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BRIEF COMMUNICATION

Jasmonic acid-induced morphological changes are reflected in auxin metabolism of beans grown *in vitro*

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Abstract

Auxins were analysed in roots, stems with apical shoots, and leaves of beans (*Phaseolus vulgaris* L. cv. Zorin) cultured for three weeks on media supplied with different concentrations of jasmonic acid (JA). Morphology changes induced by applied JA were reflected in tissue-specific changes of auxins.

Additional key words: free indole acetic acid, IAA-conjugates, *Phaseolus vulgaris*.

Jasmonates and auxins have been shown to control various aspects of plant growth and development. Some effects of both plant growth regulators are similar. For example, auxins appear to be the primary phytohormones involved in forming adventitious roots, but they inhibit the growth of main roots (Cleland 1999), as was demonstrated for jasmonic acid (JA) in potato node cultures (Ravnikar *et al.* 1992). Both JA and IAA (indole-3-acetic acid) promote the growth and division of cells, and have also been implicated in microtubulus rearrangement, the important step in cell elongation (Ravnikar *et al.* 1992, Leyser 1998). These data indicate a relationship between JA and auxins in regulating the growth and development of plants. In the present study we aimed to evaluate the morphological effects of exogenously applied JA in relation to endogenous auxin metabolism.

Pathogen free bean (*Phaseolus vulgaris* L. cv. Zorin) apical shoots, isolated from 12 to 14-d-old seedlings, were grown on MS medium supplemented with 1, 10 and 100 μM jasmonic acid (*Apex Organics*, Devon, UK). Medium without jasmonic acid (JA) was used as the control. Cultures were kept at 23 ± 2 °C, with a 16-h photoperiod, irradiance of 55 - 70 μmol m^{-2} s^{-1}. After 3 weeks, the plants were divided into leaves, stems with apical buds and roots. The plant material was homogenised in liquid nitrogen, freeze-dried and stored at -20 °C.

The lyophilised powder (generally 100 mg), supplied with antioxidant butylated hydroxytoluene (BHT), was extracted in 6 cm^3 of 5 mM K-phosphate buffer, pH 6.5. After 1 h at 4 °C, the extract was filtered and the volume adjusted with the same buffer to 30 cm^3. For the purification of auxins with *Sep-Pak* C_{18} columns (*Waters*, Milford, USA) we used the modified method of Nordström and Eliasson (1991) as described in Benedičič *et al.* (1999). The 10 cm^3 of sample extract were first run through the column conditioned at pH 6.5 and washed with 6 cm^3 of 5 mM K-phosphate buffer, pH 6.5. The eluate was acidified with 1 M H_3PO_4 to pH 2.5 and applied to a second column conditioned at pH 2.5. The column was rinsed with 2 cm^3 of double-distilled water and eluted with 2 cm^3 of 80 % methanol.

The concentrated eluate (0.4 cm^3) was analysed by HPLC (*Waters*) on a C_{18} column (*Nova-Pak*, 3.9 × 150 mm, *Waters*). Solvent A was 1 % acetic acid; solvent B was 100 % methanol. The flow rate was 1 cm^3 min^{-1}. The column was eluted using a linear gradient of 10 to 55 % methanol over 30 min. A fluorescence detector (*Waters 474 Scanning Fluorescent Detector*, excitation at 254 nm, emission at 360 nm) and photodiode array detector (*Waters 996*, absorption at 280 nm) were used for detecting auxins. They were quantified by fluorimetry, comparing the peak areas with those of known amounts of IAA and indole-3-acetyl aspartic acid (IAA-Asp). The detection limit for IAA was about 4 ng. Recoveries were

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Abbreviations: IAA - indole-3-acetic acid; IAA-Asp - indole-3-acetyl aspartic acid; JA - jasmonic acid.
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determined by adding 833 Bq [^{3}H]IAA (0.56 - 1.13 TBq mmol^{-1}, *Amersham*, UK) before extraction. Losses were also evaluated by nonradioactive standards that had gone through the whole process of extraction and pre-purification. The IAA quantities reported were corrected for losses during work-up. The calculated auxin contents are the means of two to three replicate measurements. Student's test was used to assess the significance of differences between plants grown on control medium and on media supplemented with JA.

The HPLC fractions containing IAA were evaporated to dryness and dissolved in 0.050 cm^3 ethrel diazomethane. Analysis of IAA was carried out using *AutoSpecQ* mass spectrometer (*Micromass*, Manchester, UK) coupled with HP 5890 series gas chromatograph (*Hewlett-Packard*, Waldbronn, Germany). Gas chromatography was performed on a *HP-5MS* (30 × 0.25 mm) fused silica capillary column. Injection was at 250 °C following a temperature program from 160 to 270 °C in 8 min. Mass spectrometer conditions were: GC/MS transfer line temperature and ion source temperature 250 °C, ionisation 70 eV and source electron current 150 μA.

IAA conjugates were hydrolysed under nitrogen with 1 M NaOH at room temperature for 1 h for ester-linked IAA, or 7 M NaOH at 100 °C for 3 h, for amide-linked conjugates (Bialek and Cohen 1992). Biologically active auxins were determined by the oat coleoptiles bioassay (Larsen 1961).

The effect of JA on the growth of bean shoot cultures was concentration dependent. The plants grown on 10 μM JA had a higher number of shorter roots with more lateral roots than the control plants. On the other hand, application of 100 μM JA to the medium resulted in a 73 % reduction of the growth of stems and 77 % reduction of leaves while the growth of the roots was completely inhibited.

In all tissue analysed, IAA and four of its derivatives were detected: IAA itself (retention time 21 min), IAA-Asp (retention time 14 min) and the substances eluted at the 9th, 13th and 18th min (Fig. 1). The peaks with retention time of 9, 13 and 18 min had auxin-like spectra. Strong alkaline hydrolysis indicated that the substance with retention time 13 min had the characteristic of free auxins and that the substances, which eluted at the 9th and 18th min were amide-linked conjugates. They were also biologically active. According to several other studies, certain exogenously applied IAA conjugates mimic IAA in several bioassays, which suggests either that they are themselves auxins, or that the plant or tissue hydrolyses them to release free IAA (reviewed in Bartel 1997). In all bean tissue analysed, amide conjugates constituted more than 90 % of the auxins. These results are in accordance with others who found that in legumes amide conjugates predominated (reviewed in Bialek *et al.* 1992). In our study ester-linked conjugates were not detected. The largest amount of IAA-Asp was present in roots while the largest amount of free IAA was detected in the leaves (Fig. 1, Table 1). IAA-Asp is one of the more prevalent IAA conjugates in plants. It may serve less as a storage form of IAA than as a precursor for irreversible oxidation to IAA in catabolic pathways (reviewed in Normanly and Bartel 1999). Generally, the highest contents of IAA are found in regions of active cell division, but young leaves

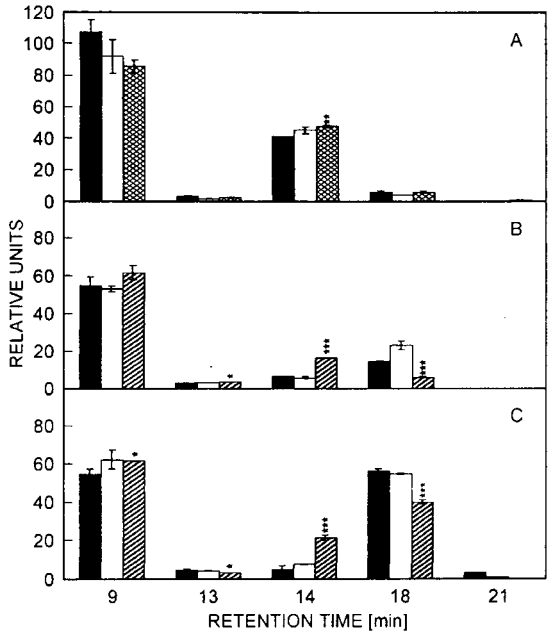

Fig. 1. Relative amounts of auxin-like substances separated by HPLC and detected with fluorescence detector in roots (*A*), stem with apical buds (*B*) and leaves (C) of control and jasmonic acid (JA) treated bean plants (*black columns* - control, *empty columns* - 1 μM JA, *checked columns* - 10 μM JA, *diagonal lines* - 100 μM JA. IAA-Asp and IAA, retention time 14 and 21 min respectively. Means of two or three replicates ± SD. Differences significant at: * - $P < 0.05$, ** - $P < 0.01$, *** - $P < 0.001$.

Table 1. The amount of endogenous IAA-Asp and IAA in different organs of control and JA-treated bean plants. Means of two to three replicates ± S.D. Differences significant at: ** - $P < 0.01$, *** - $P < 0.001$.

Organs	JA [μM]	IAA-Asp [ng g^{-1}(d.m.)]	IAA [ng g^{-1}(d.m.)]
Roots	0	82.8 ± 0.07	< 0.4
	1	92.1 ± 4.51	< 0.4
	10	95.4 ± 1.62**	0.7 ± 0.11
Stems	0	13.2 ± 0.16	< 0.4
	1	11.1 ± 3.33	< 0.4
	100	33.3 ± 0.07***	< 0.4
Leaves	0	10.9 ± 3.38	1.8 ± 0.08
	1	16.4 ± 0.25	0.5 ± 0.02
	100	42.6 ± 2.85***	< 0.4

are another rich source of IAA (reviewed in Cleland 1999, Kowalczyk and Sandberg 2001). The low amount of IAA in the roots could be the result of growth conditions in tissue culture. In the bean seedlings grown in non-sterile conditions in vermiculite, we have detected the same types of auxins but in higher amounts than in beans grown *in vitro* (Benedičič *et al.* 1999).

Application of 1 μM JA, which did not change the morphology of plants, had no effect on the amount of auxin-like substances, while application of 10 μM JA, which increased the roots number, resulted in a significant increase of IAA-Asp and IAA in root tissue (Fig. 1, Table 1). The role of auxins in lateral root development has been demonstrated in many studies (reviewed in Nag *et al.* 2001). Treatment with 100 μM JA, which resulted in the reduced growth of bean stem and leaves, was also reflected in auxin metabolism. Under this growth condition the increase of IAA-Asp and the decrease of the amide-linked IAA conjugate eluting at 18^{th} min was observed in both green tissues while in the leaves a decrease of free auxins was also detected.

Applied JA could affect auxin metabolism through multiple pathways. Titarenko *et al.* (1997) has found that one of the genes induced by JA (*JR3*), was 96 % identical with *IAR3*, which encoded an IAA amide-hydrolase. In Chinese cabbage, JA was supposed to be involved in the upregulation of three enzymes (tryptophan oxidizing enzyme, nitrilase, myrosinase) important for IAA synthesis (Grsic *at al.* 1999). The other possible but more indirect mode of JA effect on auxins might be through other hormones. JA increased ethylene synthesis, which is also supposed to be involved in adventitious root formation and root growth inhibition (reviewed in Cleland 1999). In potato, JA induced changes in cytokinin metabolism (Dermastia *et al.* 1994). The ratio between auxins and cytokinins is also an important factor for shoot and root formation in tissue cultures.

In conclusion, morphology changes induced in beans grown *in vitro* by applied JA, were also reflected in tissue-specific changes of IAA metabolism. JA may act more directly, for example on gene expression and enzyme activation involved in auxin metabolism and its transport, and/or in more indirect ways by interaction with other hormones.

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