

Effect of aromatic cytokinins and explant position on shoot multiplication of *Asparagus officinalis* L.

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Summary: *Asparagus officinalis* has been widely studied, but little information is available about its *in vitro* response to exogenous cytokinin during shoot multiplication. To study the effects of different cytokinins on shoot multiplication of *A. officinalis* 'Grolim', *in vitro* culture was initiated from shoot segments cultured on media with Murashige and Skoog medium. Effects of different aromatic cytokinins (6-benzylaminopurine, 6-benzylaminopurine riboside and meta-topolin) applied in four concentrations (0.5, 1.0, 1.5, 2.0 mg/l) on shoot multiplication of 'Grolim' were tested. Effect of explant position (vertically or horizontally) on the shoot multiplication outcome was also studied. Both the length and the number of newly developed shoots were significantly affected by explant position and cytokinin content of the medium. The highest numbers of shoots (4.9) were produced in the presence of 0.5 mg l⁻¹ 6-benzylaminopurine riboside when explants were paced horizontally onto the medium. Although the longest shoots (41.5 mm) developed on explants placed vertically onto medium supplemented with 2.0 mg l⁻¹ meta-topolin, the lengths of shoots developed on medium with 0.5 mg l⁻¹ 6-benzylaminopurine riboside were also adequate in both explant position (29.5 and 33.6 mm placed horizontally and vertically, respectively).

Key words: shoot multiplication, 6-benzylaminopurine, 6-benzylaminopurine riboside, meta-topolin, asparagus

Introduction

Members of *Asparagus* genus have been cultivated for over 2000 years as a vegetable and medicinal herb (*A. racemosus*, *A. verticillatus*, *A. adscendens* and *A. curillus*). Some species of the genus *Asparagus* is known as ornamental plants (*A. plumosus*, *A. densiflorus*, *A. virgatus*, *A. myriocladus* and *A. retrofractus*). Economically the most important species of *Asparagus* is garden *Asparagus* (*A. officinalis*), which is a popular vegetable in the market (Stajner et al., 2002). *Asparagus officinalis* is a perennial, dioecious species with male and female plants, and a well-known vegetable widely cultivated all over the temperate world (Desjardins, 1992).

Many researchers have investigated the possibilities of micropropagation of *A. officinalis* (Murashige et al., 1972; Yang & Clore, 1974; Yang, 1977) and found the Murashige and Skoog (1962) MS-based culture media to be optimal for asparagus.

In the experiments of Sarabi & Almasi (2010) asparagus explants were grown on MS medium supplemented with 6 % sucrose and effects of 1-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BA) concentrations on callus induction and shoot development were tested. Their results showed that 0.015 mg/l NAA and 0.5 mg/l BA gave the highest number of plantlets.

Fonnesbech et al. (1979) tested the effects of cytokinins on the growth and development of lateral shoot tips of

Asparagus plumosus cultured on a modified MS medium. They found that applying of 1-pyrenoylbenzoic acid (PBA) was superior for shoot development, especially in the range between 0.02 and 2 mg/l comparing to the zeatin, kinetin, BA and N6-[2-isopentenyl]adenine (2iP).

The positive effect of ancymidol, which is an inhibitor of gibberellin (GA₃) synthesis, on *in vitro* development of *A. officinalis* was reported by Chin (1982). Khunachak et al. (1987) also described that ancymidol was one of the main factor affecting the shoot and root development and more than auxins and cytokinins.

Recently, Stajner (2013) also found the MS basal medium supplemented with 1.07 μM NAA, 0.93 μM kinetin, 0.44 μM BA and 0.39 μM ancymidol to be optimal for shoot multiplication of *A. officinalis*.

Materials and methods

Young spears emerging from field-grown *A. officinalis* were used as explants for *in vitro* culture establishment. The upper part (2–3 cm long) of each spear was cut off, washed in running tap water and surface sterilized with concentrated Clorox solution with the addition of a few drops of Tween 20 and Dodenal for 10 minutes. The spear segments were then washed in sterile distilled water. This was followed by 70% ethyl alcohol treatment for 30 seconds. Finally they were thoroughly rinsed in sterile distilled water 3 times.

Explants were placed on the media containing MS macro- and micro elements supplemented with 25 mg l⁻¹ Fe-EDTA, 100 mg l⁻¹ myo-inositol, 100 µl l⁻¹ vitamin B₁, 500 µl l⁻¹ vitamin B₆, 500 µl l⁻¹ vitamin B₃, 3% sucrose, 5.7 g l⁻¹ agar-agar, 20 µl l⁻¹ 1-naphthaleneacetic acid (NAA) and different types and concentrations of cytokinins (BA, 6-benzylaminopurine riboside (BAR) and meta-topolin (TOP) at 0.5, 1.0, 1.5, 2.0 mg l⁻¹ levels.

Effect of positions of shoots was also tested. Explants were placed either horizontally or vertically onto the media.

The media were adjusted to pH 5.7 and autoclaved at 121 °C and 10⁵ Pa for 20 minutes. Five explants were placed on 40 ml of medium in Kilner jar (400 ml, 75 mm inside diameter and 85 mm long). Cultures were grown at 22±2°C, 16 h photoperiod provided by warm-white lamps at a PPF of 105 µmol s⁻¹m⁻² for four weeks.

After four weeks of culture, the length of newly developed shoots was measured and the rate of shoot multiplication was determined by counting the number of new shoots per explants. Statistical analyses were made by ANOVA followed by Tukey's test using SPSS 13.0 for Windows programme.

Results and discussion

Effect of explant position

Both the lengths and the number of newly developed shoots were significantly affected by the position of explants. The multiplication rate was significantly higher ($P < 0.05$) when explants were placed horizontally comparing to the explants placed vertically (3.5 and 2.4 in the average of all cytokinin treatments, respectively). However, no significant differences could be detected when shoots were cultured on media containing TOP in 1.5 mg l⁻¹ concentration or BAR above 0.5 mg l⁻¹ (Figure 1 A-C).

In contrast, the shoots were significantly longer ($P < 0.05$) when developed on explants placed vertically onto media comparing to those developed on explants placed horizontally onto the media (32.6 and 21.0 mm in the average of all cytokinin treatments, respectively). Differences in the lengths of shoots developed on different positioned explants were proven to be significant in the majority of treatments except for shoots cultured on media containing BA and BAR at the least concentration (0.5 mg l⁻¹). Moreover, in the case of BAR and TOP the higher concentration of cytokinin was applied in the media the greater differences in the shoot lengths could be observed (Figure 2 A-C).

Effects of cytokinins

When explants were cultured horizontally the most newly developed shoots were observed on media supplemented with BA, although it was not significantly higher than those observed on media containing TOP (4.0 and 3.6 shoots per explant in the average of all concentration, respectively). However, applying BAR in the media resulted significantly fewer shoots (2.9 per explant) comparing to the media supplemented with BA and TOP. No significant differences could be detected between the propagation rates when explants were placed vertically analyzing data in the average of all concentration (Table 1). The lengths of newly developed shoots were significantly affected by cytokinin types; the longest shoots developed on explants placed vertically and cultured on media containing TOP. Inhibitory effect of BA on shoot length could be detected in both explant positions (Table 1).

In the average of all cytokinin types the highest multiplication rate (3.7 shoot per explant) was observed

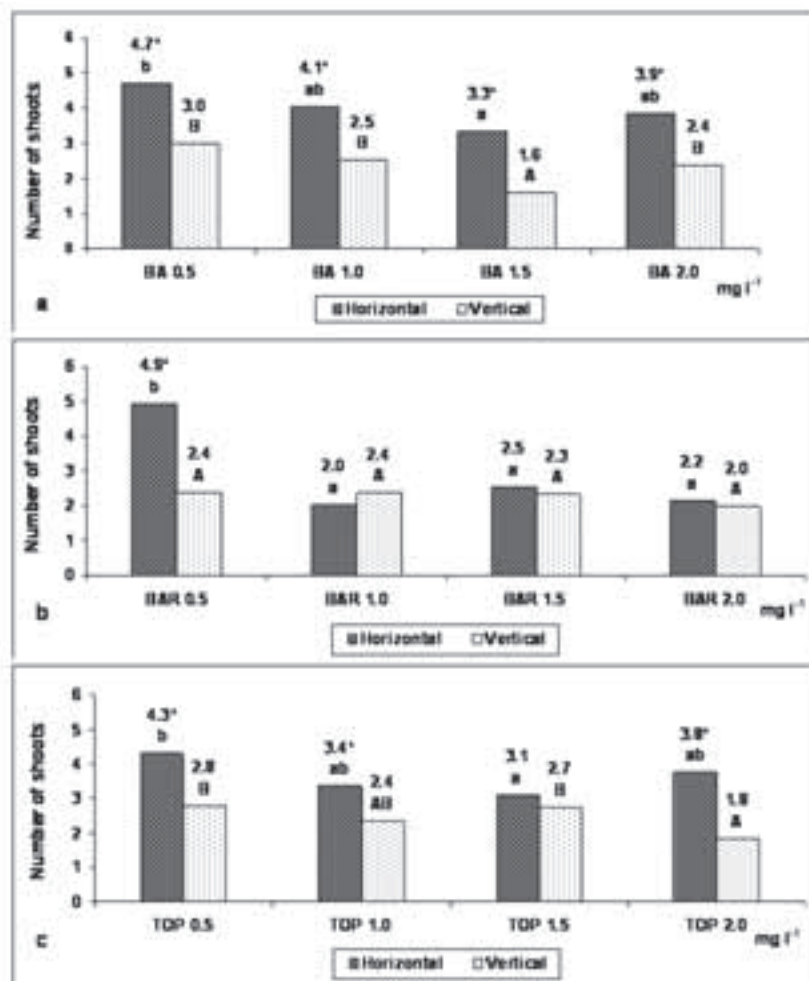


Fig. 1. The effects of explant positions and cytokinin concentrations on the multiplication rate of *Asparagus officinalis* 'Grolim'. Significant differences ($P < 0.05$) between different explant positions are indicated by * at the same cytokinin treatment. Significant differences ($P < 0.05$) between different cytokinin concentrations are indicated by small letters in the case of horizontal position of explants and by capital letters in the case of vertical position of explants

at the lowest cytokinin level (0.5 mg l⁻¹), and higher concentrations (1.0; 1.5 and 2.0 mg l⁻¹) significantly decreased (P<0.05) the shoot numbers down to 2.6 shoot per explant. The highest inhibitory effect of higher level of BAR could be detected if explants were horizontally cultured (Figure 1 B). Similarly, the shoots developed on explants cultured horizontally were also inhibited by higher levels of cytokinins in their growth (Figure 2 A-C). Mehta and Subramanian (2005) also found that the lowest level of growth regulators (KIN and NAA) resulted in the highest propagation rate and the longest shoots on nodal explants of *Asparagus adscendens*. They supposed that *A. adscendens* is one of the species, which have endogenous hormones in adequate amount for *in vitro* regeneration. Similarly, Koda and Okazawa (1980) detected remarkable cytokinin production in *in vitro* cultured *Asparagus officinalis* shoot apex. However, they observed setback in the growth of shoots, which could be restored by exogenous zeatin. Even though the *in vitro* cultured *Asparagus plumosus* shoot tips also preferred the lowest level (0.2 mg l⁻¹) of cytokinins (Kin, BA, ZEA, 2iP and PBA) but cytokinin was essential for survival of *in vitro* cultures (Fonnesbech et al. 1977).

In contrast, the shoots developed on explants cultured vertically were significantly longer on media containing 1.5 or 2.0 mg l⁻¹ BA comparing to the shoots obtained on media with 0.5 and 1.0 BA mg l⁻¹. Applying TOP in 1.5 and 2.0 mg l⁻¹ concentrations resulted in the longest shoots (40.3 and 41.5 mm, respectively) on vertically cultured explants, and their lengths did not differ significantly from results obtained on media with 0.5 and 1.0 TOP (39.1 and 35.5 mm, respectively). When media were supplemented with BAR the longest shoots developed at the highest BAR level (39.7 mm), while shorter shoots were harvested from media containing 1.0 mg l⁻¹ BAR.

Considering the multiplication rate the best result (4.9 newly developed shoots per explant) was achieved when explants were placed horizontally onto the medium containing 0.5 mg l⁻¹ BAR. In this case the lengths of shoots were adequate (29.5 mm) for further propagation or acclimatization, although the longest shoots (41.5 mm) developed on explants placed vertically onto medium contained 2.0 mg l⁻¹ TOP.

Results suggest that BAR and TOP can be used efficiently in *Asparagus officinalis in vitro* shoot multiplication, resulting similar multiplication rate as in case of applying BA but with longer shoots.

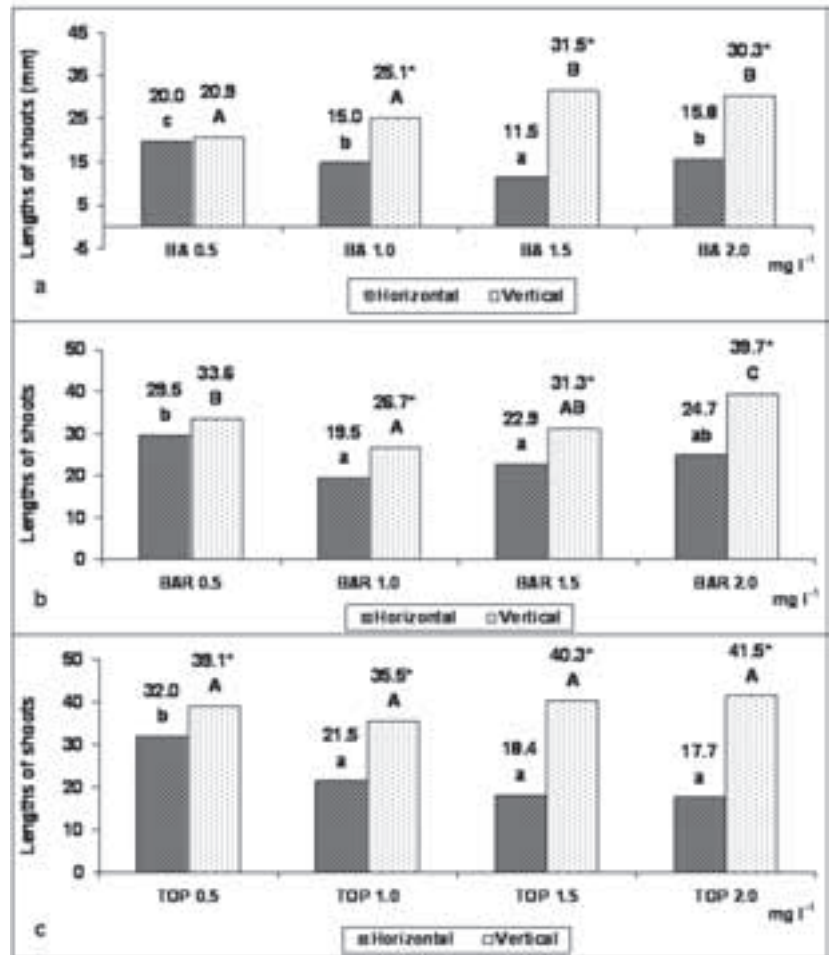


Fig. 2. The effects of explant positions and cytokinin concentrations on the length of shoots of *Asparagus officinalis* 'Grolim'. Significant differences (P<0.05) between different explant positions are indicated by * at the same cytokinin treatment. Significant differences (P<0.05) between different cytokinin concentrations are indicated by small letters in the case of horizontal position of explants and by capital letters in the case of vertical position of explants

Table 1. The main effects of the cytokinin types on the multiplication rate and shoot length of *A. officinalis* 'Grolim'. Significant differences (P<0.05) between different cytokinin treatments are indicated by small letters within a column

Explant position	Horizontally		Vertically	
	Multiplication rate	Lengths of shoots (mm)	Multiplication rate	Length of shoots (mm)
BA	4.0 b	15.9 a	2.3 a	26.2 a
BAR	2.9 a	25.4 c	2.4 a	32.5 b
TOP	3.6 b	22.9 b	2.4 a	39.0 c

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