Shoot Organogenesis and Plant Regeneration of *Aloe saponaria*

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Abstract: The medicinal values of *Aloe* make it a very popular genus, with various commercial products of Aloe being available throughout the world nowadays. In the current study, a micropropogation system was developed from cultures of *Aloe saponaria* meristem explants. Cytokinins [BAP (benzylaminopurine) and kinetin], along with the combined application of BAP and auxins, had a significant influence on shoot initiation and growth. Shoot initiation increased with increasing BAP and kinetin concentrations up to concentrations of 2 mg/l. BAP performance was optimal for initial shoot regeneration at 2 mg/l; with 5.4 times greater shoot initiation and 2.6 times greater shoot length per explant compared to the control treatment. BAP combined with any concentration of IBA generated higher shoot initiation and shoot growth compared to the control treatment. Among the treatment combinations, that of BAP with 0.5 mg/L IBA produced the highest shoot growth (37% higher) per explant and the greatest shoot length compared to the control. This result indicates that the micropropagation of *Aloe* might be as effective as using meristems for plant regeneration.
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1. Introduction

Aloe has a long ethnobotanical and medicinal history worldwide. *Aloe* occurs naturally in Africa, with approximately 400 species of shrubby succulent plants belonging to the genus. *Aloe saponaria* is one of the important species of *Aloe* that is used worldwide, and is known as the soap *Aloe* and African *Aloe*. Sap from the leaves produces sudsy foam in water, which is used as a soap substitute. *A. saponaria* has lower aloin content than other *Aloe* species, and therefore has a less bitter taste (Li et al., 2003). The ethanol extract of *A. saponaria* has pharmacological properties, including anti-oxidant, antinociceptive, and anti-inflammatory activity (Yoo et al., 2008).

Plant regeneration through organogenesis generally involves the induction and development of a shoot from explant tissue, followed by transfer to a different medium for the induction of root formation and development. Research has demonstrated that successful organogenesis in many plant species is achieved by the correct establishment of medium components, selection of a suitable explant, and control of the physical environment (Brown and Thorpe, 1986; Thorpe, 1990).

Aloe species are often propagated by removing the offsets, which are produced around the base of mature plants. However, this technique is a rather slow way of multiplication to meet the growing demand for this product. Therefore, a variety of studies have been undertaken on the *in vitro* micropropagation of *Aloe* species for multiple propagation (Campestrini *et al*., 2006; Liao *et al*., 2006)

In this study, we report the development of a simple method for highly efficient plant regeneration from the meristem explants of *A. saponaria* using cytokinins and auxins.

2. Material and Methods

2.1 Shoot Organogenesis from Meristem Explants

Meristem explants from 2-year-old *A. saponaria* were surface-sterilized with 70% (v/v) ethanol for 30 s, and then with 1% (v/v) sodium hypochlorite solution for 10 min, and finally rinsed 3 times in sterilized water. The sterilized meristem explants were cut aseptically at the ends, to obtain 4 sections of approximately 0.7 cm in size. Meristem explants were placed on 25 ml of culture medium in a Petri dish (100 × 25 mm). Seven explants were cultured in each Petri dish. The basal medium consisted of the salts and vitamins of Murashige and Skoog medium (MS; Murashige and Skoog, 1962), and was solidified with 0.7% (w/v) Phytagar. The pH of the medium was adjusted to 5.8 before adding Phytagar. The medium was sterilized by autoclaving at 1.1 kg cm^{-2} (121°C) for 20 min. For shoot regeneration from meristem explants, the MS

medium was supplemented with 0, 0.5, 1, 2, and 4 mg/l kinetin and BAP (6-benzylamino purine). To improve shoot regeneration, the culture medium was optimized by testing the effect of different concentrations (0, 0.1, 0.5, and 1.0 mg/l) of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and naphthalene acetic acid (NAA) on shoot formation and growth. Cultures were maintained at 25 ± 1°C in a growth chamber with a 16-h photoperiod under standard cool white fluorescent tubes (35 µmol $s^{-1} \cdot m^{-2}$) for 6 weeks.

2.1. Rooting of Regenerated Shoots

Regenerated shoots (approx. 1 cm long) were placed in MS medium. The medium was solidified with 7 g/l Phytagar, and then 30 ml was dispensed into Magenta boxes, with 4 shoots being cultured in each box. Regenerated shoots were incubated at 25°C in a growth chamber with a 16-h photoperiod under standard cool white fluorescent tubes (35 µmol s^{-1} m^{-2}) for 5 weeks. After 6 weeks, the rooted plants were washed with sterile water to remove Phytagar, 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 20/18°C for 2 weeks. These hardened plants were then transferred to a greenhouse.

2.3. Statistical Analysis

Data is expressed as the mean ± standard deviation of the 30 explants that were examined.

3. Results

3.1 Effect of Cytokinins on Shoot Regeneration

For *in vitro* plant regeneration and micropropagation of *A. saponaria*, a simple and effective protocol was developed. Previous research has confirmed that cytokinins play an important role in plant organ initiation (Shani *et al*., 2006; Perilli *et al*., 2010). Here, we investigated how different cytokinins affect the efficiency of shoot organogenesis in *A. saponaria*. To induce shoot growth, meristem explants of *A. saponaria* were cultured on MS media with different concentrations of BAP and kinetin. BAP and kinetin significantly influenced shoot regeneration. In addition, shoot initiation and shoot growth varied with cytokinin (BAP and kinetin) concentrations. Furthermore, the number of shoots per explant was higher when using BAP rather than kinetin in the medium. Shoot initiation increased with increasing BAP and kinetin concentrations up to 2 mg/l, after which point both shoot formation and growth decreased. BAP performance was optimal for initial shoot regeneration at 2 mg/l, with this concentration in MS media producing the greatest number of shoots (2.7/explants) and the highest shoot length (1.8 cm); however, when BAP concentrations exceeded 2 mg/l, both shoot formation and shoot length decreased. After 2 weeks of culture on the medium supplemented with BAP, shoot primordia developed adjacent to the cut surface (Figure 1A). Cells of the epidermis proliferated to produce shoots directly (direct shoot organogenesis), without an intermediate callus phase. Regenerated shoots developed from shoot primordia within 4 weeks (Figure 1B). After 6 weeks of culture, fully developed shoots were produced from the meristem explants (Figure 1C).

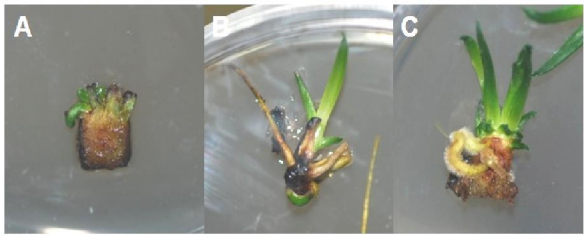

Figure 1. *In vitro* shoot organogenesis and plant regeneration from meristem cultures of *Aloe saponaria*. (A) Shoot primordia emerging from a leaf explant after 2 weeks of cultivation on MS solid medium supplemented with 2.0 mg/l BAP. (B) After 4 weeks of culture, fully developed shoots were produced from the meristem explant culture. (C) After 6 weeks of culture, shoot regeneration from the meristem explant culture was observed. Magnification A–C: ×2.

BAP at a concentration of 2 mg/l produced 5.4 times more shoots per explant compared to the control treatment. Shoot length was also 2.6 times higher in the 2 mg/l BAP treatment compared to the control. A similar trend in shoot formation and shoot growth was obtained for kinetin; however, slightly lower shoot formation and shoot length was obtained compared to when using BAP. Kinetin concentrations of 2 mg/l produced the highest shoot formation (2.5/explants) and greatest shoot length (1.5 cm); however, higher kinetin concentrations caused shoot formation and growth to decline (Table 1).

3.2. Combined Effects of BAP and Different Auxin Concentrations

To enhance shoot organogenesis, young leaf explants from *in vitro*-grown *A. saponaria* meristem explants were cultured for 6 weeks in media containing 2 mg/l of BAP and various concentrations

Table 1. Effect of different BAP and kinetin concentrations on the shoot regeneration and growth of *A. saponaria* meristem explants after 6 weeks of culture. The values are the mean ± SD.

Cytokinins* (mg L^{-1})	Number of shoots per explant**	Shoot length[a] (cm)
Control 0.0	0.5 ± 0.0	0.7± 0.1
BAP 0.5	1.3 ± 0.1	1.3 ± 0.1
BAP 1.0	2.3 ± 0.1	1.5 ± 0.1
BAP 2.0	2.7 ± 0.2	1.8 ± 0.2
BAP 4.0	2.1 ± 0.1	1.6 ± 0.1
Kinetin 0.5	1.2 ± 0.1	1.0 ± 0.1
Kinetin 1.0	2.1 ± 0.1	1.3 ± 0.1
Kinetin 2.0	2.5 ± 0.2	1.5 ± 0.2
Kinetin 4.0	1.9 ± 0.2	1.4 ± 0.1

*Basal medium consisted of MS salts and vitamins, and 30 g/l sucrose, solidified with 7 g/l Phytagar.
**From 30 tested explants.
[a]Values represent the mean ± standard deviation of 50 shoots.

of different auxins (IAA, IBA, or NAA). Shoot initiation and shoot growth was highly influenced by the combined use of BAP and auxins (Table 2). BAP combined with any IBA concentration produced higher shoot initiation and shoot growth compared to the control treatment (Table 2). Among the treatment combinations, the combination of BAP and 0.5 mg/l IBA produced the highest shoot initiation per explant (3.7), and the same combination produced the tallest shoots (2.6 cm). This treatment combination (BAP and 0.5 mg/l IBA) produced 37% taller shoots compared to the control.

Table 2. Effect of MS medium supplementation with 2 mg/l BAP and different auxin concentrations on shoot regeneration and of *A. saponaria* meristem explant after 6 weeks of culture. The values are the mean ± SD.

Auxin* (mg/l)	Number of shoots per explant**	Shoot length[a] (cm)
Control 0.0	2.7 ± 0.2	1.8 ± 0.2
IAA 0.1	2.6 ± 0.2	1.8 ± 0.2
IAA 0.5	2.9 ± 0.2	1.9 ± 0.1
IAA 1.0	3.1 ± 0.3	2.2 ± 0.2
IBA 0.1	3.3 ± 0.2	2.3 ± 0.1
IBA 0.5	3.7 ± 0.3	2.6 ± 0.2
IBA 1.0	3.4 ± 0.3	2.4 ± 0.2
NAA 0.1	3.2 ± 0.3	2.1 ± 0.1
NAA 0.5	2.2 ± 0.2	1.5 ± 0.1
NAA 1.0	1.9 ± 0.1	1.1 ± 0.1

*MS medium supplementation with 2 mg/l BAP and different auxin concentrations
**From 30 tested explants.
[a] Values represent the mean ± standard deviation of 50 shoots

4. Discussion

Several studies have reported the *in vitro* multiplication of *Aloe* species. However, limited information is available regarding *A. saponaria*. Studies have shown that young inflorescences serve as reliable explants for the regeneration of *A. arborescens* and *A. vera* (Velcheva *et al*., 2005; Richwine *et al*., 1995). In another study, seedling derived explants were used for the micropropagation of *A. polyphylla* (Abrie and van Staden, 2001), while adventitious bud formation using decapitated shoots induced multiple shoots in *A. barbadensis* (Meyer and van Staden, 1991). Furthermore, previous studies have used different plant growth regulators for the micropropagation of *Aloe*. TDZ and BA showed efficient shoot initiation in *A. arborescens* (Velcheva *et al*., 2005). Zeatin riboside stimulated shoot regeneration in *A. barbadensis* (Richwine *et al*., 1995). Moreover, 2,4-dichlorophenoxyacetic acid (2,4-D) with basal MS medium was successfully used to culture calluses and induce multiple shoots in *A. vera* (Roy and Sarkar, 1991). Natali *et al*. (1990)

reported that basal media supplemented with only cytokinins could not promote the *in vitro* regeneration of *A. barbadensis*. In contrast, in the current study, we successfully induced shoot initiation from *A. saponaria* using basal media supplemented with cytokinins. To the best of our knowledge, the current study presents the first trials for the *in vitro* tissue culture of *A. saponaria*, demonstrating the high efficiency of BAP. In conclusion, the current study presents the first report of a highly efficient system for the *in vitro* micropropagation of *A. saponaria*, which might help to open the way for the genetic manipulation of *Aloe*.

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