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Stimulatory Effects of Jasmonic Acid on Potato Stem Node and Protoplast Culture

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Abstract. The effect of jasmonic acid (JA), in a wide range of concentrations (0.01–100 μM), on the development of potato plantlets (*Solanum tuberosum* L.cv. *Vesna*) was investigated in order to discriminate between physiological and supraoptimal effects of this growth regulator in vitro. Concentrations from 0.1–1 μM significantly increased the length of developed plants. Application of JA in these concentrations resulted in a very well differentiated root system with many lateral branches. With increasing JA concentrations, the main roots shortened and thickened. Concentrations higher than 10 μM led to the compaction of the stem, roots, and root hairs, giving a stunted appearance to the plants. The effect of JA on cell wall regeneration and callus formation was observed in potato leaf protoplast culture. JA at concentrations from 0.01–1 μM stimulated cell division and microcalli development.

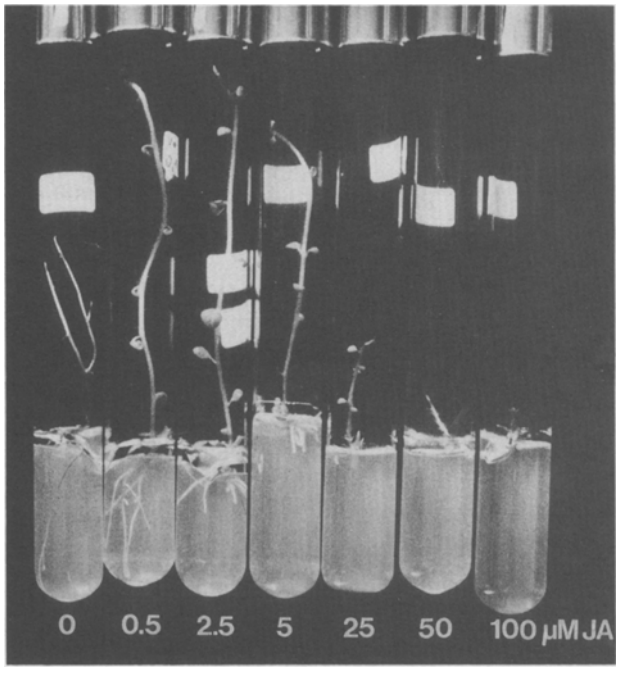

Fig. 1. The influence of JA on bud and root development after 2-week culture of stem nodes.

Jasmonic acid (JA) and other jasmonates are considered to be a new group of plant growth regulators widely distributed within the plant kingdom (Meyer et al. 1984). In the last 10 years, different physiological activities of JA have been described (Sembdner et al. 1989 and references therein). In particular, the effect of JA as an accelerator of leaf senescence has been well documented (Parthier 1990).

Recently, Weidhase and coworkers (1987) proved that jasmonates induce the synthesis of specific polypeptides in barley. Similar polypeptides were also detected in a large number of other plant species (Herrmann et al. 1989). Furthermore, jasmonic acid induces the expression of vegetative storage protein genes (Anderson et al. 1989, Staswick 1990). Anderson and coworkers (1989) found JA effective in increasing VSP levels in cell cultures of soybean at concentrations as low as 1 μM, without reducing the amount of chlorophyll per cell. They suggested that the senescence-inducing properties of JA may be due to the use of toxic concentrations. However, incorporation of JA in the meristem growth induction medium in concentrations of 0.5–10 μM increased the number of potato meristems which developed buds without senescent symptoms (Ravnikar and Gogala 1990). Therefore, we tentatively agree with Anderson and coworkers (1989) that concentrations usually used in JA experiments (10–100 μM) are supraoptimal.

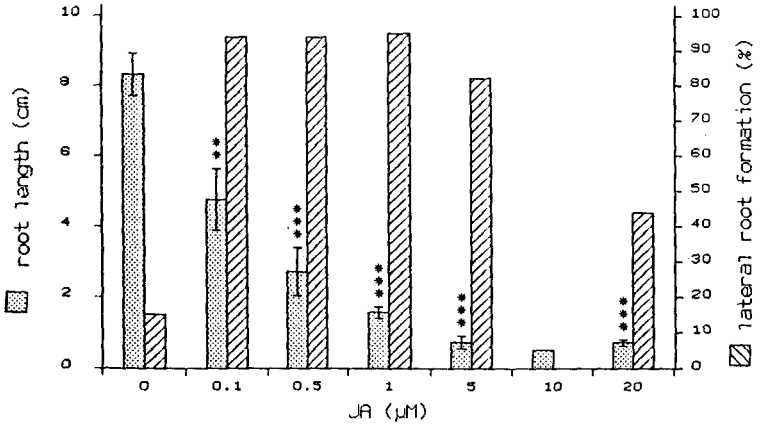

Fig. 2. The effect of JA on the length of the main root ⊡ and the percentage of nodes with lateral root formation ▨ after 2-week culture of stem nodes.

The aim of the work reported in this paper was, therefore, to determine the effects of physiologically meaningful concentrations of JA on development of potato axillary buds and adventitious roots in stem node culture. A wide concentration range was added to the culture medium in the absence of any other phytohormone; the emphasis was placed on concentrations below 10 μM.

Considering the high concentration of endogenous JA in young and rapidly dividing plant tissues (Knöfel et al. 1984, Sembdner and Klose 1985), our aim was also to establish how JA influences cell wall resynthesis and cell division in potato protoplast cultures.

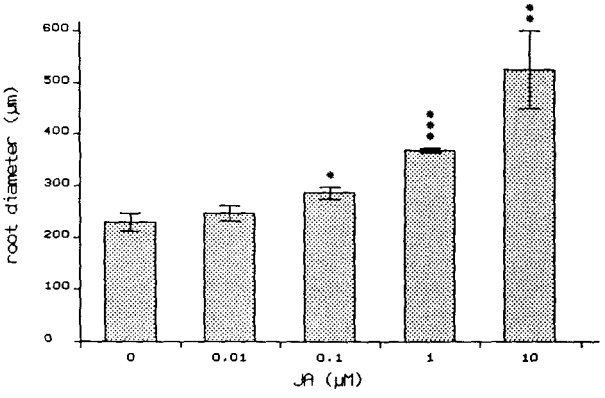

Fig. 3. The influence of JA on the main root diameter after 4-week culture of stem nodes.

Materials and Methods

Stem Node Culture

Stem cuttings with one leaf node were taken from 8-week-old healthy plants (*Solanum tuberosum* L. cv. *Vesna*), which were propagated in vitro. Murashige and Skoog medium (1962) was supplemented with jasmonic acid (±)JA (SA Firmenich, Geneva, Switzerland) in concentrations of 0.01–100 μM. Medium without JA was used as the control medium. All media were adjusted to pH 5.7–5.8 before autoclaving.

Cultures were kept at 25 ± 2°C, with a photoperiod of 16 h at 5.5–12.0 Wm^{-2} (Sylvania Gro Lux F40T12 and fluorescent LV 20 lights).

Axillary buds and adventitious roots developed on the stem cuttings. Every 2 weeks the lengths of shoots and main roots were measured, while stem nodes and main and lateral roots were counted.

After 3–4 weeks of node culture, roots were cut off and fixed in 0.1% glutaraldehyde in 0.1 M phosphate buffer (pH 6.9) for 1 h, dehydrated in graded ethanol series, followed by methyl ethanol and xylol. They were then embedded in wax, and cut through the middle of the main root. Wax was eliminated with xylol. Afterward, the roots were dried to the critical point (Boman-900 EX) and sputter-coated with gold. They were examined on a Jeol JSM-840A scanning electron microscope. The diameter of the main root without root hairs was measured.

Student's *t*-test and a χ^2 contingency table (2 × 2) with Fisher's correction (De Fossard 1976) were used to calculate the levels of significant differences between the control medium (without JA) and media supplemented with JA. Values were significantly different at $p = 0.05$ (*), $p = 0.01$ (**), and $p = 0.001$ (***). Twenty stem nodes were used for each concentration of JA and the control medium; all experiments were repeated three or more times.

Protoplast Culture

Protoplasts were isolated from leaves of 11-week-old plants, grown on modified MS medium (Murashige and Skoog 1962), without added plant hormones at 20 ± 1°C, illumination of 10 Wm^{-2} and a photoperiod of 16 h. Cut-off leaves were incubated in enzyme medium: 0.1% macerozyme, 0.5% cellulase, macroelements of medium A (Shepard and Totten 1977), 0.3 M sucrose, 0.1 M mannitol, 5 mM MES. After 14–16 h of incubation on a shaker at 24 ± 2°C in the dark, the degraded tissue was first poured through a crude nylon net and then through a combined 115 μm/60 μm nylon filter (Nybolt PA-115/32, Nybolt PA-60/33).

Protoplasts were purified by repeated centrifugation (10 min, 100 *g*) in a flotation medium: macroelements of medium A (Shepard and Totten 1977), 0.3 M sucrose, 5 mM MES. Viability of

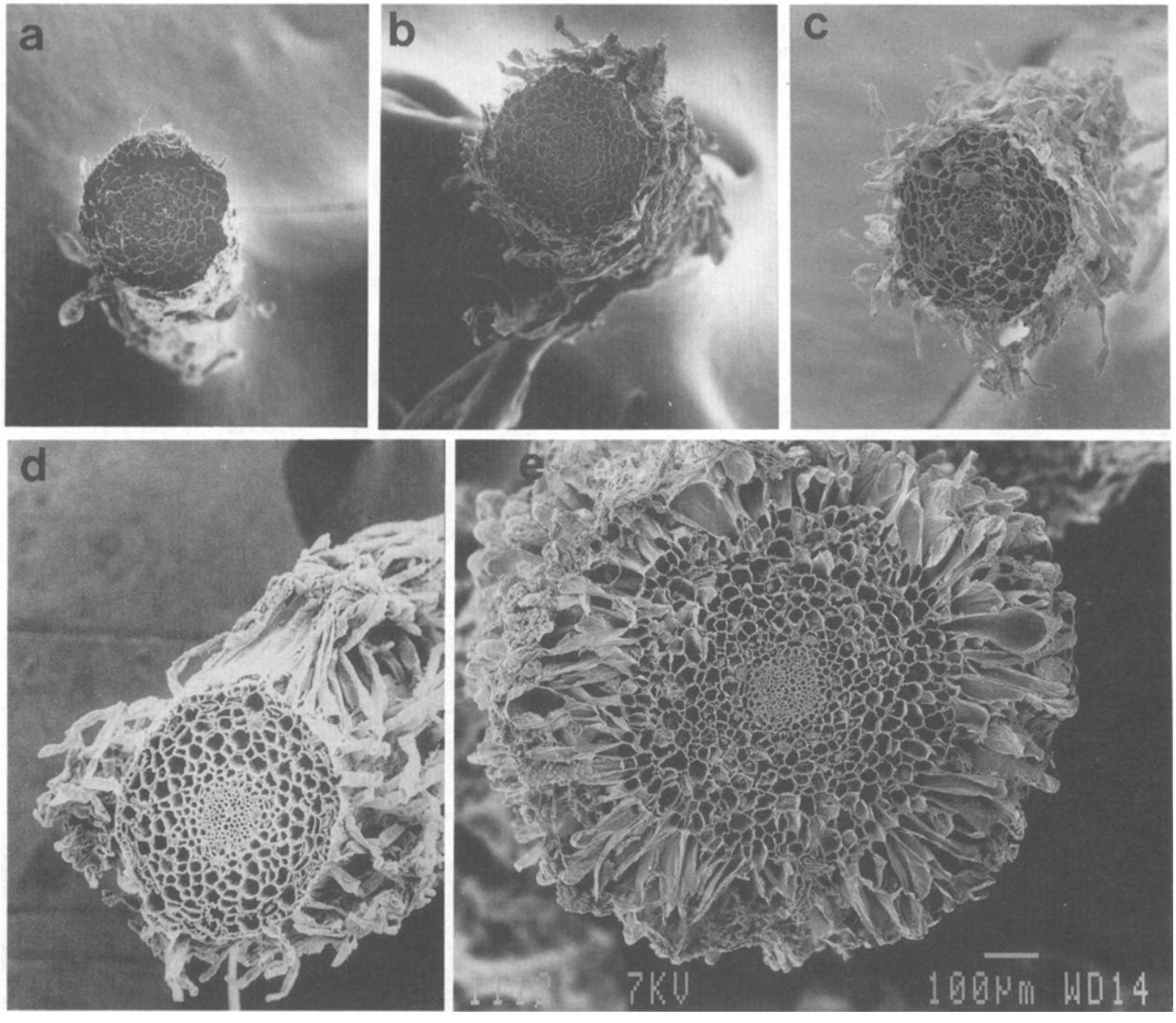

Fig. 4. The increase of main root diameter under the influence of JA after 4-week culture. **(a)** Control medium (without JA); **(b)** medium with 0.01 µM JA; **(c)** medium with 0.1 µM JA; **(d)** medium with 1 µM JA; **(e)** medium with 10 µM JA.

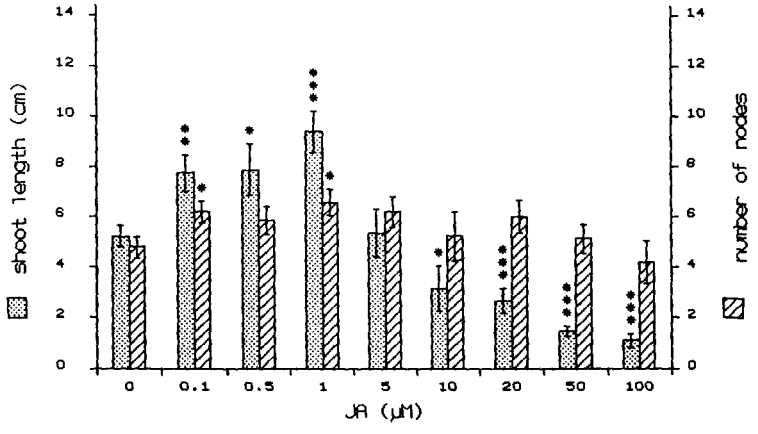

Fig. 5. The influence of JA on shoot length ⊡ and number of nodes ▨ after 4-week culture.

isolated protoplasts was determined by vital staining with 0.01% fluorescein diacetate (FDA). The protoplasts were diluted in modified liquid medium B (Shepard and Totten 1977) to a final concentration of 5×10^4 protoplasts per milliliter.

Culture media contained 2.2 μM BA (6-benzyladenine) and 4.5 μM 2,4-D (2,4-dichlorophenoxyacetic acid) and were supplemented with different concentrations of (±)JA (0, 0.01, 0.1, 1 μM). The pH of all media was adjusted to 5.7 before sterilization. Protoplast cultures were maintained in the dark at 24 ± 2°C. After 6 days, ¼ volume of fresh medium was added, and the cultures were exposed to a 16 h photoperiod.

Samples of cultured protoplasts were taken for observation of cell wall synthesis and first cell divisions after 5 days of incubation. At day 10, the percentage of developed microcalli (aggregates of three or more cells) was estimated. Samples were observed by light microscopy. Cell wall resynthesis was probed by the addition of 0.001% calcofluor white (CF-fluorescent brighter). The experiment was repeated twice.

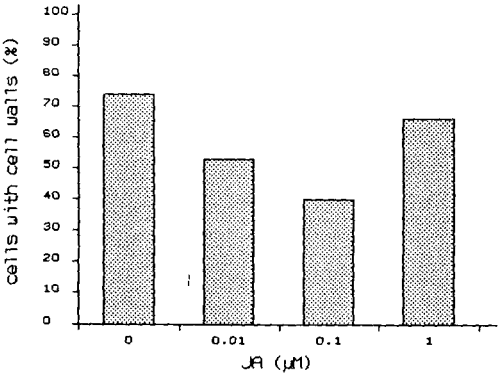

Fig. 6. Percentage of cell wall formation in total protoplast populations after 5 days of culture in media with different concentrations of JA, observed after treatment with CF.

Results

Stem Node Culture

After 2 weeks of stem node culture, large differences could be observed in explants grown on the control medium or on the medium supplemented with JA (Fig. 1). Dramatic morphological changes appeared on roots. With increasing concentrations of JA, the length of the main roots shortened (Fig. 2) and their diameter increased (Fig. 3), while root hairs gradually shortened and multiplied. JA concentrations ≥10 μM caused, in the main root, an increased cell number and the disorganization of the cell layers (Fig. 4). After 4, 6, and 8 weeks, the relations between the length and the diameter of roots grown on the control medium or on the media supplemented with different concentrations of JA sustained constantly. While the longitudinal growth of the main root was reduced, lateral root branching was induced by JA (Fig. 2). A negative correlation between the number of main roots and the number of lateral roots could be observed. Concentrations higher than 10 μM inhibited formation of both the main and the lateral roots.

The shoot length significantly increased at concentrations of 0.1–1 μM JA (Fig. 5). The addition of JA at concentrations higher than 10 μM resulted in stunted plantlets, although the number of nodes did not differ from the control plantlets.

Protoplast Culture

Isolated protoplasts were incubated in liquid medium for 5 days, and thereafter were checked by light microscopy. The regeneration of cell walls was delayed at concentrations of 0.01 and 0.1 μM JA, compared to protoplasts incubated in the control medium and in the medium containing 1 μM JA (Fig. 6).

After 10 days of culture, all viable cells in all media had formed cell walls. JA-stimulated cell divisions, especially in media with 0.01 and 0.1 μM JA, resulted in the highest number of microcalli. In the control medium, some cell divisions (but very few microcalli) were observed (Fig. 7).

Discussion

It is obvious that JA in concentrations from 0.01–1 μM stimulates the development of axillary buds and adventitious roots on potato stem cuttings. Even concentrations of JA as low as 0.01 μM significantly increase lateral branching of roots and elongation of axillary buds. Stimulation of shoot growth was previously observed on grapevine stem node culture (Ravnikar et al. 1990).

Inhibition of cytokinin-induced callus formation by JA is found in soybean callus culture (Ueda and Kato 1982), in potato meristem culture (Ravnikar and Gogala 1990), and in bean meristem culture (Ravnikar et al. 1990). The retardation of shoot and root growth at higher concentrations does not seem to be the consequence of reduction in cell divisions in view of the rather constant number of nodes. Histological examinations of roots decreased in length (Fig. 4) showed marked cell multiplication in diameter. The reduction of the shoot elongation processes and the loss of the chlorophyll at concentrations higher than 10 μM of JA (Parthier 1990), therefore, are due to the supraoptimal concentrations applied.

Evidence of a stimulatory effect of JA is also confirmed in protoplast culture. In our experiments, we observed the effect of JA on the development of

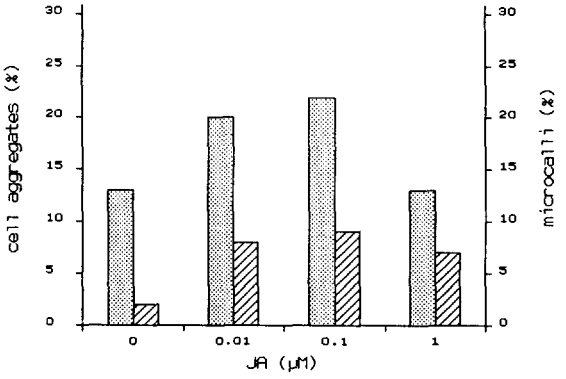

Fig. 7. Percentage of cell divisions and microcalli on media containing different concentrations of JA after 10 days of culture is shown in the left column. Percentage of microcalli (aggregates of three or more cells) in the same samples is shown separately in the right column.

protoplasts in combination with other growth regulators in the media (2.2 μM BA and 4.5 μM 2,4-D). However, very low concentrations of added JA (0.01 and 0.1 μM) resulted in an increase in the frequency of cell division and microcallus formation.

Low concentrations of JA (0.01 and 0.1 μM) cause a delay in cell wall resynthesis in the first days of protoplast culture. This might be due to previously observed direct or indirect effects of JA on physiological processes in plants, such as its influence on several enzyme activities (see Parthier 1990 for references), changes in gene expression and protein synthesis patterns (reviewed by Staswick 1990), and alterations of biophysical properties of protoplast membranes (Vilhar et al. 1991).

Our results suggest that endogenous JA may play an important role in the regulation of plant development, and that it might coregulate cell division in rapidly dividing tissues.

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