Reevaluation of Effects of Aminoethoxyvinylglycine on Growth of In Vitro Pear Shoots©

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INTRODUCTION

Aminoethoxyvinylglycine (AVG), an inhibitor of ethylene, has been used for blocking ethylene biosynthesis and revealing the responses of plants to it. The chemical 1-methylcyclopropene (1-MCP) is able to block ethylene receptors and is functionable at very low concentrations in cut flowers (Serek et al., 1995) and fruits (De Wild et al., 1999). At the end of 2010, 1-MCP was permitted for use as an inhibitor of overripening with apple, pear, and persimmon by Ministry of Agriculture, Forestry and Fisheries of Japan.

Ethylene is said to cause plant tissues responsive reactions at very low concentrations and to be promoted or inhibited by auxin. In in vitro culture, rose shoots required different concentration of ethylene depending on rooting process: an adequate amount of ethylene was needed for root emergence and root formation but more ethylene was necessary for root growth (Kepczynski et al., 2006). In apple microcuttings, it was reported that ethylene was not involved in auxin (IBA)-dependent root formation (Harbage and Stimart, 1996), whereas Ma et al. (1998) reported ethylene inhibited root formation of apple shoots. Ethylene appeared to promote shoot formation in peach rootstocks (Dimasi-Theriou and Economou, 1995) while it inhibited organogenesis and growth of kiwi fruit explants, except for rooting (Arigita et al., 2003).

As mentioned above, even in plant tissue cultures of fruit trees, the conclusions about the role of ethylene differed between the reports. The response to ethylene varied with plant materials, plant sections, developmental stages, and applied ethylene concentrations. It is also unclear whether ethylene is the cause or effect.

Recently, Soeno et al. (2010) reported that AVG was identified as an inhibiter of auxin biosynthesis by a genomics-based approach in *Arabidopsis* and it had a strong anti-auxin activity independently of ethylene biosynthesis. Aminoethoxyvinylglycine is known to inhibit ethylene by inhibiting pyridoxal phosphate (PLP) (Yang and Hoffman, 1984) that mediated the reaction of *S*-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC). In indole-3-acetic acid (IAA) biosynthesis, tryptophan aminotransferase that converts L-tryptophan to indol-3-pyruvic acid is known to require PLP as coenzyme (Shi et al., 2002), so that the inhibition of IAA biosynthesis is attributable to it.

Many researchers investigating the relations between ethylene and in vitro plant growth used AVG as an ethylene inhibitor, so we think that we should reevaluate an effect of AVG from the point of view of both ethylene and auxin. Furthermore, by using AVG, we are able to make plants defective in auxin and to provide novel insight into auxin biosynthesis and action, and uncover structural characteristics of auxin biosynthesis inhibitors (Soeno et al., 2010). The objective of this study was to reveal whether AVG, 1-MCP, and ACC affected growth and organogenesis of 'La France' pear (*Pyrus communis* L.) in vitro shoots.

MATERIALS AND METHODS

The 'La France' in vitro shoots proliferated by MW medium (Tetsumura et al., 2008), a mixture of equal ratio of MS (Murashige and Skoog, 1962) and WPM (Lloyd and McCown, 1980) was used in this study. All media contained 0.5 μM indole-3-butyric acid (IBA), 10 μM benzyladenine (BA), 0.8% (w/v) agar (Wako Pure Chemical Industries, Ltd., Tokyo, Japan), 2% (w/v) sorbitol. The pH of each medium was adjusted to 5.7 with KOH before autoclaving at 1.1 $kg \cdot cm^{-2}$ for 15 min at 121 °C. The growth regulators AVG and ACC were filter-sterilized and added to the media after autoclaving. The 1-MCP was treated as follows; a microtube with polymer absorbing 1-MCP was kept upright into medium in a conical flask, and 100 μl of water was poured into the microtube for evolution of 1-MCP, and then the flask was immediately sealed with parafilm (Pechiney Plastic Packaging, U.S.A.). The AVG (1 and 100 μM), ACC (1 μM, 10 μM, and 100 μM), and 1-MCP (1 $\mu l \cdot L^{-1}$) were added to the medium or the flask solely or in combination.

After culturing 4 to 7 weeks, the shoots were cut to ca. 1 cm of the apical section containing five to six young leaves, and the three sections were placed on 20 ml MW medium per each conical flask, and then the flasks were covered with aluminum foil. Some conical flasks containing the medium with AVG at 1 μM and 100 μM were made airtight by being wrapped with parafilm. Cultures were maintained under 16-h photoperiod with 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD and 25±2 °C. Number of leaves with lamina, >8 mm long, shoot number, and shoot length of the shoot showing the best growth in each flask were recorded every 7 days. The length of the shoot was converted to the point as based on the shoot length conversion table (Table 1).

For ethylene assays, the flasks were sealed with parafilm 24-h prior to the measurement. Subsequently a 1-ml gas sample of the headspace of flask was taken by a syringe and injected into a gas chromatograph (GC-8A, Shimadzu, Kyoto, Japan) equipped with flame ionization detector and stainless steel column packed with 50–80 mesh Porapack Q.

Table 1. Shoot length conversion table.

Shoot length (cm)		Converted value (point)
~ 0.99	⟶	0.5
1.0 ~ 1.19	⟶	1.1
1.2 ~ 1.39	⟶	1.3
≀		≀
1.8 ~ 1.99	⟶	1.9
2.0 ~ 2.19	⟶	2.1
≀		≀

RESULTS AND DISCUSSIONS

The most ethylene production from 'La France' shoots on the control medium was observed at Day 49 (Fig. 1). As active cellular division increased ethylene production (Abeles et al., 1992) and leaf number of the control rapidly increased from Day 49 (Fig. 2). However, the shoot length and the shoot number did not show rapid increases from Day 49. The organogenesis of the control was not associated with ethylene production. Aminoethoxyvinylglycine at 100 μM prevented all organogenesis (Fig. 2).

Little ethylene was detected from the flasks with the medium containing AVG 1 μM and 100 μM. However, a certain amount of ethylene was detected from the airtight flask containing AVG. Hence, it was suggested that covering with an aluminum foil lid kept a little bit of ventilation and ethylene accumulation hardly occurred in the flasks. The greatest leaf number was produced by 1 μM AVG, but the shoot length on the medium with 1 μM AVG was shorter than that of the control. This inhibition of shoot elongation might result from the inhibition of auxin biosynthesis by AVG.

Cultures treated with ACC (1 μM and 10 μM) produced much calluses without decreasing the leaf number, the shoot elongation, and the shoot number. 'La France' shoots are probably insensible to increased amount of ethylene. The shoots in the 1-MCP grew as well as those in the control. The growth inhibition by 100 μM AVG was overcome by neither ACC nor 1-MCP.

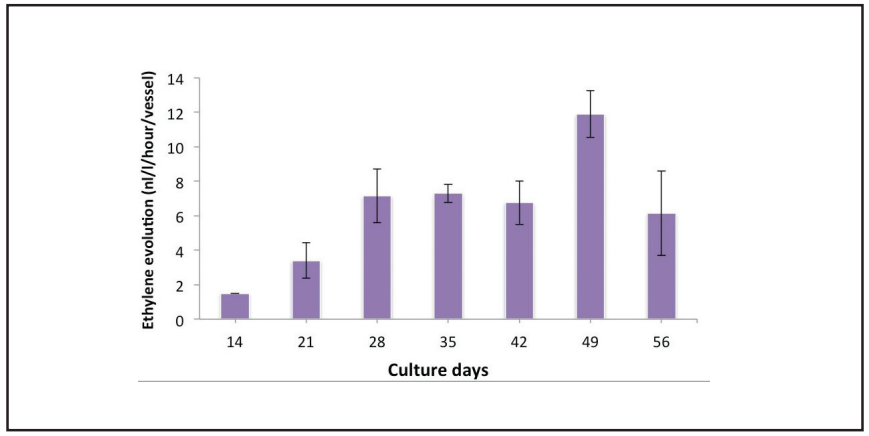

Figure 1. Ethylene evolution from *Pyrus communis* 'La France' shoots on MW medium [a mixture of equal ratio of Murashige and Skoog (MS) medium and Lloyd and McCown (WPM) medium]. Vertical bars represent ± se. N = 5.

CONCLUSION

In conclusion, ethylene had little effect on organogenesis of 'La France' shoots and the effect of AVG on the shoot growth may be caused by the inhibition of IAA biosynthesis. Further investigation, that is, whether an addition of auxin to the medium can overcome the inhibitory effect of AVG on the organogenesis, should be conducted.

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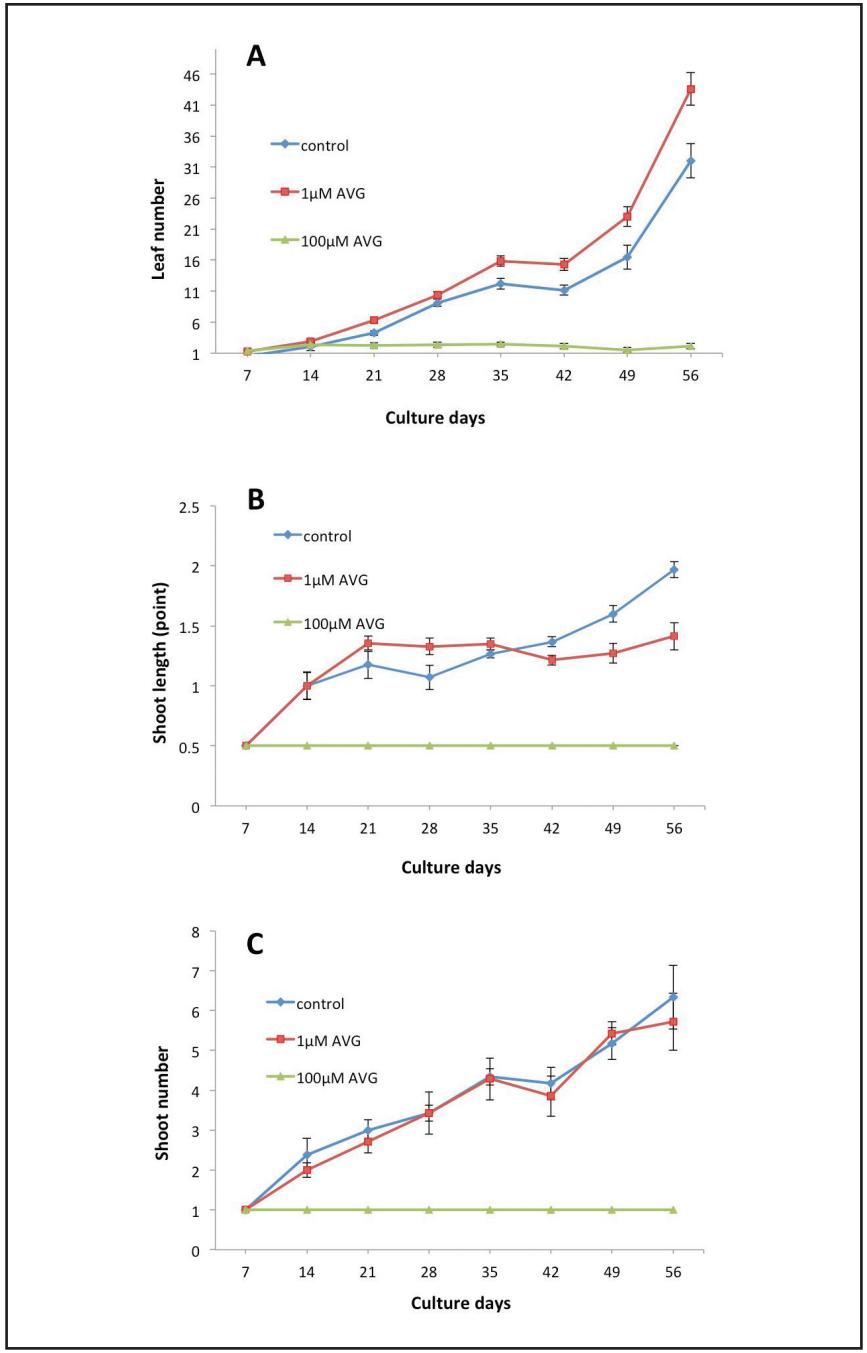

Figure 2. The effect of AVG on leaf number (A), shoot length (B) and shoot number (C) of *Pyrus communis* 'La France' shoots. Vertical bars present ± se. N = 8.

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