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BRIEF COMMUNICATION

# Effect of jasmonic acid on *in vitro* explant growth and microtuberization in potato

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## Abstract

The shoot fresh mass, root length and root numbers of two potato (*Solanum tuberosum* L.) cultivars Favorita and Helanwuhua were increased significantly by the application of 0.2 - 2 mg $dm^{-3}$ jasmonic acid (JA) in the Murashige and Skoog medium. However, the growth of potato explants was inhibited by JA at high concentrations (20 - 50 mg $dm^{-3}$). Chlorophyll content in explant leaves decreased with an increase in the concentration of JA. In leaves treated with 0.2 mg $dm^{-3}$ JA acid peroxidase activity increased, while in the leaves treated with more than 2 mg $dm^{-3}$ JA peroxidase activity decreased. Under the dark, the microtuber numbers, fresh mass and percentage of big microtubers of two potato cultivars were not promoted by the application of 0.2 - 50 mg $dm^{-3}$ JA.

*Additional key words*: chlorophyll content, fresh mass, peroxidase activity, plantlet, root development, *Solanum tuberosum*.

Jasmonic acid (JA) and other jasmonates are plant growth regulators widely distributed within the plant kingdom (Ulloa *et al*. 2002). Recently, JA has been found to have various effects on the growth and development of plants, such as inhibition of seed germination (Corbineau *et al.* 1988, Bin *et al.* 2001, Huang *et al.* 2002, Kumari and Sudhakar 2003) and root growth (Wang *et al.* 2002), stimulation of floret opening (Zeng *et al.* 1999) and bulb formation (Ravnikar *et al.* 1992), defense response in leaves (Repka *et al.* 2001, 2004), and promotion of flower and fruit development (Wilen *et al.* 1991, Czapski and Saniewski 1992). Tuberization in potato was controlled by the tuberonic acid and its glucosides which had a close relation with JA in structure (Koda and Okazawa 1988, Koda *et al.* 1988, Yoshihara *et al.* 1989). In addition, *in vitro* microtuberization provided an adequate experimental model for the physiological and metabolic studies of tuberization and the screening of potential potato genotypes (Li *et al.* 2004, Zhang *et al.* 2005a,b). The purpose of this work was to investigate the effects of different concentrations of JA on the growth and development of potato plantlets and microtubers under *in vitro* conditions.

*In vitro* plantlets of two commercial potato (*Solanum tuberosum* L.) cultivars, Favorita and Helanwuhua, were propagated using single nodal cutting on the propagation Murashige and Skoog MS (1962) medium containing 30 g $dm^{-3}$ sucrose and 8 g $dm^{-3}$ agar (pH 5.8). Plantlets were cultured at temperature of 25 °C and 16-h photoperiod (irradiance of 100 μmol $m^{-2}$ $s^{-1}$). Five single-node explants obtained from propagated plantlets were transferred into each culture vessel with propagation MS medium supplemented with different concentrations of JA (0, 0.2, 2, 20 and 50 mg $dm^{-3}$), and cultured under the same condition as described above. Shoot length and fresh mass, leaf number, root length and fresh mass were recorded for *in vitro* plantlets after 40 d, and the chlorophyll content and peroxidase (POD) activity of shoots were determined. Chlorophyll content was analyzed spectrophotometrically with a *UV-2450* spectro-

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*Abbreviations*: BAP - benzylaminopurine; FM - fresh mass; GA - gibberellin; JA - jasmonic acid; MS medium - Murashige and Skoog medium; POD - peroxidase.
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photometer (*Shimadzu*, Tokyo, Japan) according to the method of Zhang (1992), and POD activity was measured by the guaiacol reduction method as previously described by Zhou and Leul (1998).

In a further experiment, five two-node explants obtained from propagated plantlets were transferred into each Erlenmeyer flask with 30 $cm^3$ liquid MS media. The flasks were also cultured at 25 °C under a 16-h photoperiod. After 3 weeks, the liquid MS medium was drained and substituted by liquid induction MS medium, which was supplemented with 80 g $dm^{-3}$ sucrose, 5 mg $dm^{-3}$ benzylaminopurine (BAP), 1 g $dm^{-3}$ activated carbon and different concentrations of JA (0, 0.2, 2, 20 and 50 mg $dm^{-3}$). The explants were incubated for 60 d in darkness at 20 ± 1 °C. After harvested, microtuber fresh mass, the number and percentage of big microtubers (diameter is more than 5 mm) were recorded per flask. JA, MS basal salts and other chemicals were obtained from *Sigma*, St. Louis, USA, or from *Wako Pure Chemical*, Osaka, Japan.

The growth of potato plantlets cultured for 40 d on the propagation MS medium was promoted by the adding of 0.2 - 2.0 mg $dm^{-3}$ JA (Fig. 1, Table 1). Under the above mentioned photoperiod, JA of 2.0 mg $dm^{-3}$ significantly increased fresh mass of shoot and root, root number and root length of two cvs. Favorita and Helanwuhua. However, higher JA concentrations inhibited markedly the growth of plantlets. Compared with the control, 20 mg $dm^{-3}$ JA decreased root length and root fresh mass of Favorita and all growth parameters determined of Helanwuhua, while 50 mg $dm^{-3}$ JA inhibited completely the plantlet growth of two cultivars tested. However, there were no significant changes in the leaf number of Favorita under 0 - 50 mg $dm^{-3}$ JA.

Chlorophyll contents of leaves decreased progressively with an increase in JA concentrations (Table 1). At 20 and 50 mg $dm^{-3}$ JA, the chlorophyll contents of Helanwuhua were only half and one-fourth of the control, while that of Favorita were just only one-fourth and one-tenth of the control. The lower concentration of JA treatments (up to 0.2 mg $dm^{-3}$) tended to increase the POD activity of potato plantlets, while increasing of JA concentrations over 2.0 mg $dm^{-3}$ decreased the POD activity in both cultivars (Table 1).

Under the dark, JA of 0.2 - 50 mg $dm^{-3}$ decreased the fresh mass of microtubers and percentage of big


Fig. 1. Effect of different concentrations of JA (0, 0.2, 2, 20 and 50 mg $dm^{-3}$, left to right) on the *in vitro* growth of explants in two potato cultivars (Favorita and Helanwuhua).

Table 1. Effect of different concentrations of JA [mg $dm^{-3}$] on leaf and root number, shoot and root length [cm] and fresh mass [g], chlorophyll content [mg $g^{-1}$(FM)], and POD activity [$A_{470}$ $g^{-1}$(FM) $min^{-1}$] of potato leaves *in vitro*. Means followed by the same letter are not significantly different at the $P < 0.05$; nd - not determined due to too small explants.

| | Favorita | | | | | Helanwuhua | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 0.2 | 2.0 | 20.0 | 50.0 | 0 | 0.2 | 2.0 | 20.0 | 50.0 |
| Leaf number | 8.33a | 9.00a | 9.67a | 10.33a | 9.00a | 7.33b | 8.00b | 10.00a | 3.00c | 1.33d |
| Shoot length | 4.43c | 6.27b | 8.07a | 4.23c | 0.70d | 7.50a | 8.80a | 8.83a | 0.80b | 0.20b |
| Shoot FM | 0.05bc | 0.06b | 0.15a | 0.06bc | 0.02c | 0.10b | 0.12b | 0.21a | 0.03c | 0.01c |
| Root FM | 0.09c | 0.10b | 0.12a | 0.02d | 0.00c | 0.10b | 0.12a | 0.12a | 0.02c | 0.00c |
| Root number | 4.00bc | 6.00b | 7.67a | 3.33bc | 0.00c | 5.67b | 8.67ab | 12.00a | 0.67c | 0.00c |
| Root length | 13.20b | 13.13b | 16.37a | 1.70c | 0.00c | 8.90b | 14.50a | 13.37a | 0.37c | 0.00c |
| Chl content | 0.28a | 0.26b | 0.21c | 0.07d | 0.02e | 0.280a | 0.22b | 0.23b | 0.15c | 0.08d |
| POD activity | 17.17b | 19.34a | 15.58c | 7.16d | nd | 11.16b | 12.65a | 9.31c | 8.77c | nd |

Table 2. Effect of different concentrations of JA on number of microtubers, FM of microtubers and percentage of big microtubers formed *in vitro*. Means followed by the same letter are not significantly different at the $P < 0.05$.

| | Favorita | | | | | Helanwuhua | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 0.2 | 2.0 | 20.0 | 50.0 | 0 | 0.2 | 2.0 | 20.0 | 50.0 |
| Microtuber number [flask$^{-1}$] | 11.30a | 11.50a | 11.50a | 10.80a | 10.70a | 26.00a | 23.50a | 16.50b | 16.30b | 16.70b |
| FM of microtubers [g flask$^{-1}$] | 2.18a | 1.75b | 1.73b | 1.73b | 1.54b | 2.19a | 1.94a | 0.88b | 0.67b | 0.71b |
| Big microtubers [%] | 71.41a | 55.86b | 51.89b | 50.56b | 42.35b | 44.23a | 41.92a | 15.38b | 11.54b | 12.00b |

microtubers in Favorita, while no difference with regard to number of microtubers per flask was observed between JA treatments and the control (Table 2). A significant decrease in the number of microtubers, fresh mass and percentage of big microtubers was observed in Helanwuhua treated with JA of 2.0 - 50 mg dm$^{-3}$. No significant difference with regard to these three parameters was observed between 0.2 mg dm$^{-3}$ JA and the control.

Morphological and histochemical studies using light microscopy and transmission electron microscopy (TEM) analysis of leaves from treated plants revealed that JA also affected subcellular organelles of mesophyll cells (Ulloa *et al.* 2002). The results of Sembdner and Parthier (1993) showed that jasmonate-induced promotion of leaf senescence, which was characterized by chlorophyll degradation and accompanied by degradation of RuBPcase, increases in cellular respiration rate, and of protease and peroxidase activities as well as a reduction of photosynthetic activity. In this study, peroxidase activities were measured in view of the possible involvement of jasmonates in plant multiple defense responses (Repka 2001, Repka *et al.* 2004). This experiment also found that the chlorophyll content decreased with the increasing of JA concentration, and lower JA concentration promoted POD activity significantly. However, higher JA concentration reduced the POD activity, which might be speculated that the cell organization had been damaged by applying higher level of JA. By contrast, substantially higher concentration of methyl jasmonate still stimulated POD activity in grapevine (Repka *et al.* 2004). This might be due to the difference in their responses to jasmonates between herbaceous and woody plant species, and further research is needed in this aspect.

Formation of potato tuber was affected by many factors such as photoperiod and temperature, but hormone played a dominant role in this process (Vreugdenhil and Struik 1989, Momoh *et al.* 2002, 2004, Tang *et al.* 2003, Zhang *et al.* 2005b). Contrary views still existed for the induction of JA on potato tuberization. Some researchers considered that jasmonates and gibberellins (GA) were the important components of the signal transduction pathways in regulating potato plant morphogenesis and controlling tuber induction (Takahashi *et al.* 1994, Abdala *et al.* 2002). The results of Koda and Kikuta (2001) suggested that not only GA but also JA were the key factors determining potato maturity and either a higher level of GA or lower level of JA may predispose the cultivars to have a late-maturing habit (maturity). However, other researchers suggested that JA spraying did not induce tuberization in short-day-requiring potato species kept in non-inducing conditions (Jackson and Willmitzer 1994), and different levels of JA itself did not control the tuberization (Ulloa *et al.* 2002). In our work, the microtuber number, fresh mass and the percentage of big microtubers of two potato cultivars did not increase significantly by the treatment of different levels of JA. These results indicated that the application of 0.2 - 50 mg dm$^{-3}$ JA did not promote the *in vitro* tuberization of potato cvs. Favorita and Helanwuhua.

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