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Effect of Carbon Sources and Sucrose Concentrations on Shoot Organogenesis of *Aloe saponaria*

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In the present study, the effect of various carbon sources and sucrose concentrations on *in vitro* organogenesis of *Aloe saponaria* was investigated and a rapid micropropagation protocol was developed from *in vitro*-derived meristem explants. Meristem explants were cultured in initial shoot regeneration media with five different carbon sources (fructose, glucose, lactose, maltose, and sucrose), and sucrose as the best carbon sources for shoot regeneration and shoot elongation was investigated at five different concentrations (10, 20, 30, 40, 50 mg L^{-1}). The treatment with sucrose resulted in the highest number of shoots (2.7 ± 0.2) per explant and produced the longest shoots (16.4 ± 1.3 mm), whereas the treatment with maltose was the least efficient in promoting shoot number (1.5 ± 0.1) and shoot elongation (10.4 ± 0.9 mm). The highest shoot regeneration (3.3 ± 0.3) and the longest shoots (19.1 ± 1.5 mm) were observed in treatments with 40 g L^{-1} sucrose. Further increase in sucrose concentration delayed shoot induction, resulting in stout shoots stunted in their growth. Our results suggest that carbon sources, particularly sucrose, could be used for micropropagation and in plant transformation protocols for regeneration of *Aloe* species.

Key words: *Aloe saponaria*, Shoot organogenesis, Fructose, Glucose, Lactose, Maltose, Sucrose .

The monocotyledonous leaf succulent genus *Aloe*, a member of the Liliaceae family, presently comprises over 500 species, ranging from small shrubs to large tree-like forms[1]. Of those, five species, *A. vera*, *A. arborescens*, *A. perryi*, *A. ferox*, and *A. saponaria* are used primarily for therapeutic medicinal purposes, with *A. vera* being the most known for commercial and therapeutic uses[2]. *Aloe saponaria*, commonly known as the soap aloe, is one of the most popular species in the *Aloe* genus. Its leaves are used as a soap substitute as they are able to produce foam in water. This plant has a short stem with green-white striped leaves approximately 50 cm long. This aloe has a less bitter taste compared to other species because of lower concentration of aloin[3]. Aloe species are well characterized on the African Continent, Arabian Peninsula, Madagascar, and eastern Indian Ocean Islands[4]. *Aloe*, native to South Africa, is now spread worldwide. The genus has been used as a multipurpose folk remedy, and the products of *A. vera* have been used in the medicinal and cosmetic industries. The species has been an essential component in the traditional medicine of several contemporary cultures, such as China, India, the West Indies, and Japan[5]. *Aloe vera* plants have high water content, ranging from 99–99.5%[6]. The remaining 0.5–1.0% is the solid material comprised

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of over 75 various biologically active compounds including fat-soluble vitamins, minerals, enzymes, simple/complex polysaccharides, phenolic compounds, and organic acids.

In recent years, many researchers have studied this species because of its medicinal value. The gel extracted from aloe has been used as a natural ingredient of many products in cosmetics, topical preparation, and foods. *Aloe vera* gel has been reported to exhibit wound healing, anti-bacterial, anti-fungal, immune stimulating, and anti-inflammatory activities[7-9]. Furthermore, it has been used as an agent in treatment of peptic ulcers, diabetes mellitus, and cancer[8,10]. The ethanol extract of *A. saponaria* has pharmacological properties, including anti-oxidant, antinociceptive, and anti-inflammatory activity[11]. Research on medicinal active compounds of *Aloe* has developed in scope during the last three decades and the production or transformation of value-added compounds, which are medicinally important, still needs to be investigated.

In last decades, the demand for this aloe plant species has increased due to its medicinal properties. The commercial cultivation of *A. saponaria* is challenging because of the low seed viability, low germination rate, limited availability of raw material with high quality, and slow vegetative growth. Tissue culture technique is an alternate method for preservation of those beneficial medicinal plants[12-14]. Large scale propagation and conservation of plants by tissue culture is recognized as one of the key areas in biotechnology techniques.

Several studies investigated shoot regeneration and proliferation of different species of the *Aloe* genus[15,16]. Most of the studies used shoot tips and axillary buds as explants for regeneration of *A. vera*[17]. Moreover, the presence of the plant growth promoter is essential for regeneration[17-19] reported on axillary shoot formation using indole-3-butyric acid (IBA), whereas Roy and Sarkar[20] and Natali et al.[21] regenerated shoots on a medium containing 2,4-D and kinetin. Richwine et al.[22] examined the initiation of shoots by zeatin, and Debiasi et al.,[23] and Liao et al.[19] investigated the effects of benzyladenine, indole-3-acetic acid, and naphthaleneacetic acid on bud initiation. In plants, carbohydrates are a crucial source of carbon in biosynthesis processes. *In vitro* cell, tissue, and organ cultures of plants are not fully autotrophic, requiring a source of carbohydrates to maintain the osmotic potential of the culture media and to provide energy and carbon for developmental processes with high energy requirement, such as organogenesis, root induction, embryogenesis, emission, and shoot multiplication[24]. Hence, sugars have a promising effect on the growth, differentiation of cells, and physiology[25]. The objective of the present investigation is to determine the influence of carbon source such as glucose, fructose, lactose, maltose, and sucrose on shoot organogenesis using meristem explants and to evaluate different concentrations of sucrose on rapid *in vitro* propagation of *A. saponaria*.

MATERIALS AND METHODS

Plant material and culture medium

Aloe saponaria seeds were purchased from Richters Herbs (Goodwood, ON, Canada) and stored at 4°C. The seeds were surface-sterilized with 70% (v/v) ethanol for 30 s, rinsed with 2% (v/v) sodium hypochlorite solution for 10 min, and washed with sterilized distilled water three times in a laminar air flow hood. Ten seeds were inoculated on 25 mL of agar-solidified culture medium in Petri dishes (100 × 15 mm). The basal Murashige and Skoog (MS) medium consisted of salts and vitamins (Murashige and Skoog, 1962)[26], and it was solidified with 0.7% (w/v) agar. The pH of the medium was adjusted to 5.7 to 5.8 using 1 N hydro chloric acid (HCL) and 1 N potassium hydroxide (KOH) before adding agar, and then sterilized by autoclaving at 121°C for 20 min. The seeds were germinated in a growth chamber at 25°C, illumination of 35 µmol s^{-1} m^{-2} provided by standard cool-white fluorescent tubes, and a 16-h photoperiod.

In vitro shoot organogenesis

Meristem explants of *A. saponaria* were taken from plants grown *in vitro* and cut aseptically at the ends into sections approximately 0.7 cm long. Explants were placed on the medium in Petri dishes (100 × 25 mm). Each Petri dish contained approximately 25 mL of basal medium supplemented with 30 g L^{-1} sucrose, 7 g L^{-1} Phytagar, and 2 mg L^{-1} 6-benzylaminopurine (BAP). The pH of the medium was adjusted and the medium was sterilized using the same procedures

described for the germination medium, and seven explants were inoculated in each plate. For enhancement of shoot regeneration, the medium was optimized by testing the effect of 30 g L^{-1} of each carbon source (fructose, glucose, lactose, maltose, and sucrose) and different concentration (10, 20, 30, 40, and 50 g L^{-1}) of sucrose. Inoculated plates were incubated at 25 ± 1°C in a growth chamber with a 16-h photoperiod and illumination provided by standard cool-white fluorescent tubes (35 µmol s^{-1} m^{-2}) for 6 weeks.

Rooting of regenerated shoots

Regenerated *A. saponaria* shoots (~1.5 cm in length) were transferred to 1/2 MS medium in a Magenta box (Magenta LLC, Chicago, IL, USA). After 3 to 4 weeks, the regenerated shoots were transferred into the rooting medium, consisting of MS medium with 8 g L^{-1} of Phytagar and 1 mg L^{-1} of IBA. Four shoots were cultured in each culture vessel. Regenerated shoots were incubated at 25 ± 1°C in a growth chamber with a 16-h photoperiod and illumination of 35 ìmol·s^{-1}·m^{-2} provided by standard cool-white fluorescent tubes for 5 weeks. After 5 weeks, the rooted plants were washed with water to remove agar, transferred to pots containing autoclaved vermiculite, and covered with polyethylene bags for 1 week to maintain high humidity. The plants were then transferred to soil and maintained in a growth chamber with a 16-h photoperiod and a day/night temperature of 18/20°C for 2 weeks. These hardened plants were then transferred to the greenhouse.

Statistical analysis

Data were expressed as means ± standard deviation of 50 leaf explants tested.

RESULTS AND DISCUSSION

A protocol was enhanced for *in vitro* shoot organogenesis of *A. saponaria*[27], but shoot development efficiency using this protocol was not sufficient. We used various carbon sources, i.e., fructose, glucose, lactose, maltose, and sucrose to study the effectiveness of shoot organogenesis in *A. saponaria*. Different types of carbohydrates have been found to play substantial roles in micropropagation of *A. saponaria*. Among the five different carbon sources used in this study, addition of sucrose resulted in the highest number of shoots and longest shoots. The highest shoot number (2.7 ± 0.2 per explant) and the longest shoots (16.4 ± 1.3 mm) were observed in explants cultured on carbon media (MS media with 2 mg L^{-1} BAP) supplemented with 30 g L^{-1} sucrose, followed by glucose (Table 1). The treatment with maltose showed the lowest efficiency in shoot regeneration (1.5 ± 0.1) and shoot elongation (10.4 ± 0.9 mm) in *A. saponaria*. Sucrose produced 1.6-fold greater number of shoots per explant and 1.3-fold longer shoots than the lowest producing carbon sources maltose.

To determine the optimum concentration for increased regeneration in *A. saponaria*, the generation medium (MS medium with BAP at 2 mg L^{-1}) was supplemented with different concentrations of sucrose (10, 20, 30, 40, and 50 g L^{-1}). Increasing the concentration of sucrose increased the shoot number and shoot length; the

Table 1. Effect of carbon sources on shoot regeneration and growth of *Aloe saponaria* explants after 6 weeks of culture on regeneration medium (Murashige and Skoog medium with 2.0 mg L^{-1} 6-benzylaminopurine)

Carbon sources (30 g L^{-1})	No. of shoots per explant*	Shoot length* (mm)
Fructose	2.0 ± 0.2	12.5 ± 1.2
Glucose	2.5 ± 0.3	14.8 ± 1.4
Lactose	1.7 ± 0.2	11.2 ± 1.1
Maltose	1.5 ± 0.1	10.4 ± 0.9
Sucrose	2.7 ± 0.2	16.4 ± 1.3

* Values represent the mean ± standard deviation of 50 shoots

Table 2. Effect of sucrose concentration on shoot regeneration and growth of *Aloe saponaria* explants after 6 weeks of culture on regeneration medium (Murashige and Skoog medium with 2.0 mg L^{-1} 6-benzylaminopurine).

Sucrose (g L^{-1})	No. of shoots per explant*	Shoot length* (mm)
10	1.8 ± 0.2	12.9 ± 0.9
20	2.2 ± 0.2	14.5 ± 1.1
30	2.7 ± 0.2	16.4 ± 1.3
40	3.3 ± 0.3	19.1 ± 1.5
50	3.2 ± 0.4	18.7 ± 1.2

* Values represent the mean ± standard deviation of 50 shoots

highest shoot number (3.3 ± 0.4) and shoot length (19.1 ± 1.5 mm) were observed in treatments with 40 g L^{-1} sucrose. Thereafter, further increase of sucrose concentration reduced the shoot number and shoot length (Table 2).

The results suggested that the inclusion of carbon sources (carbohydrates) is important for enhanced shoot development; shoot regeneration of *A. saponaria* is affected by both the type and concentration of sugar in the culture medium (Tables 1 and 2). The highest shoot regeneration and shoot elongation were obtained in media supplemented with sucrose at 40 g L^{-1}. Similar results were reported for *Echinacea angustifolia*[28]. A few studies reported that sucrose at 3% level was the optimal concentration to achieve the highest level of shoot regeneration and shoot elongation[29,30]. Sucrose enhanced shoot regeneration of *A. saponaria* compared to fructose and glucose[31] and stimulated shoot organogenesis of *Prunus domestica* and increased shoot number compared to glucose[32]. Previous report suggested that glucose, fructose, and maltose elicit very low levels of shoot regeneration and shoot elongation[33], and similarly, the effect of different monosaccharides used as carbon source on regeneration was relatively low compared to sucrose[34]. However, *in vitro* beech cultures showed high adventitious shoot regeneration and axillary branching in treatments with glucose[35]. In other plants, including *Alnus glutinosa* [36], *A. cremastogyne*[37], *Corylus avellana*[38], *Prunus mume*[39], *Juglans regia*[40], and *Rosa*[41], the best shoot regeneration was obtained in treatments with glucose and fructose compared to sucrose. Many studies reported that 3% sucrose enhanced the most the micropropagation of plants, whereas in the present study, we showed that the highest level of shoot regeneration and elongation of meristem explants of *A. saponaria* were achieved in treatments with 4% sucrose.

CONCLUSION

Previously established protocols for shoot organogenesis and plant regeneration from meristem explants were not successful in the regeneration of *A. saponaria*. In the present study the effect of carbon source and sucrose concentrations on *in vitro* regeneration of *A. saponaria* was studied. It was observed that sucrose was the best carbon source and that 4% sucrose was the optimum concentration for healthy shoot regeneration and elongation. This protocol can be helpful for large scale proliferation of *Aloe* plant species and thus contribute to effective conservation of the species. The results presented herein will facilitate research on genetic enhancement of *A. saponaria*.

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