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Morphology of Potato (*Solanum tuberosum* L. cv. Sante) Stem Node Cultures in Relation to the Level of Endogenous Cytokinins

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Abstract. Stem node culture of the potato (*Solanum tuberosum*) cv. Sante was used to examine the phenotypical alterations due to different levels of endogenous cytokinins. The altered phenotype, which dramatically deviates from the control phenotype, was induced after treatment of plantlets with 1 μM jasmonic acid. Plantlets grown on the medium supplemented with jasmonic acid were taller, with well developed root systems, expanded leaves, thickened stems, and they showed hyperhydric symptoms. Their cytokinin content was about half that of the control plantlets. Morphologic characteristics corresponding to transgenic plants that overproduce cytokinins, including release of axillary buds and inhibited rooting, correlated with the high cytokinin levels in control plants.

Key Words. Cytokinins—Jasmonic acid—Morphology—Potato

The effect of cytokinin activity on the plant morphology is based mainly on experiments with exogenous cytokinins or on transgenic plants that overproduce cytokinins. But direct correlations between endogenous cytokinin levels and different phenotypes of untransformed plants have not been demonstrated.

Recently, we reported some morphologic features and cytokinin determinations of potato stem node cultures of cv. Ulster Sceptre and cv. Vesna (Dermastia et al. 1994a, 1994b). The potato cultivar Sante exhibits in vitro a phenotype that differs consistently from those of Ulster Sceptre and Vesna. Moreover, it shows many characteristics of transgenic plants that carry the cytokinin biosynthetic *ipt* gene from T-DNA of *Agrobacterium* and have elevated cytokinin levels. Such transgenic plants exhibit reduced apical dominance, reduction of height, inhibition of root formation, increased chlorophyll content, and delayed senescence (Brzobohatý 1994 and references therein).

Tissue culture experiments have clearly shown that potential of plant growth regulators provided in the medium to alter greatly the developmental fate of cells (Singh et al. 1992, Skoog and Miller 1957). Indeed, after treatment with the plant growth regulator jasmonic acid, dramatic phenotypical changes occurred in plantlets of cv. Sante (Kovač and Ravnikar 1994). Two different morphologic potato forms at the same developmental stage provide an appropriate system for examining the relationship between growth habit and cytokinin content. In the present paper we report that alterations in morphology correlate with changes in endogenous cytokinin concentration.

Materials and Methods

Stem Node Culture

Potato plants (*Solanum tuberosum* L. cv. Sante) were propagated in vitro by stem node cuttings. Murashige and Skoog (1962) medium was supplemented with 1 μM (±) jasmonic acid (JA), (Apex Organics, UK). Medium without JA was used as the control. All media were adjusted to pH 5.7–5.8 before autoclaving. Cultures were kept at 20 ± 2°C, with a photoperiod of 16 h at 50 μmol m^{-2} s^{-1} (Osram L18W 20 lamps).

Morphometric Measurements

Leaf area was measured as the product of lamina length and width at the widest part. Stem diameters were measured at the sixth internode using a stereomicroscope.

Abbreviations: JA, jasmonic acid; Z, *trans*-zeatin; ZR, *trans*-zeatin riboside; ZRMP, zeatin riboside 5′-monophosphate; Z-9-G, *trans*-zeatin *N*-9-glucoside; DHZ, dihydrozeatin; DHZR, dihydrozeatin riboside; DHZRMP, dihydrozeatin riboside 5′-monophosphate; DHZ-9-G, dihydrozeatin 9-glucoside; iP, *iso*-pentenyladenine; iPA, *iso*-pentenyladenosine; iP-9-G, *iso*-pentenyladenine 9-glucoside; HPLC, high performance liquid chromatography.

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Cytokinin Isolation and Determination

After 4 weeks of culture in vitro, whole plantlets were frozen and processed further exactly as described (Dermastia et al. 1995, Dermastia and Ravnikar 1995, Kovač and Žel 1995). Briefly, the cytokinin metabolites were isolated and analyzed by a modified immunoaffinity chromatographic system (Nicander et al. 1993). The polyclonal antibodies raised against *trans*-zeatin riboside- and *iso*-pentenyladenosine-bovine serum albumin conjugates used for the immunoaffinity purification bound the following cytokinins: Z, ZR, ZRMP, Z-9-G, DHZ, DHZR, DHZRMP, DHZ-9-G, iP, iPA, and iP-9-G, if the gel binding sites were present in a large excess compared with the sample size. Samples were applied to a HPLC Supelcosil LC 18 DB column (250 × 4 mm, 5-μm spherical particles) in a starting buffer of 1 mM triethylamine (pH 7), containing a 10% mixture of methanol:acetonitrile (1:1, v/v). The elution was performed by increasing the concentration of the organic solvent to 20% over 25 min, followed by an increase from 20 to 30% for 30–40 min. The retention times (in min) for nine cytokinin standards were checked (ZRMP and DHZRMP, 10.54; Z-9-G, 16.1; DHZ-9-G, 17.8; Z, 21.7; DHZ, 23.2; ZR, 27.9; DHZR, 30.3; iP-9-G, 42.3; iP, 51.5; iPA, 55.2), and the corresponding HPLC fractions were collected for further identification. HPLC peaks were quantified by integrating the areas at 265 nm and compared with the areas of known standard quantities. Recoveries were determined by adding [^{3}H]iPA to the extract buffer before homogenization and determining radioactivity by liquid scintillation counting. Recoveries of 60–70% were obtained after elution of the immunoaffinity columns and HPLC separation. No correction was made for the losses.

The modified *Amaranthus* bioassay (Biddington and Thomas 1973, Kovač and Žel 1994, 1995) was used for confirming free bases and ribosides following HPLC separation, but there was little or no correlation between the cytokinin concentration calculated from HPLC peak area and that estimated from bioactivity. The bioactivities of DHZR and iPA were detectable, but they were evidently underestimated. In the case of Z-9-G, DHZ-9-G, and iP-9-G there were no responses to the *Amaranthus* bioassay. Thus, the purity of HPLC fractions corresponding to the retention times of 9-glucoside standards was estimated by scanning the interval between 220 and 300 nm with a Hewlett Packard 8452A diode array spectrophotometer (Nicander et al. 1993). The sample spectra were compared further with the spectra of the standards.

Results and Discussion

Qualitative determinations have demonstrated that two cytokinin-free bases Z and DHZ and their ribosides and 9-glucosides are generally present in potato tissues (Dermastia et al. 1994b, 1995, Dermastia and Ravnikar 1995), and they were also shown in the plantlets grown from stem node cultures of cv. Sante. In agreement with results using plantlets of cv. Ulster Sceptre (Dermastia et al. 1994b), iP was not detected, but its riboside and 9-glucoside forms represented a great part of all cytokinins detected (Table 1). ZR was the most abundant cytokinin identified in Sante tissue culture, although it accounted for only 15% of detected cytokinins in cv. Ulster Sceptre (Dermastia et al. 1994b). ZR has been demonstrated as principal cytokinin metabolite in potato by earlier studies of Turnbull and Hanke (1985) using radioimmunoassay combined with HPLC for the analysis of zeatin-type cytokinins. They also detected some

Table 1. Concentration (pmol g^{-1}) and distribution (%) of cytokinins in control and JA-treated potato plantlets. Concentration results are the mean of three independent experiments ± S.E. BD, below detection limit. The quantitation is based on the integration of HPLC peaks areas at 265 nm.

	Control		JA treated	
	Concentration	%	Concentration	%
Z	10.0 ± 2.7	2.8	5.9 ± 1.8	2.9
ZR	164.2 ± 11.7	46	64.6 ± 17.4	32.6
Z-9-G	21.6 ± 4.3	6	9.3 ± 0.3	4.7
DHZ	9.5 ± 5.0	2.7	11.7 ± 6.3	5.9
DHZR	17.5 ± 8.8	4.9	16.4 ± 0.3	8.2
DHZ-9-G	35.5 ± 1.5	9.9	21.3 ± 3.8	10.7
iP	BD		BD	
iPA	55.8 ± 13.7	16	36.4 ± 11.6	18.3
iP-9-G	44.2 ± 14.4	12	32.7 ± 2.9	16.5
Total concentration	358.3 ± 22.3		198.3 ± 17.6	

Z-9-G, which was identified unambiguously in immunoaffinity-purified extracts of *Solanum* sprouts by gas chromatography-mass spectrometry, as well (Nicander et al. 1993). Their findings are consistent with our determinations. There are also reports about the presence of nucleotides in potato roots, underground stems, tubers (Turnbull and Hanke 1985) and sprouts (Nicander et al. 1993). However, in stem node culture of potato cv. Sante the HPLC sample peak area corresponding to the retention times of nucleotide standards ZMP and DHZMP were high, but their UV spectra were not clear. The responses of these fractions to *Amaranthus* were much lower, as reported for nucleotides (Kovač and Žel 1994). Regarding sufficiently high cross-reactivity of the antibodies used in our immunoaffinity chromatographic system with nucleotides, it is most likely that those substances are present in the above-mentioned fractions.

The plantlets of cv. Sante grown on the control medium contained an about twofold higher concentration (i.e. 358 pmol/g dry weight) of cytokinins (Table 1) compared with those reported in cv. Vesna (i.e. 161 pmol/g dry weight) (Dermastia et al. 1994a) or cv. Ulster Sceptre (i.e. 185 pmol/g dry weight) (Dermastia et al. 1994b). The plantlets of cv. Sante had smaller leaves, poorly developed root systems (Fig. 1*B* and Table 2), and a higher chlorophyll concentration (Kovač and Ravnikar 1994). They showed pronounced sprouting of the axillary buds after 7 weeks of culturing (Fig. 1*C*). Sprouting appears to be typical for plant tissues with an elevated cytokinin content (Van Loven 1993). Application of cytokinins affects the transport of indoleacetic acid and releases buds from apical dominance. It was suggested that changes in the cytoki-

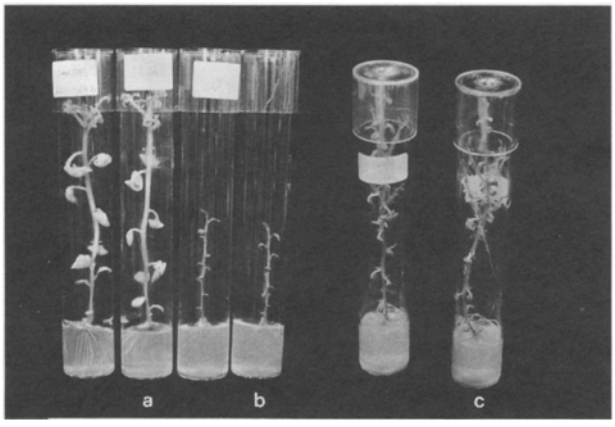

Fig. 1. Different phenotypes of potato cv. Sante. **(A),** 4-week-old plantlets treated with JA. **(B),** 4-week-old control plantlets. **(C),** 3-month-old control plantlets with developed axillary buds.

Table 2. Morphologic characteristics of control and JA-treated potato plantlets cultured for 4 weeks. Results are expressed as the mean of three independent experiments ± S.E. In each experiment at least 15 plantlets were used. Calculation of the significance of differences between control and JA-treated samples was based on Student's t test. N.S., not significant.

	Control	JA treated	*p*
Height (cm)	5.28 ± 0.2	9.5 ± 0.2	<0.001
Node number	9.1 ± 0.3	9.6 ± 0.3	N.S.
Leaf area (mm^2)	36.5 ± 12.0	180 ± 28.6	<0.01
Stem diameter (mm)	1.9 ± 0.1	2.6 ± 0.05	<0.002
Plant fresh weight (mg)	297 ± 60.3	856.5 ± 144.2	<0.002
Plant dry weight (mg)	25.3 ± 4.4	47.4 ± 7.9	N.S.

nin concentration modify the nutrient distribution pattern, which allows axillary buds to grow. Relatively minor changes of three- to tenfold in cytokinin and auxin levels are sufficient to alter quantitative aspects of plant development significantly (Brzobohatý et al. 1994).

After JA treatment the cytokinin concentration in plantlets decreased to 198 pmol/ng/g dry weight (Table 1). Simultaneously, prominent alterations in morphology were observed. The plantlets had enlarged root systems with many lateral roots (Fig. 1*A*). It has been shown that reduced expression of *ipt* gene and a lower level of cytokinins allowed root formation (Yusibov et al. 1991). Although the number of nodes was not changed (Table 2), the plantlets of cv. Sante grown on the medium supplied with JA showed an increase in height. This finding correlates with transgenic plants with high cytokinin levels which displayed a reduced height that was due to a decrease in internode length rather than node number (Brzobohatý et al. 1994). There are controversial reports about the involvement of cytokinins in stem thickening (Van Loven et al. 1993), but in the plantlets of cv. Sante this phenomenon was observed in the plantlets with lower cytokinin content.

We recently reported (Kovač and Ravnikar 1994) that JA-treated plantlets of cv. Sante showed hyperhydric symptoms with an abnormal morphologic appearance (Debergh et al. 1992). Hyperhydricity was expressed especially on the leaves, which were brittle and light green, containing increased amount of water. Furthermore, there was a significant decrease of chlorophyll a, and b, in the leaves. A comparable decrease of carotenoid content was accompanied by an activation of the xanthophyll cycle (Kovač and Ravnikar 1994), a metabolic pathway seen frequently in stressed photosynthetic tissues (Demming-Adams and Adams 1996). Hyperhydric properties of the plantlets were similar to that caused by stress action of JA (Koda 1992). It was suggested that stress-related properties of JA are already expressed at 1 μM concentration in cv. Sante (Kovač and Ravnikar 1994). Stress conditions elicited by JA could affect cytokinin activity and have an inhibitory effect on chlorophyll and carotenoid biosynthesis, which is known to be stimulated by cytokinins (Kusnetsov et al. 1994).

However, the hyperhydric symptoms of the JA treatment were not notable in the plantlets of cv. Ulster Sceptre (Kovač and Ravnikar 1994), and these plants grew even more vigorously than the controls (Dermastia et al. 1994b). It has been shown that the enhanced growth of JA-treated potato plantlets of cv. Ulster Sceptre correlates with an increased level of biologically active cytokinin ribosides without apparent alterations in overall cytokinin levels (Dermastia et al. 1994). In the JA-treated plantlets of cv. Sante, we did not observe obvious changes in the ratio among cytokinin metabolites (Table 1), although the total cytokinin concentration was decreased significantly.

In conclusion, this study confirms the correlation between elevated endogenous cytokinin levels and induction of bud release and inhibition of root growth. However, the phenotype of the plant is not modified only by the cytokinin level. It has been shown that the altered cytokinin level does not necessarily correlate with the same response to the treatment. Moreover, the absolute concentration of cytokinins in the plants is not the crucial factor controlling their development, and the local changes in cytokinin level can have global consequences for the plant (for review, see Binns 1994). Probably, diminished levels of cytokinins with simultaneous changes in the ratio to the other plant growth regulators and the applied in vitro procedure contribute to the observed morphology of the potato plantlets of cv. Sante. Regarding other potato cultivars it seems possible that the effect of the different factors varies as a function of the sensitivity of the plant.

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