A trans-zeatin riboside in root xylem sap negatively regulates adventitious root formation on cucumber hypocotyls

Takeshi Kuroha1, Hisashi Kato2, Tadao Asami2, Shigeo Yoshida2, Hiroshi Kamada1 and Shinobu Satoh1,3

1 Institute of Biological Sciences, University of Tsukuba, Ibaraki 305-8572, Japan
2 RIKEN (The Institute of Physical and Chemical Research), 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

Received 14 March 2002; Accepted 3 July 2002

Abstract

Shoot cultures of cucumber were used to analyse the roles of root-derived substances in adventitious root formation on hypocotyl tissues. Xylem sap collected from the roots of squash had a strong inhibitory effect on the formation of hypocotyl adventitious roots. Double-solvent extraction followed by fractionation with both normal and reverse phase column chromatographies and analysis by liquid chromatography/tandem mass spectrometry identified trans-zeatin riboside (ZR) as the primary suppressor of adventitious root formation. ZR was the predominant cytokinin present in the xylem sap, occurring at a concentration of $2 \times 10^{-8}$ M. Application of ZR at concentrations from $3.16 \times 10^{-9}$ M effected inhibition of adventitious root formation. These results suggest that ZR transported from roots via xylem sap may act as an endogenous suppressor of hypocotyl adventitious root formation in planta.

Key words: Adventitious root suppression, cucumber, Cucumis sativus, Cucurbita, cytokinin, squash, xylem sap, zeatin riboside.

Introduction

The formation of adventitious roots from the stems or hypocotyls of most plants is stimulated by the removal of root tissue, and appears to be controlled by various factors transported from both the roots and shoots. It is well established, for example, that basipetally transported auxin, which accumulates at the basal end of hypocotyl and stem cuttings in tomato and wild cherry, plays a stimulatory role during adventitious root formation (Maldiney et al., 1986; Label et al., 1989). Similarly, removal of the shoot apex decreases both the level of endogenous auxin in the basal part of a cutting and the number of adventitious roots in pea (Nordström and Eliasson, 1991), and auxin-overproducing mutants of Arabidopsis produce numerous adventitious roots from the hypocotyls of intact plants (Boerjan et al., 1995). Consequently, endogenous auxins that are produced in the shoot apex, transported basipetally and accumulate in the cut end of a stem are believed to induce adventitious root formation.

Ethylene is synthesized as a stress response to wounding in most plants, and promotes adventitious root development in cut tissues. Inhibitors of ethylene biosynthesis and perception reduce the number of adventitious roots on cuttings of sunflower and mung bean (Robbins et al., 1983, 1985), while ethylene-insensitive tomato and petunia plants produce fewer adventitious roots than wild-type cuttings (Clark et al., 1999).

Although it has been reported that root extracts have an inhibitory effect on adventitious root formation in bean (Libbert, 1956), and that removal of the root stimulates adventitious root formation (Socker, 1965), the endogenous root-derived suppressor of adventitious root formation has not yet been identified.

When exogenous chemical applications, rather than endogenous stimulators and suppressors, are considered,
stimulatory effects on adventitious root formation have been reported (Haisig and Davis, 1994), especially by plant hormones such as the auxins (Went, 1939; Basu et al., 1969; Shibaoqa, 1971) and ethylene (Zimmerman et al., 1933; Mensualisodi et al., 1995). There are fewer reports of adventitious root inhibition as a consequence of exogenous chemical treatment (Fellenberg, 1966; Mitsuhashi et al., 1969), but these too include effects of plant hormones such as gibberellic (Brian et al., 1960; Fabijan et al., 1981) and cytokinin (Humphries, 1960; Bollmark and Eliasson, 1986). Both the studies of endogenous and exogenous factors leading to the control of adventitious root development suggest that plant hormones probably play a critical role.

It is reasonable to suppose that a root-derived suppressor would be transported to the hypocotyl through the xylem via the transpiration stream. Squash plants, from which a large amount of xylem sap can be obtained (300–3000 ml plant−1 in 2 d), were used as a source of xylem sap. Squash plants are widely used as a rootstock for commercial cucumber production, as they ensure protection from soilborne disease. Cucumbers graft well to squash rootstock, and produce many adventitious roots from their own hypocotyl tissues as well, providing an excellent study system.

When hypocotyls of cucumber seedlings were cultured in squash root xylem sap fractions, inhibition of adventitious root formation was observed (Satoh et al., 1998). A significant concentration of a novel amino acid, methoxybenzylglutamine [N5-(4-methoxyphenyl)methyl-L-glutamine], was identified in the inhibitory fraction of the root xylem sap (Satoh et al., 1998; Inouye et al., 1999). Chemically synthesized methoxybenzylglutamine inhibited the formation of adventitious roots at a concentration of 5×10−3 M, but not 5×10−4 M (Satoh et al., 1998), but the endogenous concentration of methoxybenzylglutamine was estimated to be 10−6 M in root xylem sap. The endogenous concentration is far too low to be effective in above-ground organs, even if concentrated in the shoots via transpiration by several hundred times. These results suggest that methoxybenzylglutamine is a poor candidate for being the sole suppressor of adventitious root formation, and that another suppressor is likely to be present in root xylem sap.

In the search to identify the endogenous suppressors of the formation of adventitious roots, double-solvent extraction was used, followed by normal and reverse phase column chromatographies and analysis by liquid chromatography/tandem mass spectrometry (LC/MS/MS). These techniques revealed a trans-zeatin riboside (ZR), one of the cytokinins, which acts as an inhibitory factor in root xylem sap. The negative regulation of adventitious root formation by ZR transported from roots to shoots in planta will be discussed.

**Materials and methods**

**Plant materials and chemicals**

Seeds of interspecific hybrids of squash Cucurbita maxima Duchesne×C. moschata Duchesne cv. Shintosa-ichigou, commonly used as the commercial rootstock for cucumber, and seeds of cucumber, Cucumis sativus cv. Shimoshirazu-jibai were obtained from the Sakata Seed Co. (Kanagawa, Japan). For the identification and quantification of cytokinins using LC/MS/MS, ZR, cis-ZR, Z, cis-Z, and [3H]Z were purchased from Apex Organics (Devon, UK). For the bioassay on the formation of adventitious roots, ZR, Z and BA were purchased from Sigma (St Louis).

**Collection of root xylem sap**

Squash plants, cv. Shintosa-ichigou, were grown in the field for 2–3 months (May to August), and the stems were cut off 15–30 cm above the soil level. After the first drops of exudates were discarded, the cut surface was washed with distilled water; further exudates were collected in flasks over ice and stored at −30 °C as root xylem sap.

**Fractionation of the inhibitory factor in root xylem sap**

Root xylem sap (5.0 l) was filtered under reduced pressure with a glass filter (110 mm ID; Whatman, Maidstone, UK). The filtrate was subjected to reverse phase chromatography on a silica gel ODS-Q3 open column (80 ml volume; Wako, Osaka, Japan) (Fig. 1). The bound substances were eluted sequentially with 200 ml of 20%, 50% and 100% (v/v) acetonitrile and the samples were evaporated to completely remove acetonitrile. Eluate (150 ml) of the 20% acetonitrile sample with inhibitory activity was partitioned with 150 ml of butanol (Fig. 1). This step was repeated three times and the results combined. The butanol and aqueous phases were separately evaporated at 40 °C, and solubilized in 5 ml of water. The solution was centrifuged at 3000 g for 10 min. The supernatant was subjected to reverse phase chromatography on an ODS-80 Tscolumn (4.6 mm ID×15 cm; Tosoh, Tokyo, Japan) in an HPLC system (Tosoh), and

![Root xylem sap (5 L)](https://academic.oup.com/jxb/article-abstract/53/378/2193/426533)

![Silica gel ODS-Q3 open column](https://academic.oup.com/jxb/article-abstract/53/378/2193/426533)

![Flow through 20% acetonitrile 50% acetonitrile 100% acetonitrile](https://academic.oup.com/jxb/article-abstract/53/378/2193/426533)

![Partitioning](https://academic.oup.com/jxb/article-abstract/53/378/2193/426533)

![Aqueous phase Butanol phase](https://academic.oup.com/jxb/article-abstract/53/378/2193/426533)

![ODS-80 Ts column (HPLC system)](https://academic.oup.com/jxb/article-abstract/53/378/2193/426533)

![Fraction No. 4 (35-39 min)](https://academic.oup.com/jxb/article-abstract/53/378/2193/426533)

![Spherisorb W silica column (HPLC system)](https://academic.oup.com/jxb/article-abstract/53/378/2193/426533)

![Fraction No. 7 (10-11 min)](https://academic.oup.com/jxb/article-abstract/53/378/2193/426533)

![ODS-80 Ts column (HPLC system)](https://academic.oup.com/jxb/article-abstract/53/378/2193/426533)

![Fraction No. 2 (18-22 min)](https://academic.oup.com/jxb/article-abstract/53/378/2193/426533)

**Fig. 1.** Purification procedure for the adventitious root inhibitory factor from squash root xylem sap.
eluted with a linear gradient of acetonitrile (from 0% to 60% (v/v) in 30 min) at a flow rate of 0.5 ml min\(^{-1}\) with monitoring absorbance at 280 nm (Fig. 1). The pooled fractions were evaporated at 40 °C, to remove acetonitrile, and used for the bioassay.

The eluate from 35–39 min with inhibitory activity was dissolved in 80% (v/v) acetonitrile and applied to normal phase chromatography on a Spherisorb W silica column (4.6 mm I.D.×25 cm; Waters, Tokyo) in an HPLC system, with a monitoring absorbance of 280 nm (Fig. 1). The mobile phase was isocratic and consisted of 80% (v/v) acetonitrile at a flow rate of 0.5 ml min\(^{-1}\). The pooled fractions were used for the bioassay following evaporation. The inhibitory fraction at the retention time from 10–11 min was again subjected to reverse phase chromatography on the ODS-80Ts column in the HPLC system (Fig. 1). The mobile phase was isocratic and consisted of 0.1% (v/v) acetic acid solution mixed with 10% acetonitrile (v/v) at a flow rate of 0.5 ml min\(^{-1}\). The inhibitory activity at the retention time from 18–22 min was recovered with a single UV-peak on HPLC analysis.

**Identification and quantification of the inhibitory factor**

The fraction showing inhibitory activity at the retention time from 10–11 min with normal phase chromatography on the Spherisorb W silica column was evaporated to near dryness and subsequently dissolved in 0.5 ml water. An aliquot of the sample was applied to a reverse phase CapselPak MG 18 column (2.0 mm I.D.×250 mm; Shiseido, Tokyo) and eluted with 40% (v/v) acetic acid solution mixed with 15% acetonitrile (v/v) at a flow rate of 0.2 ml min\(^{-1}\). Ion peaks were monitored using LC/MS/MS (Applied Biosystems Sciex API-2000, Toronto). The detection of various cytokinin species was performed using selected reaction monitoring modes (ZR, mass-to-charge ratio (m/z) of 352/220; trans-zeatin (Z), m/z=220/136; trans-zeatin-O-glucoside (ZOG), m/z=382/220; trans-zeatin-O-glucoside riboside (ZORG), m/z=514/382; trans-zeatin riboside-S'-monophosphate (ZMP), m/z=432/220; isopentenyladenosine-S'-monophosphate (pPMP), m/z=416/204). The conditions for MS detection of cytokinins were determined by infusing a mixture of authentic cytokinins (ZR, cis-ZR, Z, cis-Z).

Quantification of cytokinins in root xylem sap was performed by the LC/MS/MS system as follows. Each root xylem sap sample (0.5 l) was lyophilized and extracted with 80% (v/v) methanol, to which \(^3\)HJZ was added as an internal standard. The methanol extract was applied to an Oasis™ HLB extraction cartridge (Waters, Tokyo) and eluted with 40% (v/v) methanol containing 0.1% acetic acid. The eluate was evaporated, the resulting residue was redissolved into an aliquot of methanol and applied to a reverse phase CapselPak MG 18 column under the same conditions as described above for the identification of the inhibitory factor. Cytokinins were quantified using the selected reaction monitoring mode (ZR and cis-ZR, m/z=352/220; Z and cis-Z, m/z=220/136). The internal concentration of each cytokinin was calculated using the recovery value of the corresponding internal standard.

**Induction of the formation of adventitious roots**

Cucumber seedlings cv. Shimoshirazu-jibai were grown in an artificial soil (Kurehakagaku, Tokyo, Japan) for 6 d under white fluorescent light (150 μmol m\(^{-2}\) s\(^{-1}\)) at a photoperiod at 28 °C. The hypocotyl of each seedling was cut off 4 cm below the cotyledon using a razor blade; seedlings were cultured with 1 ml of the solution used for the bioassay in the presence of 1/20 strength MS medium on an ODS-80Ts column. The butanol phase partitioned from the 20% acetonitrile eluate (by open column chromatography on silica gel ODS-Q3) was applied to the ODS-80Ts column of the HPLC system and eluted with a linear gradient of acetonitrile (from 0–30% in 60 min); absorbance at 280 nm was monitored (A). The pooled fractions (Nos 1–14) were evaporated and used for the bioassay in the presence of 1/20 strength MS medium (B). The concentrations were adjusted to 10 times those of the original xylem sap. Error bars represent SE with n=5. The mean number of adventitious roots formed without the fractions, shown as control, was 12.4 (SE=2.4).

In a previous study, inhibitory activity in squash root xylem sap was observed only in the 20% acetonitrile eluate on an ODS-80Ts column of the butanol phase fraction (Satoh et al., 1998). Therefore, the inhibitory fraction was prepared using double-solvent extraction coupled with reverse phase chromatography; the butanol phase partitioned from the 20% acetonitrile eluate (using open column chromatography on a silica gel ODS-Q3) was subjected to an ODS-80Ts column by reverse phase chromatography (Fig. 2A, B). The inhibitory activity of fraction No. 4 was significantly stronger than that of fraction No.11, indicating that fraction No. 4 was used for each bioassay and all experiments were repeated at least twice.
included the main active compounds in xylem sap. Therefore, fraction No. 4 was further subjected to normal phase chromatography on a Spherisorb W silica column in the HPLC system (Fig. 1). The inhibitory activity of fraction No. 7 (10–11 min) was similarly detected (Fig. 3A, B).

Identification and quantification of ZR as an inhibitory factor

To identify the substance in the inhibitory fraction of root xylem sap, mass spectrometry (MS) and tandem mass spectrometry (MS/MS) was performed. Fraction No. 7 (10–11 min) from a Spherisorb W silica column, which showed the inhibitory activity (Fig. 3A, B) was applied to a reverse phase CapselPak MG 18 column and analysed by MS. The molecular mass of the major component of the fraction was found to be 351, indicating a [M+H+] ion at m/z=352. An ion peak at m/z=220 was also detected as a minor component (data not shown). After the fragmentation of the peak at m/z=352 by MS/MS, this peak produced the component ion at m/z=136. These results and the standard m/z of ZR (352/220) and Z (220/136) indicated that zeatin riboside was a major component of this fraction.

This fraction was further analysed by liquid chromatography/tandem mass spectrometry (LC/MS/MS). In the total ion chromatograms using normal mode, two major peaks were detected at the retention time (around 8 and 9 min, Fig. 4, upper profile of total). In the ion chromatograms using selected reaction monitoring mode for ZR (m/z=352/220) and Z (m/z=220/136), two major peaks were detected at the same retention time as two peaks in the total ion chromatograms (Fig. 4, upper profiles of ZR and Z). The retention times of these major peaks were identical to those of authentic trans-ZR and cis-ZR (Fig. 4, lower profiles). In contrast, ion peaks of iPMP, ZMP, ZOG, and ZOGR were not detected in the ion chromatograms using the selected reaction monitoring mode for each cytokinin (data not shown).

To determine which peak in the chromatogram on LC/MS/MS had the inhibitory activity, the fraction from a Spherisorb W silica column was subjected to reverse phase chromatography on an ODS-80Ts column of an HPLC system under the same conditions as reverse phase chromatography on the CapselPak MG 18 column (Fig. 1), except for the flow rate and the concentration of acetonitrile in the mobile phase (see Materials and methods); the pooled fractions were used for the bioassay. Inhibitory activity was detected in fraction No. 2 (18–22 min) (Fig. 5). This peak was identical with that of trans-ZR in the HPLC chromatograph on LC/MS/MS. Weak inhibition was also observed in fraction No. 4 (24–40 min), but the activity was not significant (Fig. 5).

Inhibitory effect of cytokinins on the formation of adventitious roots

To confirm that ZR had an inhibitory effect on the formation of adventitious roots, cytokinins (ZR, Z, BA) were tested by bioassay. All cytokinins clearly showed concentration-dependent inhibitory activities for the adventitious root formation on the cucumber hypocotyls (Fig. 6). ZR and Z had strong inhibitory activities at concentrations between 3.16×10^{-9} M and 10^{-5} M (Fig. 6), but BA showed weaker inhibitory activity at 3.16×10^{-9} M (Fig. 6).

Quantification of cytokinins in root xylem sap

To evaluate whether the concentration of endogenous ZR in root xylem sap is sufficient to inhibit adventitious root formation, relative quantities of endogenous cytokinins were determined. LC/MS/MS analysis showed that the concentration of endogenous ZR in root xylem sap was estimated to be 2.0×10^{-8} M (SD=0.1×10^{-8} M) (Table 1). The concentration of cis-ZR was also estimated to be 6.6×10^{-9} M (SD=0.5×10^{-9} M), approximately three times lower than trans-ZR (Table 1). The concentrations of
trans-Z and cis-Z were less than 1/10th of those of trans-ZR and cis-ZR (Table 1).

Discussion

In the present study, ZR has been identified from squash root xylem sap as the main suppressor of the formation of hypocotyl adventitious roots in cucumber. Cytokinin is a phytohormone that is involved in such processes as cell division, photosynthesis, chloroplast differentiation, senescence, and nutrient metabolism (Mok, 1994). Naturally occurring cytokinins are adenine derivatives with a side chain at the N\(^6\)-position. Isopentenyladenine (iP), Z and cis-Z were less than 1/10th of those of trans-ZR and cis-ZR (Table 1).
dihydrozeatin (DZ) are the predominant cytokinins found in the tissues and calli of higher plants. ZR is a riboside of Z, which is thought to be one of the most biologically active compounds, along with isopentenyladenosine (iPA) and dihydrozeatin riboside (DZR), ribosides of iP and DZ. ZR showed significant inhibitory effects on adventitious root formation at concentrations from 3.16×10⁻⁹ M to 10⁻⁴ M (Fig. 6). In previous reports, cytokinins such as BA and kinetin were shown to inhibit the formation of adventitious roots in bean (Humphries, 1960), Pinus radiata (Smith and Thorpe, 1975) and rose (De Vries and Dubois, 1988). Z, the predominant active cytokinin in plants, inhibits the formation of adventitious roots in pea, as does BA (Bollmark and Eliasson, 1986). As far as is known, the inhibitory effect of ZR has not been reported, despite the current evidence showing that ZR and Z exhibit the same strong inhibition of adventitious root formation (Fig. 6). It is possible that ZR was converted to Z in the hypocotyl, which then affected the inhibition; in intact plants, the ZR conversion mechanism following translocation from roots to shoots is unknown. BA, which is not dominant as an endogenous cytokinin, had a weaker inhibitory activity on the formation of adventitious roots than ZR and Z (Fig. 6), perhaps as a consequence of different metabolism and/or transport within the plant.

As with species such as Urtica dioica (Beck and Wagner, 1994) and pea (Beveridge et al., 1997), ZR was the most abundant cytokinin in squash root xylem sap, at an endogenous concentration estimated to be 2.0×10⁻⁸ M (SD=0.1×10⁻⁸ M) (Table 1). This concentration is sufficient to inhibit the formation of adventitious roots by cucumber hypocotyls (Fig. 6).

By contrast, a cis-isomer of ZR, cis-ZR, was also detected at an estimated concentration of 6.6×10⁻⁹ M (SD=0.5×10⁻⁹ M) (Table 1). Since no isomerization of the internal standard [²H]Z was observed in root xylem sap (data not shown), extraction artefacts such as tRNA breakdown and isomerization during extraction, resulting in the formation of the cis-isomer of cytokinins, are unlikely to have influenced the results. Earlier work has shown cis-ZR in xylem sap from chickpea (Emery et al., 1998), wheat and oats (Parker et al., 1989), Emery et al. (1998) suggested that because cis-isomers of cytokinin are predominant in chickpea, activity would be expected in this species. The present results failed to show activity in the cis-ZR fraction (Fig. 5), although considerable cis-ZR was present in root xylem sap from squash (Table 1), supporting a report of lower biological activity of the cis-isomer than the trans-isomer in bioassays (Kaminek, 1982).

While Z was observed in root xylem sap at a concentration of 2.4×10⁻¹⁰ M (SD=0.1×10⁻¹⁰ M) (Table 1), and has a strong inhibitory capability, the concentration was insufficient to inhibit the formation of adventitious roots (Fig. 6). These results lend further support to the hypothesis that ZR is the only appropriate candidate as a root-derived endogenous suppressor of adventitious root formation.

Root tips are the major sites of cytokinin biosynthesis and the source of cytokinins translocated via the xylem to control shoot development (Letham, 1994). This current work would suggest that much of the ZR carried by the transpiration stream from the roots is taken up by hypocotyl tissues to inhibit the formation of adventitious roots, and never reaches the uppermost regions of the shoot. Other reports support the hypothesis about the distribution of ZR from root to shoot. When radioactive ZR was supplied to root-pruned plants from the cut end, the largest proportion of extractable radioactivity was detected in the stem, with more than 50% in lupin stems (Jameson et al., 1987) and 40% in tobacco stems (Singh et al., 1992). Of course, root-pruning is expected to lead to a decrease in the ZR level in the stem, which has been shown to occur in both tomato (Maldiney et al., 1986) and pea (Bollmark et al., 1988) 1 d after cutting.

The mechanism for the negative regulation of adventitious root formation by ZR remains unclear. The inhibition of adventitious root formation by exogenously applied cytokinins occurs during the induction phase of root cell division (Eriksen, 1974; Fabijan et al., 1981; Bollmark and Eliasson, 1986; De Klerk et al., 1995). During the induction phase, auxins induce adventitious root formation in apple cuttings (De Klerk et al., 1995) and seem to interact antagonistically with cytokinins to control the initiation of adventitious roots. Similarly, auxins and cytokinins have antagonistic effects during lateral root formation (Böttger, 1974), and in controlling the gene expression of a cdc2-like protein, which regulates the G₂-M transition of the cell cycle as mitotic cyclin (John et al., 1993). The control of the expression of a cdc2-like protein might be involved in the inhibitory effects of ZR in the formation of adventitious roots, particularly if cell cycling were inhibited.

These results imply that ZR prevents the formation of adventitious roots on hypocotyls or stems in the normal,

| Compound | Concentration (nM ±SE) |
|----------|------------------------|
| trans-ZR | 20±1                   |
| cis-ZR   | 6.6±0.5                |
| trans-Z  | 0.24±0.01              |
| cis-Z    | 0.38±0.01              |

Table 1. Cytokinin levels in the xylem sap collected from squash roots

Cytokinins were quantified with LC/MS using selected reaction monitoring mode (trans-Z, cis-Z, m/z=220/136; trans-ZR, cis-ZR, m/z=352/220). All results were calculated using the recovery value of the internal standard in the given sample. Results shown represent the means of five replications under the same conditions.
non-wounded condition. When the roots are cut off, a rapid decrease in the ZR level would occur, along with an accumulation of auxin and the synthesis of ethylene in the basal part of the cut hypocotyls or stems, resulting in the formation of adventitious roots from the hypocotyls or stems.

Further support for this study’s hypothesis comes from a report that transgenic tobacco plants with reduced cytokinin content, due to the overexpression of a cytokinin oxidase gene, showed an increase in lateral and adventitious roots (Werner et al., 2001). Moreover, transgenic tobacco plants overexpressing a zeatin O-glucosyltransferase gene showed increased adventitious roots on their lower stems, suggesting that the reduction of active cytokinin by O-glucosylation leads to a lower cytokinin/auxin ratio (Martin et al., 2001).

Because weaker inhibitory activities were also observed in the other fraction than the ZR fraction (Fig. 2), it is possible that substances other than ZR have auxiliary roles in the negative regulation of adventitious root formation; these remain unidentified.

Acknowledgements

This work was supported, in part, by a Grant-in-Aid from the ‘Research for the Future’ Program, of the Japanese Society for the Promotion of Science (JSP-RFTF97L00601).

References

Basu RN, Bose TK, Roy BN, Mukhopadhyay A. 1969. Auxin synergists in rooting of cuttings. Physiologia Plantarum 22, 649–652.
Beck E, Wagner BM. 1994. Quantiﬁcation of the daily cytokinin transport from the root to the shoot of Urtica dioica L. Botanica Acta 107, 342–348.
Beveridge CA, Murfet IC, Kerhoas L, Sotta B, Miginiac E, Rameau C. 1997. The shoot controls zeatin riboside export from pea roots. Evidence from the branching mutant rms4. The Plant Journal 11, 339–345.
Boerjan W, Cervera M-T, Delarue M, Beeckman T, Dewitte W, BoÈttger M. 2001. The shoot controls zeatin riboside export from the xylem sap of squash root. Phytochemistry 51, 425–428.
Bollmark M, Eliasson L. 1986. Effects of exogenous cytokinins on root formation in pea cuttings. Physiologia Plantarum 68, 662–666.
Bollmark M, Kußt B, Eliasson L. 1988. Variation in endogenous cytokinin content during adventitious root formation in pea cuttings. Journal of Plant Physiology 132, 262–265.
Böttger M. 1974. Apical dominance in roots of Pisum sativum L. Planta 121, 253–261.
Brian PW, Hemming HG, Lowe D. 1960. Inhibition of rooting of cuttings by gibberellic acid. Annals of Botany 24, 407–419.
Clark DG, Gubrium EK, Barrett JE, Nell TA, Klee HJ. 1999. Root formation in ethylene-insensitive plants. Plant Physiology 121, 53–59.
De Klerk GJ, Keppel M, Ter Brugge J, Meekes H. 1995. Timing of the phases in adventitious root formation in apple cuttings. Journal of Experimental Botany 46, 965–972.
De Vries DP, Dubois LAM. 1988. The effect of BAP and IBA on adventitious root formation of ‘Amanda’ rose single-node softwood cuttings. Scientia Horticulturae 34, 115–121.
Emery RJN, Leport L, Barton JE, Turner NC, Atkins CA. 1998. cis-Isomers of cytokinins predominate in chickpea seeds throughout their development. Plant Physiology 117, 1515–1523.
Eriksen EN. 1974. Root formation in pea cuttings. III. The influence of cytokinin at different developmental stages. Physiologia Plantarum 30, 163–167.
Fabijan D, Taylor JS, Reid DM. 1981. Adventitious rooting in hypocotyls of sunflower (Helianthus annuus) seedlings. II. Action of gibberellins, cytokinins, auxins and ethylene. Physiologia Plantarum 53, 589–597.
Fellenberg G. 1966. Die Hemmung auxininduzierter Wurzelbildung an etiolierten Erbsenepicotylen mit Histon und Antimetaboliten der RNS- und Proteinsynthese. Planta 71, 27–42.
Haißig BE, Davis TD. 1994. An historical evaluation of adventitious rooting research to 1993. In: Davis TD, Haißig BE, eds. Biology of adventitious root formation. New York, London: Plenum Publishing Corporation, 275–331.
Humphries EC. 1960. Inhibition of root development on petioles and hypocotyls of dwarf bean (Phaseolus vulgaris) by kinetin. Physiologia Plantarum 13, 659–663.
Inouye Y, Wakahoi T, Satoh S. 1999. N6-(4-methoxyphenyl)methyl-L-glutamine in xylem sap from squash root. Phytochemistry 51, 425–428.
Jameson PE, Letham DS, Zhang R, Parker CW, Badenoch-Jones J. 1987. Cytokinin translocation and metabolism in lupin species. I. Zeatin riboside introduced into the xylem at the base of Lupinus angustifolius stems. Australian Journal of Plant Physiology 14, 695–718.
John PCL, Zhang K, Dong C, Diederich L, Wightman F. 1993. Related proteins in control of cell cycle progression, the switch between division and differentiation in plant development, and stimulation of division by auxin and cytokinin. Australian Journal of Plant Physiology 20, 503–526.
Kamienke M. 1982. Mechanisms preventing the interference of tRNA cytokinins in hormonal regulation. In: Wareing PF, ed. Plant growth substances 1982. New York: Academic Press, 215–224.
Label PH, Sotta B, Miginiac E. 1989. Endogenous levels of abscisic acid and indole-3-acetic acid during in vitro rooting of wild cherry explants produced by micropropagation. Plant Growth Regulation 8, 325–333.
Letham DS. 1994. Cytokinin as phytohormone—site of biosynthesis, translocation and function of translocated cytokinin. In: Mok DWS, Mok MC, eds. Cytokinins: chemistry, activity and function. Boca Raton, FL: CRC Press, 57–80.
Libbert E. 1956. Untersuchungen über die physiologie der adventivwurzelbildung. II. Die korrelative Beeinflussung der adventivwurzelbildung durch andere organs, insbesondere durch die wurzel. Planta 48, 157–189.
Maldiney R, Pelèse F, Pilate G, Sotta B, Sossountzov L, Miginiac E. 1986. Endogenous levels of abscisic acid, indole-3-acetic acid, zeatin and zeatin-riboside during the course of adventitious root formation in cuttings of Craigella and Craigella lateral suppressor tomatoes. Physiologia Plantarum 68, 426–430.
Martin RC, Mok DWS, Snets R, Van Onckelen HA, Mok MC. 2001. Development of transgenic tobacco harboring a zeatin O-glucosyltransferase gene from Phaseolus. In vitro Cellular and Developmental Biology—Plant 37, 354–360.
Mensualidi A, Panizza M, Tognoni F. 1995. Endogenous ethylene requirement for adventitious root induction and growth.
in tomato cotyledons and lavandin microcutting in vitro. *Plant Growth Regulation* **17**, 205–212.

Mitsuhashi M, ShibaoKa H, Shimokoriyama M. 1969. Morphological and physiological characterization of IAA-less-sensitive and IAA-sensitive phase in rooting of *Azukia* cuttings. *Plant Cell Physiology* **10**, 867–874.

Mok MC. 1994. Cytokinins and plant development—an overview. In: Mok DWS, Mok MC, eds. *Cytokinins: chemistry, activity and function*. Boca Raton, FL: CRC Press, 155–166.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum* **15**, 473–497.

Nordström A-C, Eliasson L. 1991. Levels of endogenous indole-3-acetic acid and indole-3-acetylaspartic acid during adventitious root formation in pea cuttings. *Physiologia Plantarum* **82**, 599–605.

Parker CW, Badenoch-Jones J, Letham DS. 1989. Radioimmunoassay for quantifying the cytokinins cis-zeatin and cis-zeatin riboside and its application to xylem sap samples. *Journal of Plant Growth Regulation* **8**, 93–105.

Robbins JA, Kays SJ, Dirr MA. 1983. Enhanced rooting of wounded mung bean cuttings by wounding and ethephon. *Journal of the American Society for Horticultural Science* **108**, 325–329.

Robbins JA, Reid MS, Paul JL, Rost TL. 1985. The effect of ethylene on adventitious root formation in mung bean (*Vigna radiata*) cuttings. *Journal of Plant Growth Regulation* **4**, 147–157.

Satoh S, ShibaoKa T, Wakahoi T, Inouye Y. 1998. Inhibition of the formation of adventitious roots on cucumber hypocotyls by the fractions and methoxybenzylglutamine from xylem sap of squash root. *Journal of Plant Research* **111**, 541–546.

ShibaoKa H. 1971. Effects of indoleacetic, p-chlorophenoxyisobutyric and 2,4,6-trichlorophenoxyacetic acids on three phases of rooting in *Azukia* cuttings. *Plant Cell Physiology* **12**, 193–200.

Singh S, Letham DS, Palni LMS. 1992. Cytokinin biochemistry in relation to leaf senescence. VIII. Translocation, metabolism and biosynthesis of cytokinins in relation to sequential leaf senescence of tobacco. *Physiologia Plantarum* **86**, 398–406.

Smith DR, Thorpe TA. 1975. Root initiation in cuttings of *Pinus radiata* seedlings. II. Growth regulator interactions. *Journal of Experimental Botany* **26**, 193–202.

Soekarjo R. 1965. On the formation of adventitious roots in cuttings of *Coleus* in relation to the effect of indoleacetic acid on the epinastic curvature of isolated petioles. *Acta Botanica Neerlandica* **14**, 373–399.

Went FW. 1939. The dual effect of auxin on root formation. *American Journal of Botany* **26**, 24–29.

Werner T, Motyka V, Strnad M, Schmülling T. 2001. Regulation of plant growth by cytokinin. *Proceedings of the National Academy of Sciences, USA* **98**, 10487–10492.

Zimmerman W, Crocker W, Hitchcock AE. 1933. Initiation and stimulation of roots from exposure of plants to carbon monoxide gas. *Contributions from the Boyce Thompson Institute* **5**, 1–17.