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BRIEF COMMUNICATION

Effect of gibberellic acid combined with saponin on shoot elongation of *Asparagus officinalis*

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Abstract

Effect of gibberellic acid (GA_3) combined with saponin on shoot elongation of *Asparagus officinalis* was evaluated in tissue culture. Addition of saponin to GA_3 supplemented Murashige and Skoog (MS) basal medium showed a dose depended effect on shoot length of *Asparagus officinalis*. However, increasing concentration of saponin above 3.0 mg dm^{-3} decreased shoot length and showed yellowing and thinning of shoots. Saponin (3.0 mg dm^{-3}) + GA_3 (0.2 mg dm^{-3}) mixture treated by variable duration of sonication (0, 1, 3, 5, 7, 9, 11, 13 and 15 min) were evaluated for shoot elongation on MS basal medium. The highest shoot lengths (14.4 ± 0.3 and 15.1 ± 0.1 cm) were found after 5 and 7 min sonication.

Additional key words: growth regulators, sonication, tissue culture.

Saponins are amphiphilic high molecular mass glycosides, consisting of a hydrophobic aglycone linked to a hydrophilic sugar moiety (Armah *et al.* 1999). The precise mechanism of interaction of saponins with the cell membranes are not fully understood (Morrissey and Osbourn 1999, Oleszek *et al.* 1999). Biological activity of saponins might be related to their ability to join sterols in the biological membranes and thereby to cause a loss of their integrity (Nishikawa *et al.* 1984, Keukens *et al.* 1995, Morrissey and Osbourn 1999). Saponins such as those found in *Quillaja saponaria* could be good candidates for the delivery of biomaterials through the biological membranes (Bishnu and Wiesman 2006), which could be exploited for the plant growth regulators uptake in plant tissue culture. Present investigation exploits the possibility of saponin mediated delivery system of GA_3 in *Asparagus officinalis* shoot elongation in tissue culture.

In present investigation, *Asparagus officinalis* seeds were procured from Herbal Garden, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India. Seeds were initially submerged in 70 % (v/v) ethanol solution for 1 min, then surface disinfested with 0.1 % (m/v) $HgCl_2$ for 15 min, washed several times with sterile water and cultured on half strength Murashige and Skoog (1962; MS) medium without plant growth regulators. The pH of MS medium was adjusted to 5.8 prior to sterilization at 120 °C for 20 min. Epicotyls were cut into 0.5 cm segments from 20- to- 25-d-old seedlings and cultured on MS basal medium containing 1.0 mg dm^{-3} 2,4-dichlorophenoxyacetic acid (2,4-D) for induction of callus. After 4 weeks, calli were transferred to 1.0 mg dm^{-3} 6-benzylaminopurine (BAP) + 0.5 mg dm^{-3} kinetin (Kin) supplemented medium for shoot induction. Microshoots (3.0 cm long) harvested after 4 weeks on shoot induction media were used for screening of shoot elongation. MS basal medium augmented with saponin (*Quillaja saponeria*, *Sigma-Aldrich*, St. Louis, USA) and gibberellic acid (GA_3; *Himedia*, Mumbai, India) alone and together, were evaluated for shoot elongation. Sonication of variable duration (0, 1, 3, 5, 7, 9, 11, 13, and 15 min) was also applied for preparation of saponin + GA_3 formulation. For sonication, *Sonicator X 100* (*Panomics*, Fremont, CA, USA) of 40 kHz was used.

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Abbreviations: 2,4-D - 2,4-dichlorophenoxyacetic acid; BAP - 6-benzylaminopurine; GA_3 - gibberellic acid; IBA - indole 3-butyric acid; Kin - kinetin; MS - Murashige and Skoog.
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Water soluble saponin alone or with GA_3 were subjected to sonication by placing the rod (6 mm) in beaker in such a way that it would disperse equal amount of energy through out the samples. Ice cubes were used in water bath to maintain the temperature. After sonication, these solutions were supplied to MS basal media.

Every experiment was repeated twice and every treatment consisted of three repetitions. Every repetition included 30 microshoots with the same height (3.0 cm). Shoot length was measured after 4 weeks growth on MS basal medium with different treatments. Statistical analysis was performed with *JMP* software v. *6* (SAS 2007) using the Tukey-Kramer HSD test for determining significant differences among treatments at $P \leq 0.05$.

Table 1. Effects of different concentrations of saponin in MS basal medium on shoot length of *Asparagus officinalis* and subsequent rooting on 0.5 mg dm^{-3} IBA supplemented MS basal medium. Means ± SE of three repeated experiments. Each treatment consisted of three replicates. Means in column followed by different letter are significantly different at $P \leq 0.05$ according to Tukey-Kramer HSD.

Saponin [mg dm^{-3}]	Shoot length [cm]	Rooting [%]
0	5.0 ± 0.1ab	100
1	4.8 ± 0.1ab	100
2	4.9 ± 0.3ab	100
3	5.6 ± 0.2a	100
4	5.0 ± 0.1ab	100
5	4.0 ± 0.1b	40

Table 2. Effect of saponin + GA_3 supplemented MS basal media on shoot length of *Asparagus officinalis* and subsequent rooting on 0.5 mg dm^{-3} IBA supplemented MS basal media. Means ± SE of three repeated experiments. Each treatment consisted of three replicates. Means in column followed by different letter are significantly different at $P \leq 0.05$ according to Tukey-Kramer HSD.

Saponin [mg dm^{-3}]	GA_3 [mg dm^{-3}]	Shoot length [cm]	Rooting [%]
0	0	4.9 ± 0.2c	100
0	0.2	8.0 ± 0.1ab	100
1	0.2	8.0 ± 0.2ab	100
2	0.2	8.1 ± 0.2ab	100
3	0.2	9.0 ± 0.1a	100
4	0.2	8.4 ± 0.5a	70
5	0.2	7.0 ± 0.1b	55

The optimum concentrations of saponin and GA_3 for shoot elongation were found. GA_3 induced maximum shoot length (8.0 cm) at concentration 0.2 mg dm^{-3}, whereas saponin maximized shoot length (5.6 cm) at 3.0 mg dm^{-3}. Increasing concentration of saponin expressed deleterious effects on shoot (Table 1). Further, a constant concentration of GA_3 (0.2 mg dm^{-3}) were combined with variable concentration of saponin to see their synergistic effects on shoot elongation. Addition of saponin to GA_3 showed a dose depended effect on shoot length (Table 2). Increasing concentration of saponin up to 3.0 mg dm^{-3} increased shoot length (9.0 cm) while higher concentration of saponin did not enhance shoot elongation, besides showing yellowing and thinning of shoots. Yellow pale thin shoots could not survive further for rooting. Sonication can help in rearrangement of biomolecules (Oldenburg *et al.* 2005). Microscopic study of the saponin has shown that formation of self-assembled nanosized vesicles (Bishnu and Wiesman 2006) can be useful for the delivery of different compounds (Gregoriadis *et al.* 1998). Saponin + GA_3 formulation treated by sonication for 0, 1, 3, 5, 7, 9, 11, 13 and 15 min were evaluated for shoot elongation on MS basal medium. The longest shoots (14.4 ± 0.3 and 15.1 ± 0.1 cm) were found after 5 and 7 min sonication (Table 3). Longer duration of sonication upto 15 min significantly decreased shoot length without effecting rooting percentage (Table 3). These results strengthened the hypothesis that saponin nanosized vesicles act as delivery system for GA_3 to plant cells.

Table 3. Effect of sonication of variable duration to saponin + GA_3 formulation on shoot length of *Asparagus officinalis* and subsequent rooting on 0.5 mg dm^{-3} IBA supplemented MS basal media. Means ± SE of three repeated experiments. Each treatment consisted of three replicates. Means in column followed by different letter are significantly different at $P \leq 0.05$ according to Tukey-Kramer HSD.

Sonication [min]	Saponin [mg dm^{-3}]	GA_3 [mg dm^{-3}]	Shoot length [cm]	Rooting [%]
0	0	0	4.8 ± 0.3g	100
0	3.0	0	5.4 ± 0.4g	100
0	3.0	0.2	9.9 ± 0.1cd	100
1	3.0	0.2	10.0 ± 0.1cd	100
3	3.0	0.2	12.1 ± 0.2b	100
5	3.0	0.2	14.4 ± 0.3a	100
7	3.0	0.2	15.1 ± 0.1a	100
9	3.0	0.2	10.9 ± 0.3c	100
11	3.0	0.2	9.1 ± 0.2de	100
13	3.0	0.2	8.3 ± 0.1ef	100
15	3.0	0.2	7.5 ± 0.1f	100

Recent study proved that *Quillija saponin* preparation can effectively deliver 2,4-D across isolated cuticle membranes of *Citrus grandis* (Bishnu and Wiesman 2006). In fact, the specific ability of saponins to form pores in membranes has contributed to their use in physiological research. Our finding open up a new avenue for further investigation for saponin mediated delivery system in plant tissue cultures.

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