Role of cytokinins in growth correlations between roots and stems in pea (*Pisum sativum* L.) seedlings

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ABSTRACT

Pea seeds were swollen in solutions with different concentrations of cytokinin benzyladenine (BA). As compared with controls, a low concentration of BA (0.05 mg/l) did not affect the growth of stems and roots of twelve-day-old seedlings but concentrations of 0.20–4.00 mg/l showed an inhibiting effect; this inhibition was proportional to the concentration of BA. In controls the ratio between the lengths of stems and roots was equal to 1.14, while in seedlings influenced by BA in concentration of 0.20 mg/l its value was significantly lowered to 0.91. Also in six-day-old pea seedlings cultivated under *in vitro* conditions, BA concentrations of 0.70 and 3.30 mg/l inhibited the growth of roots and epicotyls and significantly increased the production of ethylene (by 150 and 330%, respectively). Three hours after the amputation of the root of five-day-old pea seedlings the level of cytokinin trans-zeatin increased in the apical part of the stem. Within the interval of 48 hours after the amputation of the root the concentration of this plant hormone gradually decreased again. The initial increase in the zeatin level in the stem indicates that the absence of the root induces a reduction of growth-inhibiting effects of roots, which negatively influences the zeatin level in the stem apex at the beginning of germination.

Keywords: benzyladenin; trans-zeatin; ethylene

The recent literary data concerning growth correlations in pea seedlings paid attention above all to the phytohormonal principles of the apical dominance over lateral buds (Blažková et al. 1999, Procházka and Truksa 1999, Li and Bangerth 2003 and others). However, growth correlations existing between roots and stem are not practically mentioned. Lately, this correlation was studied by Haver and Schuch (2001) in petunias. After the reduction of roots, these authors observed an enhanced apical dominance of the stem, which was associated with an increase in the ratio of auxins to cytokinins. Dostál (1941) was the first author who studied root/stem correlations in pea seedlings. In his experiments it was demonstrated that the excision of radicle and/or young root resulted in the growth stimulation of plumule and/or young epicotyl. Tan et al. (1984) studied this initial growth-inhibiting effect of the radicle of pea seedlings with regard to changes in IAA activity in the apical part of the stem (epicotyl) after the excision of root of seedlings and found out that the level of IAA increased within 12 hours after this excision.

Only small attention was paid to the role of cytokinins in root/stem correlations in pea seedlings. Klíčová et al. (1986) studied the effect of radicle and/or plumule excision on the level of cytokinins in the plumule and/or radicle in pea seedlings. However, these authors only tried to estimate so-called cytokinin-like substances using the Amaranthus-bioassay 24 and 48 hours after the excision of radicle and/or plumule.

Auxin-rich tissues produce large amounts of ethylene, which retroactively inhibits the polar transport of auxins and causes a thickening of the stem and a retardation of its elongation growth. Yu and Yang (1979) mentioned that the level of auxin is directly proportional to the production of ethylene, which is responsible for different responses attributed in plants to high auxin levels. BA also increases the production of ethylene (Li 1997, Rashotte et al. 2005); a synergism of IAA, cytokinin and ethylene was also observed (Li and Bangerth 1992).

The aim of this study was to obtain a deeper insight into the role of cytokinins in the root/stem correlations in pea seedlings. The effect of exog-

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Supported by the Ministry of Education of the Czech Republic, Project No. 432100001.

enous cytokinin (BA) on the growth correlation existing between the root and the stem was studied both under *in vivo* and *in vitro* conditions with regard to the production of ethylene. The level of endogenous cytokinin trans-zeatin in the stem apex of pea seedlings was estimated in different time intervals after the excision of the root.

MATERIAL AND METHODS

Pea (*Pisum sativum* L.) seeds, cv. Oskar were used in these experiments. To obtain the seedlings, pea seeds were swollen for 24 hours either in water or in water solutions of benzyladenine (BA) – (for concentrations see Figure 1). Subsequently, they were placed into perlite moistened with water. The seeds germinated in darkness at 22 ± 2°C and 80% relative humidity for 12 days. At the end of the experiment, epicotyls and roots of seedlings were measured and their lengths were statistically analysed using the Student *t*-test.

To estimate the ethylene production of pea seedlings, the seeds were sterilised in 0.20% solution of mercuric chloride ($HgCl_2$) for 11 min and then rinsed three times for 10 min in sterile distilled water. In a flow-box, sterilised seeds were placed with tweezers either on an AS medium (0.80% agar, 1.00% saccharose) without growth regulators or with an addition of 0.70 and 3.30 mg/l BA into test tubes. The test tubes were closed with aluminium caps with a septum. On days 1, 3, 4 and 6 of the experiment, the air from test tubes was sampled to assess the content of ethylene. Lengths of epicotyls and roots of pea seedlings were measured at the end of the experiment.

Roots of five-day-old pea seedlings grown in perlite were then removed and samples of apical parts of the pea seedlings were taken 1, 2, 3, 6, 24 and 48 hours to estimate the content of trans-zeatin.

Cytokinin analysis. Extraction and purification of cytokinin was carried out using lyophilisation and homogenisation of samples in a Bieleski solution (Bieleski 1964); the debris was removed by centrifugation. The purification step passed over the system of C_{18} and DEAE-cellulose cartridges and was followed by evaporation in vacuum (for details see Lexa et al. 2003). HPLC separations were performed using an AQUASIL C_{18} reversed-phase column (250 × 2.1 mm i.d., 5 µm particle size; ThermoHypersil-Keystone, Cheshire, UK). Following solvents were used for this separation: A (100% methanol) and B (0.25% formic acid, v/v). The flow rate was 200 µl/min with an isocratic elution (A: B = 35:65, v/v); the injected volume was 10 µl. The effluent was introduced into the mass spectrometer Finnigan AQA (ThermoQuest, Manchester, UK) equipped with an electrospray interface (capillary voltage +4.5 kV, source temperature 250°C, cone voltage 5 V). Mass spectrometric data were acquired in the selected ion monitoring (SIM) mode at the molecular ion $[MH]^+$ value 220 for trans-zeatine and quantified by the method of external standard.

Estimation of ethylene production. Using a tuberculin syringe, 1 ml of air was drawn from the test tube closed with an aluminium cap with a rubber septum and analysed in a gas chromatograph of the firm FISSONS INSTRUMENT, Italy (50 m capillary column Al_2O_3 S 15 µm, ID 0.53 mm). When estimating gaseous hydrocarbons, spray, column and detector temperatures were 230°C, 40°C and 200°C, respectively (Fišerová and Hradilík 1994, Fišerová et al. 2001).

RESULTS

The effect of exogenous benzyladenine (BA) on root/stem correlations *in vivo*

Lengths of stems (epicotyls) and roots of twelve-day-old pea plants cultivated from seeds swollen in solutions containing 0.05–4.00 mg/l BA are presented in Figure 1. As compared with controls, the lowest concentration of BA, i.e. 0.05 mg/l, did not influence significantly the growth of epicotyls and/or roots; however, increasing concentrations of BA statistically and highly significantly inhibited their growth. BA reduced the epicotyl/root ratio and its effect was the highest in concentration of 0.20 mg/l. In controls, epicotyls were highly significantly longer than roots; they were significantly longer also after the application of the lowest concentration of BA, i.e. 0.05 mg/l; however, the concentration of 0.20 mg/l resulted in a correlative reversal and the roots were highly significantly longer than the epicotyls. The highest BA concentrations showed an inhibiting effect but the differences in the epicotyl/root length ratio were not significant.

Although the lowest concentration of BA, i.e. 0.05 mg/l, did not cause significant changes in the length of the epicotyls and roots as compared with control plants, it was also studied whether the lower concentrations could not show the stimulating effect on the growth of seedlings. As compared with controls, the applied BA concentrations (0.02; 0.01 and 0.005 mg/l) did not

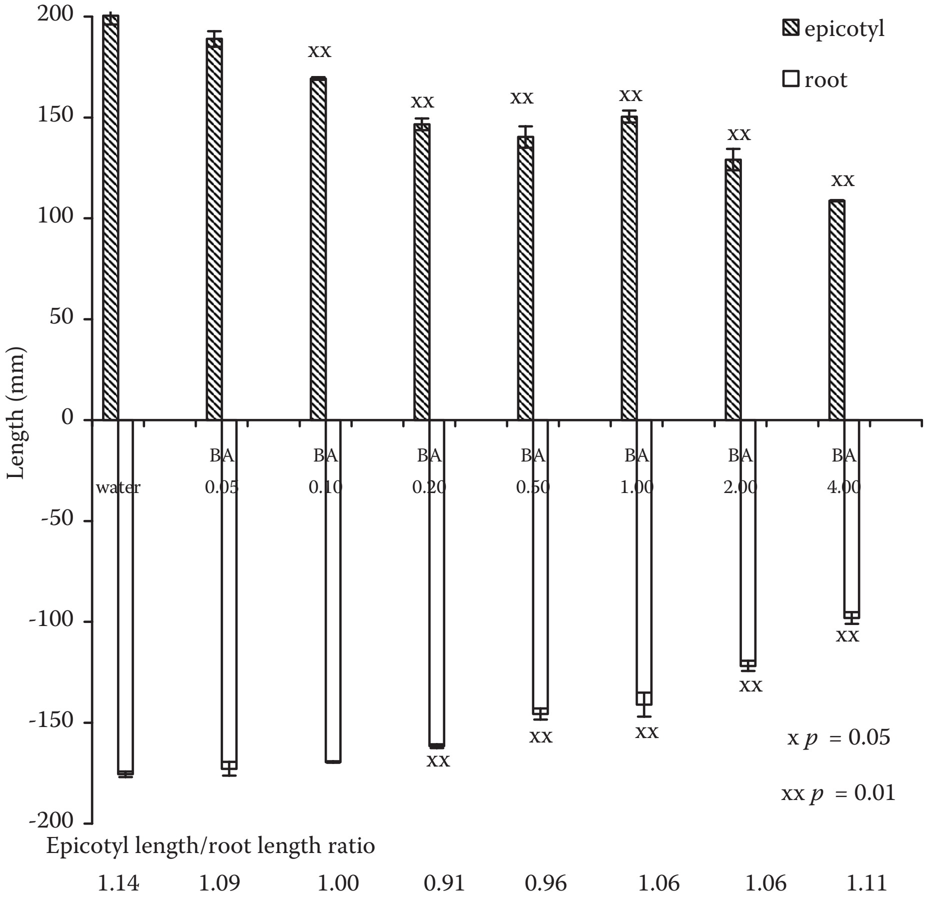

Figure 1. Lengths of epicotyls and roots in twelve-day-old pea seedlings cultivated from seeds swollen either in water or in solutions of benzyladenine (BA; 0.05–4.00 mg/l); the standard errors and the statistical significance as determined by the *t*-test are indicated

affect the elongation growth of epicotyls and/or roots.

The effect of exogenous benzyladenine (BA) on root/stem correlations *in vitro*

As soon as on day 4 of the experiment, statistically significant differences in ethylene production were observed. These differences were even higher at the end of the experiment (Figure 2). Ethylene production was directly proportional to the concentration of BA and indirectly proportional to the size of the seedling (Figure 3).

For pea seedlings, concentrations lower than 20 nl/l can be therefore considered as stimulating. Higher ethylene concentrations inhibited the growth of both the roots and the epicotyls.

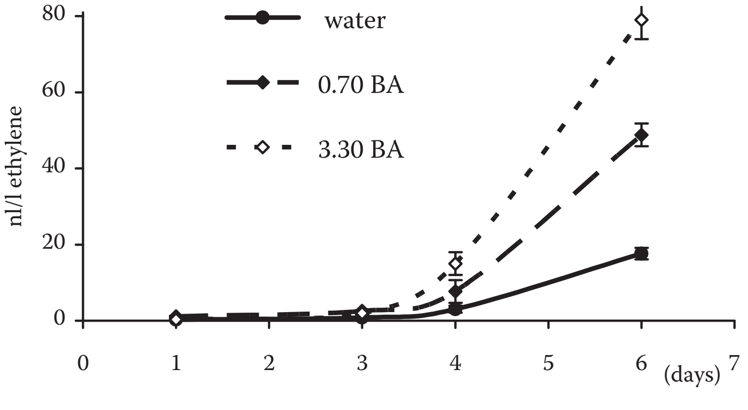

Figure 2. Production of ethylene by pea seedlings cultivated on a medium without cytokinin and on media containing 0.70 and 3.30 mg/l BA; y-axis: concentration of ethylene (in nl/l), x-axis: time of sampling in days (for both swollen and germinating pea seeds); standard errors are indicated

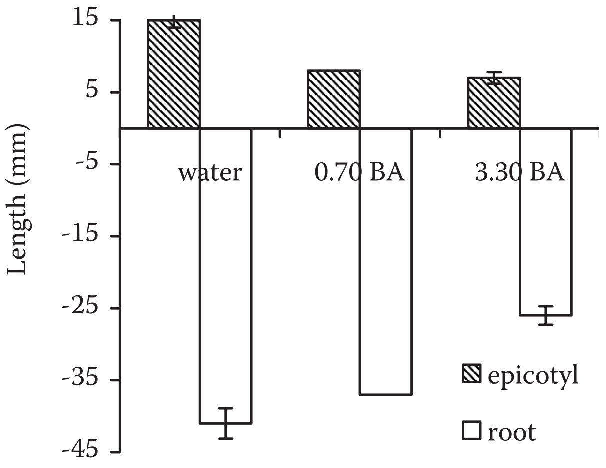

Figure 3. Lengths of epicotyls and roots of six-day-old pea seedlings cultivated on media containing 0.00, 0.70 and 3.30 mg/l of benzyladenine (BA); standard errors are indicated

The effect of root excision on the level of endogenous trans-zeatin in the stem apex

Changes in the content of trans-zeatin in epicotylar apices of pea seedlings observed at different time intervals after the root excision are presented in Figure 4. As one can see, the content of trans-zeatin began to increase within the first 3–6 hours of amputation and after 48 hours it slowly decreased again.

DISCUSSION

The application of exogenous BA, in various concentrations of which the pea seeds were left to swollen, demonstrated that, in twelve-day-old plants, a solution of 0.20 mg/l BA could induce a correlative reversal in the ratio between the root and the stem in favour of the roots. In our experiments it was found out that the effect of cytokinins on the root/stem correlation was quite opposite to that of gibberellins.

Although the exogenous cytokinin was able (albeit in only one of the applied BA concentrations) to change in pea the correlation between the root and the epicotyl in favour of the root, the exogenous gibberellins are known that they change (also in a broad spectrum of concentrations) this correlation in favour of epicotyls (Šebánek et al. 1991). These different effects of cytokinins and gibberellins may be associated with the fact that in 10 to 12-day-old pea seedlings the gibberellins biosynthesised in roots contribute to the stimulating effect of roots on the epicotyl growth. By strengthening of the elongation growth of the epicotyl they also amplify the apical dominance of the stem. On the other hand cytokinins attenuate the apical dominance (Blažková et al. 1999) and these results in an initiation of branching of the stem and in an inhibition of its elongation growth. This effect can be induced by cytokinins biosynthesised both in roots (Šebánek et al. 1991) and leaves (Miyawaki et al. 2004).

The growth of pea germinating under *in vitro* conditions was influenced in a similar way. The application of 0.70 and 3.30 mg/l BA to six-day-old seedlings inhibited the growth of both roots and epicotyls (Figures 2 and 3) on the one hand and stimulated production of ethylene on the other. These results were corroborated with the recent data about the stimulation of ethylene production by cytokinins (Rashotte et al. 2005). Concentrations of 50–80 nl/l in the air can be already considered as inhibiting; this indicates that pea seedlings are more sensitive to exogenous ethylene than radish, petunia or prunus (Radin and Loomis 1969, Dimasi-Theriou et al. 1993, Alsalihy et al. 2004).

It is known that auxins amplify the apical dominance of the stem which is weakened by the auxin inhibitors (Šebánek et al. 2000, Nakajima et al. 2001). Cytokinins attenuate the apical dominance and stimulate the growth of axillary buds of pea (Procházka and Truksa 1999). According to our results, this could be due the fact that cytokinins increase the synthesis of ethylene that is known as an exogenous inhibitor of apical dominance (Haver and Schuch 2001).

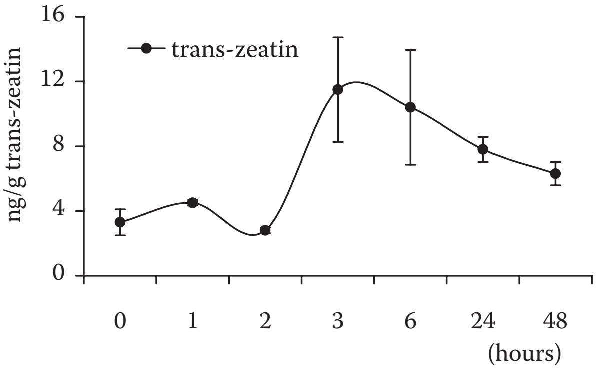

Figure 4. Changes in the levels of trans-zeatin in the apical part of epicotyls of five-day-old pea seedlings measured in different time intervals after the root excision; the content of trans-zeatin (ng/g) is plotted on the y-axis, the time interval from the root excision on the x-axis; standard errors are indicated

In pea plants, the radicle and young roots inhibit the growth of plumule and epicotyls at the beginning of germinations (Tan et al. 1984). Our results indicate that the inhibiting effect of the root on the level of endogenous cytokinin in the stem persists for as much as 5 days because the root excision causes a marked increase in the level of trans-zeatin in epicotyls within the first 24–48 hours.

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Received on April 19, 2005

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