covered with translucent plastic bags to ensure keeping high humidity around the plants. The use of this procedure during the acclimatization phase ensured that most transplanted plantlets to *ex vitro* conditions continued to grow vigorously. After two months the plastic bags were removed, 80% of the plantlets survived in the green house and showed no sign of water stress and without any morphological abnormalities or variations.

CONCLUSION

The micropropagation protocol described here in this study has made rapid and large scale production of plantlets possible, starting with small quantity of plant material. This should decrease the pressure and diminish collection of this endemic species from the wild one. That makes this method appropriate for *ex situ* conservation and for commercial production of *Solenostemma arghel* plantlets.

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