

Effects of B Vitamins and Benzylaminopurine on Adventitious Shoot Formation from Hypocotyl Segments of Snapdragon (*Antirrhinum majus* L.)

Kazuhiko SHIMASAKI and Yasufumi FUKUMOTO*

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Snapdragon (*Antirrhinum majus* L.) is an important plant for its ornamental value. Several studies have been carried out on the plant regeneration from tissue culture of snapdragon such as mesophyll protoplasts [1], suspension culture cells [2] and internode stem tissues [3]. Okubo *et al.* [4] reported vigorous formation of adventitious shoots from the basal hypocotyl segments of snapdragon, which contained the highest level of endogenous cytokinins among young seedlings. The regeneration ability or sensitivity to plant growth regulators are quite different according to genotype, times or timing of subculture, source and age of explants. Plant tissue culture media contain a variety of organic constituents and plant growth regulators. Earlier reports showed that yeast extract had a promotive effect on the growth of excised corn roots [5], and this effect could be partly replaced by thiamine. Bonner and Adicott [6] also reported that nicotinic acid, thiamine and pyridoxine were essential for the growth of isolated roots of four plant species. Pyridoxine has been shown to be indispensable for the growth of tomato roots [7]. In snapdragon, the effect of vitamins on the growth and regeneration of cultured tissues has not examined. In addition, interaction between plant growth regulators and vitamins has not been well understood. The objective of this study was to examine the effects of a plant hormone BA and three B vitamins, nicotinic acid, pyridoxine and thiamine on adventitious shoot regeneration from hypocotyl segments of snapdragon.

Seeds of *Antirrhinum majus* L. (cv. Apple Blossom) were surface-sterilized for 6 min in a sodium hypochlorite solution (1% effective chloride) containing 0.01% Tween 20, and rinsed three times with sterilized distilled water. They were sown on a double layers of filter papers (No. 2, 90 mm, Toyo, Tokyo, Japan) inside a plastic Petri dish (90×20 mm, Nipro, Osaka, Japan) containing 10 ml sterilized distilled water. The seeds were incubated at 25±1°C under a 16-hr/day photoperiod (25 μE/m²·s) with white fluor-

escent lamps for 2 weeks. Whole hypocotyl segments (ca. 5 mm long) were excised after expansion of seedling cotyledons. A basal medium consisted of MS inorganic salts [8], 100 mg/l myo-inositol, 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine-HCl, 0.1 mg/l thiamine-HCl, 2 mg/l glycine and 20 g/l sucrose was used. The medium was supplemented with various concentrations of B vitamins, with or without 0.225 mg/l BA, adjusted to pH 5.5, and solidified with 2 g/l gellan gum. For culture 500 ml jars (UM Culture Bottle, Iuchi, Osaka, Japan) each containing 50 ml of the medium were used. Cultures were incubated at 25±1°C under a 16-hr/day photoperiod (25 μE/m²·s) with white fluorescent lamps for 2 weeks. Each treatment consisted of 5 jars, each containing three explants. Percentage of hypocotyl segments forming adventitious shoots with leaves and the number of shoots were recorded 2 weeks after the initiation of culture.

Hypocotyl segments began to form adventitious shoots 7 days after the onset of culture. Most shoots were formed on the basal end of hypocotyl segments. Effects of B vitamins and BA on adventitious shoot formation from hypocotyl segments are summarized in **Table 1**. In BA-free media, absence of nicotinic acid reduced the number of shoots developed. However, both the percentage of shoot formation and the number of shoots were similar between the BA-free control medium and nicotinic acid-free medium with 0.225 mg/l BA. In BA-free media, lack of pyridoxine resulted in the lower percentage and number of shoots. However, 100% of shoot formation and more than 3.6 times as many shoots as pyridoxine-free media were obtained by adding 0.01 mg/l pyridoxine-HCl to BA-free medium. For adventitious shoot formation, The pyridoxine-HCl concentration at 0.01 mg/l was superior to that of the control medium. The highest percentage (100%) and number of shoots (5.5) were obtained by adding both 0.01 mg/l pyridoxine-HCl and 0.225 mg/l BA. Lack of thiamine-HCl in BA-free medium markedly reduced both the percentage and the number of shoots. Percentage and the number of shoots partially recovered by adding 0.

* Department of Subtropical Agriculture, Kochi University, Monobe B200, Nankoku-city, Kochi 783-8502, Japan

Table 1. Effect of B vitamins and BA on adventitious shoot formation from hypocotyl segments of snapdragon.

B vitamins and BA in culture media				0.225 mg/l BA	% of hypocotyl segments forming adventitious shoots	Number of adventitious shoots/explant
Nicotinic acid (mg/l)	Pyridoxine - HCl (mg/l)	Thiamine - HCl (mg/l)				
0.5	0.5	0.1		(Cont.* ¹)	83.3	2.0 d
0	0.5	0.1		—	58.3	1.6 e
0.5	0	0.1		—	46.1	0.8 fe
0.5	0.5	0		—	30.8	0.3 g
0.5	0.01	0.1		—	100	2.9 cd
0.5	0.5	0.01		—	61.5	1.2 efg
0.5	0.5	0.1		+	100	3.4 b
0	0.5	0.1		+	83.3	2.8 cd
0.5	0	0.1		+	66.7	2.2 d
0.5	0.5	0		+	83.3	3.4 b
0.5	0.01	0.1		+	100	5.5 a
0.5	0.5	0.01		+	100	3.4 b

Values represent the mean obtained from duplicate experiments.

*1 The control medium containing 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine-HCl and 0.1 mg/l thiamine-HCl was used as the original MS medium.

*2 Means followed by the same letter are not significantly different according to the Duncan's new multiple range test ($P=0.05$).

0.1 mg/l thiamine-HCl. Similar percentage of shoot formation was obtained by adding 0.225 mg/l BA instead of thiamine-HCl, and the number of shoots on this media was superior to that of the BA-free control medium.

This study showed that both B vitamins and BA in the culture media influenced adventitious shoot formation from snapdragon hypocotyls. Absence of any B vitamins in BA-free medium reduced adventitious shoot formation. By adding 0.225 mg/l BA to culture medium, hypocotyl segments could develop adventitious shoots without exogenously supplied nicotinic acid and thiamine. A similar phenomenon was observed with soybean cells cultured on a medium containing thiamine and BA [9]. It appeared that nicotinic acid, thiamine-HCl and pyridoxine-HCl are necessary for snapdragon tissue culture. However, the concentration of pyridoxine-HCl in original MS medium is too high for adventitious shoot formation for this snapdragon genotype. The effects of thiamine-HCl on adventitious shoot formation obtained with snapdragon hypocotyls are in agreement with a number of other studies [6, 10, 11]. BA is effective for adventitious shoot regeneration from hypocotyl segments. Pyridoxine could partially be replaced by 0.225 mg/l BA for adventitious shoot formation. However, the effect of BA seems to be independent of those of B vitamins, since addition of BA to the culture medium

could promote adventitious shoot regeneration in all treatments. The results obtained from this investigation seem to offer useful suggestions for efficient adventitious shoot regeneration in tissue culture systems of snapdragon.

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