

Article

Effect of sucrose supplementation in micro-propagation nodal culture of Sicilian Soapwort (*Saponaria sicula*)

Sara Sardashti

Department of Agronomy, Faculty of Agriculture, Zabol University, Zabol, Iran

E-mail: sarasardashti19803@gmail.com

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Abstract

Sicilian Soapwort (*Saponaria sicula*), is a perennial plant belonging to the family of carnation and a valuable medicinal and industrial plant. The origin of this plant is the Mediterranean region. The medicinal properties include blood filtering, elimination of rheumatism, gout, urinary tract and skin diseases, cure cancer, eczema and tuberculosis. The purpose of this study was to determine the *Saponaria sicula* micro-propagation using explants inter nodes in a medium containing 50 grams of sucrose in the presence of hormone levels of *Benzyl adenine* (BA) in range of (0, 0.05, 0.2 and 0.5) and naphthalene acetic acid in range of (0, 0.05, 0.2 and 0.5). Some factors such as fresh weight, root and stem length, leaf area, length and width of callus, number of stems and roots, embryo genetic plants, aerial root, root hair, green leaves, red leaves, lost leaves, number of nodes and stem produced from each node and color of callus were determined. Results showed that the free-hormone medium conducive to the increment of root length, the medium containing 0.5 mg/l BA and 0.5 mg/l naphthalene acetic acid (NAA) increased the number of red leaves, length of plant, as well as the number of stems growing from each node, the medium without BA, containing 0.5 mg/l NAA increased the number of green leaves, the number of nodes, length and width of leaves, medium containing 0.5 mg/l BA and NAA 0.05 mg per liter gave rise to dead leaves, medium containing 0.5 milligrams per liter BA and without naphthalene acetic acid affected the number of roots, plant fresh weight and length of callus, the medium 0.05 mg/l BA and 0.05 mg/l naphthalene acetic acid increased the length of leaves and the number of embryo genetic plants. The medium containing mg/liter BA with 0.2 mg/l naphthalene acetic acid had an increasing effect on hair roots and the medium containing 0.05 mg/l BA and 0.5 mg per liter NAA were effective in increasing the width of the callus.

Keywords *Benzyl adenine*; naphthalene acetic acid; *Saponaria sicula*; sucrose supplementation.

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1 Introduction

Soapwort is a small genus of some twenty herbaceous or perennial species which are native from southern Europe through southwest Asia (Fig. 1). They have terminal clusters of showy flowers in terminal racemes. It

is easily cultivated in any humus rich, well drained soil in full sun (Zargari, 1997). It develops clumps of upright stems up to a few centimeters height and its leaf stalks are derived from thin rhizomes, modified subterranean stems that are usually found underground. Creeping rhizome is reddish brown, highly branched, about 1 cm, erected stem, cylindrical, simple slightly branched, green to red (Ans, 2010). The leaves are bright green opposite, sessile, slightly hairy, simple and the tube-shaped, sweetly scented flowers are radially symmetrical and pink and appear in terminal clusters at the tip of the stems (Zargari, 1997). As an ornamental plant, soap plant grows widely in open unused areas, along roadsides and railroad tracks (Yang et al., 2012).



Fig.1 The Soapwort.

Soapwort is a diuretic drug, transpire, purifier of the blood and functions intangibly as a source of energy. It is medicinal against rheumatism, gout, urinary tract diseases, eczema as well as anemia, especially in young girls. A decoction of the herb is applied externally to treat itchy skin. Iranian and Arab physicians considered it effective in the treatment of cancer (Zargari, 1997). It has also been used as a medicine against tuberculosis, cervical lymph nodes, skin ulcers, hepatic disorders and jaundice, and as a booster of milk secretion (Henry, 1989). A positive correlation between antioxidant activity and total phenol content was found in plant samples studied, where methanol extracts from soap plant exhibited an antibacterial activity against a number of microorganisms. The mentioned extracts containing compounds possessing antibacterial characteristics can likely be used as natural sustenance in food and drug industries (Sengul et al., 2011). Also Nabinezhad (2013) conducted an experiment on the antibacterial effects of soap extract on the pathogen *E. coli*. The researcher compared the antibacterial effects of soap plant alcoholic extract to some common antibiotics, with the results confirming the antibacterial effects for the former. Plant tissue culture is an acronym for protoplast culture, tissue culture and cell culture. Using a variety of different plant organs and tissue, this technique includes growing microbe free plant materials in a sterilized environment, such as disinfected growth media inside a test tube. In the recent years, plant tissue culture has been considered a reliably important tool in proliferation and breeding of plant species. The aim of this study was conducted to Investigating In vitro micro propagation of *Saponaria sicula* by nodal culture with supplying 5% sucrose.

2 Materials and Methods

For the experiment, the internodes needed were obtained from the flower garden of Esfahan's parks and green space organization. The experimental plants were washed with tap water for 30 minutes, and then disinfected in bleach (containing 2% sodium hypochlorite) for 15 minutes. The disinfected specimens were then washed 3

times in double distilled water, under sterilized condition. After having been gone through the hygienic process, the samples were transferred into a MS growth medium containing a variety of different hormones, such as Naphthalene acetic acid, *Benzyl adenine* (0, 0.05, 0.2 and 0.5), sucrose 5%, and agar 7%, with PH= 5.7, for 70 days. All tools involved in the experiment such as containers and growth media were autoclaved in 120°C for 20 minutes. Then, after cooling off, 2 to 3 explants were carefully planted into glass containers containing growth media, with each explants plunged 0.5 cm into the medium. The growth chamber was steadily conditioned by 23°C temperature, 3000 lux bright light, 16 light and 8 dark hours, for 70 days. Each treatment was repeated three times. After 70 days, the young seedlings were taken out of growth chamber, and undergone physiological measurements as follows; the total fresh weight was measured using a fine digital scale, the length of stem, surface of leaves as well as length and width of callus were defined using a ruler. The number of stems, roots, embryogenic seedlings, aerial roots, hairy roots, green leaf, red leaves, lost leaves, the number of nodes and the number of stems per each node as well as the color of the callus were also evaluated. The study was conducted as a factorial experiment on a complete randomized design. The data were analyzed by using statistic software (SAS, 2001) and the mean comparison of the variables was conducted using Duncan's test.

3 Results

The hormone-free MS medium with 50 g of sucrose was contributing environment to measure traits like root length, the medium containing 0.5 mg/l BA and 0.5 mg/l naphthalene acetic acid for red leaf number, length and number of stems per plant node, the environment without BA, containing 0.5 mg/l NAA for the number of green leaves, nodes, leaf length and width, the medium containing 0.5 mg/l BA and 0.05 mg/l (NAA) for the number of lost leaves, the medium with 0.5 mg/l BA and without NAA for the number of roots, fresh weight and length of plant callus, the environment containing 0.05 BA and 0.05 mg per liter (NAA) for leaf length and number of embryogenic calluses, the environment with 0.05mg LBA and 0.2 mg per liter of naphthalene acetic acid for the hair root traits, the environment containing 0.05 mg/l BA and 0.5 mg per liter NAA for callus width, medium containing 0.2 milligrams per liter BA and NAA was suitable for measuring traits like the number of stems per node and the environment containing 0.2 mg/l BA and 0.5 mg per liter NAA was suitable for generating embryonic callus number. The results for interaction of hormones showed that the highest amount of plant fresh weight (1.63) pertained to the medium containing 5.0 mg/l BA + NAA as control which was statistically significantly different from other treatments at 5% level (Table 1).

Table 1 Comparison of the effect of concentration of BA on some of the characteristics of plants (50 g sucrose).

Number red leaves	Number green leaves	Number node	Number of stems per each node	Plant height	Fresh weight	Concentrations BA (ppm)
				(cm)	(g)	
1.01 ^{c*}	11.69 ^a	4.85 ^a	1.52 ^b	7.30 ^a	0.34 ^d	0
3.251 ^b	7.44 ^b	3.85 ^b	1.88 ^b	5.50 ^b	0.50 ^c	0.05
5.04 ^a	7.48 ^b	3.90 ^b	2.35 ^a	5.69 ^b	0.72 ^b	0.2
5.27 ^a	5.70 ^c	4.04 ^b	2.52 ^a	5.80 ^b	1.15 ^a	0.5

*In each column, means with the same letter are not significantly different ($P \leq 0/05$).

The maximum number of root formation was observed in the medium containing BA + 0.5 mg/l naphthalene acetic acid (n =14), and the medium containing 2.0 mg/l BA + NAA control (11.67 numbers), but the difference with other treatments was not statistically significant at the level of 5%. Fraga et al (2004) reported that the percentage of rooting in *Dianthus Gratianopolitanus* was the highest in the hormone-free medium. Root length in the medium containing control BA + NAA was the highest (33.7 cm) and showed no statistically significant difference at 5% with the other treatments. More aerial roots formed in the medium containing 0.05 milligrams per liter BA + the medium containing 0.2 mg per liter NAA (2.33 numbers), but at 5% the difference with other treatments was not statistically significant. These results showed good agreement with in Kaur and Anand's (2014) observations of Chinese carnation where white hair roots were formed by adding NAA. The height of the callus in medium containing 0.5 mg/l BA + control naphthalene acetic acid (1.97 cm), showed a statistically significant difference at the level of 5% with other treatments. Width of the callus in presence of mg/l BA + 0.5 mg/l NAA was more size-wise (1.32 cm), but it did not show any statistically significant differences with the other treatments at 5% (Table 2).

Table 2 Comparison of the effects of NAA concentration on some characteristics of plants (50 g sucrose).

Length callus	Width callus (cm)	Length root	Number roots	Number lost leaves	Number red leaves	Fresh weight	Concentrations NAA
						(g)	(ppm)
0.88 ^{a*}	0.55 ^b	3.70 ^a	8.70 ^a	2.48 ^b	3.40 ^{bc}	0.86 ^a	0
0.82 ^a	0.72 ^a	3.12 ^{bc}	4.47 ^c	3.70 ^a	4.20 ^a	0.80 ^a	0.05
0.56 ^b	0.50 ^b	3.60 ^{ab}	7.71 ^a	2.11 ^b	3.21 ^c	0.71 ^b	0.2
0.68 ^{ab}	0.61 ^b	2.86 ^c	5.92 ^b	2.18 ^b	3.80 ^{ab}	0.55 ^c	0.5

*In each column, means with the same letter are not significantly different ($P \leq 0/05$).

4 Discussion

Azadi et al. (2007) noted that an increase in the concentration of sucrose along with 1.0 mg per liter BA and NAA led to a raise in plant fresh weight in *Lilium ledebouri*. In an experiment conducted studying various levels of sucrose in *Syringa vulgaris*, researchers found that higher levels of sucrose had significantly increased the plant fresh weight (Gabryszewska, 2011). High concentration of sucrose can increase the optimal weight of potatoes (Fufa et al., 2012). Maximum plant height (9 cm) occurred on a growth medium containing 0.5 mg per liter BA + 0.5 mg/l (NAA), although it was not statistically significantly different from other treatments at the 5% level. The results of this study are congruent with those obtained by Ricardo et al. (2004) working on *Dendrobium nobile*, where higher concentrations of sucrose increased plant height. The control with 11.67 grown green leaves showed statistically significant difference versus other three levels of this hormone at 5% (Ricardo et al., 2004). For the number of leaves variable, the medium containing 0.5 mg L BA + 0.5 mg/l naphthalene acetic acid produced the maximum number of red leaves, 33.7 in which was statistically significantly different from other treatments at 5%. The maximum number of 12.67 green leaves found in the control medium with BA + 5.0 mg/l naphthalene acetic acid showed no statistically significant difference at 5 percent with other treatments. The maximum number of 8 lost leaves was found in the medium containing 0.5

mg l BA + 0.05 mg/l naphthalene acetic acid, but showed no statistically significant difference with other treatments at 5%. The maximum leaf length (3.33 cm) was found in the medium containing BA + 0.5 mg/l NAA and the medium containing 0.5 mg/l BA + 0.05 mg/l naphthalene acetic acid, which had no statistically significant difference with other treatments at the 5% level. Some researcher introduced BAP as a plant hormone responsible for leaf elongation in zinnia elegance. For leaf width, the trait was larger in size (87.0 cm) in the medium containing BA + 0.5 milligrams per liter (NAA), but showed no statistically significant difference at 5 percent with other treatments. The obtained results here are in congruence with those achieved by Kharazi et al (2010) showing exogenous application of BA led to an increase of width of leaves in *Dianthus Caryophyllus*.

The results of the current study were similar to those obtained by some researchers that working on *Gypsophila Paniculata* in 2011, where they found BA effective in callus formation. Kaur and Anand (2014) found IBA hormone effective in the formation of callus from leaf explants of Chinese Carnation. Callus growth in *Citrus sinensis* under the influence of a high concentration of sucrose, in the presence of BA, was reported by (Gilandi et al., 1977). Also Gurel and Gulsen (1998) concluded that increase in sucrose gives rise to the formation of callus. The number of nodes in the control medium containing BA + 0.5 mg per liter (NAA) outnumbered (n= 6), but showed no statistically significant difference with other treatments at 5 percent. The highest number of stems per plant node (n=3) belonged to the medium containing 0.5 milligrams per liter of BA + 0.5 mg/l naphthalene acetic acid, plus 0.2 mg/l BA + control NAA acid acetic, but the difference with other treatments was not statistically significant at 5%. Kanchanapoom et al (2011) stated that BA had increased (the number of) stems in *Gypsophila paniculata*. 0.05 ppm NAA +0.05 ppm BA and 0.2 ppm NAA +0.5 ppm BA were the only treatments that possessed embryogenic callus. Kaur and Anand (2014) suggested applying BAP and NAA for the purpose of embryo genetic plant formation. Rafique et al. (2013) found 5% sucrose effective in the formation of somatic embryos.

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