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Cytokinin Inhibition of *Arabidopsis* Root Growth: An Examination of Genotype, Cytokinin Activity, and N^6-Benzyladenine Metabolism

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Abstract. The effects of cytokinins on the in vitro growth of the roots of *Arabidopsis thaliana* seedlings were examined. Root growth was inhibited in a manner dependent upon the type of cytokinin compound, the cytokinin concentration, the *Arabidopsis* genotype, and the duration of exposure to cytokinin. For the cytokinins N^6-benzyladenine (BA), isopentenyl adenine (iP), or dihydrozeatin (DHZ), the concentration required for 50% root growth inhibition differed for each cytokinin and in each of three *Arabidopsis* genotypes tested. iP was the most active cytokinin in inhibiting the root growth of the Ler-0 genotype, whereas iP and BA had equal activity when tested with the Col-2 and Columbia genotypes. DHZ had the lowest activity of the three cytokinins tested in all three genotypes. A brief 1-day exposure of seeds to a root-inhibiting concentration of BA increased root growth compared with seedlings grown without BA; exposure to BA for 3–6 days inhibited root growth. BA metabolism was evaluated after 6 h and 1, 3, and 6 days in Columbia seedlings. BA, N^6-benzyladenosine (BAR), and N^6-benzyladenosine-5′-monophosphate (BAMP) decreased with time, whereas N^6-benzyladenine-7-β-D-glucopyranoside (BA-7-G) and N^6-benzyladenine-9-β-D-glucopyranoside (BA-9-G) accumulated in the growing seedlings. Seven aromatic cytokinins were compared at 5 μM for their effect on Col-3 root growth. BA, BAR, N^6-(*m*-hydroxybenzylamino)adenine, and N^6-(*o*-hydroxybenzylamino)adenine were highly effective in inhibiting root growth, whereas N^6-(*p*-hydroxybenzylamino)adenine produced only a slight decrease in root growth. BA-7-G and BA-9-G did not affect root growth.

Key Words. Cytokinin—Metabolism—Root growth

Plant roots are recognized as active sites of cytokinin biosynthesis, metabolism, and transport (Feldman 1984, Torrey 1976). Evidence for a role for cytokinins in root growth comes from many sources, but transgenic plants that overproduce cytokinins provide some of the strongest evidence that increased endogenous cytokinins reduce root growth (Groot et al. 1995, Binns 1994, Li et al. 1992). Cytokinin metabolism is also important to root growth because these biochemical pathways regulate active compounds, cytokinin transport, temporary storage, and irreversible inactivation (Binns 1994, Jameson 1994, Letham and Palni 1983).

Various plant systems have been used to evaluate the relationship between cytokinin chemical structure and the effects of the hormone on root growth (Bertell and Eliasson 1992, Torrey 1976, Svensson 1972, Butcher and Street 1960). *Arabidopsis* is an excellent plant for studying the relationship between root development and cytokinins because it has a simple root morphology, roots can be observed in vitro, it is amenable to genetic analysis, and exogenous compounds can be applied easily (Holding et al. 1994, Schiefelbein and Benfey 1991). Several groups have used an in vitro root growth inhibition assay to select putative cytokinin mutants in *Arabidopsis* (Deikman and Ulrich 1994, Su and Howell 1992). In these experiments *Arabidopsis* mutants were selected based on resistance to the inhibitory effect of BA on seedling root growth. However, the *ckr1* mutant of Su and Howell (1992) was later shown to be an ethylene-resistant mutant (Cary et al. 1995).

Abbreviations: BA, N^6-benzyladenine; *iP*, isopentenyl-adenine; DHZ, dihydrozeatin; BAR, N^6-benzyladenosine; BAMP, N^6-benzyladenosine 5′-monophosphate; BA-7-G, N^6-benzyladenine-7-β-D-glucopyranoside; BA-9-G, N^6-benzyladenine-9-β-D-glucopyranoside; *m*-OH BA, N^6-(*m*-hydroxybenzylamino)adenine; *o*-OH BA, N^6-(*o*-hydroxybenzylamino)adenine; *p*-OH BA, N^6-(*p*-hyrdoxybenzylamino)adenine; HPLC, high performance liquid chromatography; gFW, grams fresh weight.

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The effects of *Arabidopsis* genotype, cytokinin compound structure, and cytokinin concentration on the root inhibition assay used for mutant selection have not been reported. In addition, the uptake and metabolism of the applied cytokinin have not been examined in relation to patterns of root growth.

In the experiments reported here the effects of various isoprenoid and aromatic cytokinins, cytokinin concentration, and length of exposure to cytokinins were evaluated by measuring the growth of the primary root of young *Arabidopsis* seedlings in four genotypes. In addition, the metabolism of N^6-benzyladenine (BA), a potent root inhibitor used in mutant screens, was characterized in young *Arabidopsis* plants to determine the relationship between cytokinin metabolism and root growth.

Materials and Methods

Arabidopsis Root Growth Bioassays

Arabidopsis seedlings were grown on Murashige and Skoog (MS) media (Murashige and Skoog 1962) containing 30 g/liter sucrose and 8 g/liter agar (Gum-agar A-1296, Sigma, St. Louis, MO). Cytokinins were filter sterilized and added to autoclaved media. The media were dispensed into glass Petri plates (100 × 15 mm, 30 mL/plate).

The four *Arabidopsis* ecotypes used were Columbia CS907 (Col-2), Columbia CS908 (Col-3), Landsberg erecta CS20 (Ler-0), and Columbia (Col) from the Arabidopsis Biological Resource Center, The Ohio State University. Seeds were surface sterilized in 70% ethanol for 3 min followed by treatment with 30% bleach containing 0.01% Tween X-405 for 15 min and then rinsed three times with sterile water. The seeds were arranged on the surface of the culture media in Petri plates, and the plates were placed in a vertical orientation in a growth chamber. Growing conditions consisted of 55 $\mu M\ m^{-2}\ s^{-1}$ white light for 16 h/day at 22°C. Primary root length was measured using an Olympus SZH binocular microscope equipped with an ocular micrometer.

The activities of BA, isopentenyl adenine (iP), and dihydrozeatin (DHZ) in this root growth inhibition assay were compared at 0.008, 0.04, 0.2, 1, or 5 μM concentrations. Primary root length was measured after 6 days of growth on 10 seedlings in three replicate Petri plates for a total of 30 seedlings treatment.

Cytokinin transfer experiments were conducted using *Arabidopsis* Col seeds placed on media containing 5 μM BA in vertical Petri plates. After 1, 2, 3, 4, or 5 days on the media, seedlings were carefully transferred to basal media. Control seedlings were grown on basal media or media with BA for 6 days. Primary root length was measured daily on each individual seedling in the seven treatments. Root growth data were collected for 24 seedlings in each treatment.

The root growth of *Arabidopsis* Col-3 seedlings was measured in the presence of 5 μM concentrations of each of the following seven aromatic cytokinins: BA, N^6-benzyladenosine (BAR), N^6-benzyladenosine 5′-monophosphate (BAMP), N^6-benzyladenine-7-β-D-glucopyranoside (BA-7-G), N^6-benzyladenine-9-β-D-glucopyranoside (BA-9-G), N^6-(*o*-hydroxybenzylamino)adenine (*o*-OH BA), N^6-(*m*-hydroxybenzylamino)adenine. (*m*-OH BA), and N^6-(*p*-hydroxybenzylamino)adenine (*p*-OH BA). The length of the primary root was measured after 6 days on 10 seedlings in three replicate Petri plates for a total of 30 seedlings/treatment. The scarcity of some cytokinin compounds precluded experiments at different concentrations.

BA Uptake and Metabolism

Col seedlings were grown for 15 days on basal MS media containing 1.5 g/liter agar in Magenta jars. Five seedlings were then transferred to one well in a 24-well sterile polystyrene plate (Falcon 3047). Each well contained 1 mL of liquid MS medium with 43 MBq of [^{3}H] BA for a final concentration of 58 μM BA (labeled at an unspecified position on the benzyl ring, specific activity 740 MBq/μmol, CEA, Gif-sur-Yvette, France). The relatively high BA concentration was needed to measure BA metabolism after 6 h and was not phytotoxic; seedlings continued to grow, as demonstrated by a continuous increase in fresh weight and size during the 6-day study. The multiwell plate was placed on a platform shaker to agitate the media gently around the roots of the floating seedlings. Seedlings were removed from three wells (providing three replicates) after 6 hours, 1 day, 3 days, or 6 days of exposure to the labeled BA. The seedlings were rinsed with water, weighed, and placed in Eppendorf tubes with 500 μL of 10% perchloric acid (v/v).

The methods of extraction, HPLC separation, and compound identification were as published previously (Auer et al. 1992a, 1992b, Moffatt et al. 1991). In brief, seedlings were homogenized, the homogenate was centrifuged, and the supernatant was neutralized to pH 6. The samples were prepared for anion exchange HPLC by the addition of nonradioactive cytokinin standards. The ribotide conjugates were analyzed using an anion exchange HPLC column (Partisil 10 SAX, Whatman) with continuous monitoring of the effluent by an on-line liquid scintillation counter (IN/US B-ram, Tampa, FL) and a UV absorbance detector (Waters, Milford, MA). The *Rt* for BAMP was 19.04 min. A fraction was split from the effluent and collected. Cytokinin compounds were separated and quantified using reverse phase HPLC (LiChrospher 60 RP-select B, Merck) using the on-line liquid scintillation counter and UV absorbance detector. The *Rt* for BA, BAR, BA-7-G, and BA-9-G was 19.49, 18.15, 15.21, and 17.02 min, respectively.

The identities of the BA conjugates were checked using enzymatic or acid hydrolysis using previously established procedures (Auer et al. 1992b, Moffatt et al. 1991). Alkaline phosphatase treatment was performed on radioactive metabolites coeluting with cold standards of BAMP. In brief, an HPLC fraction (0.9 mL) was dried in vacuo and combined with 200 μL of 0.5 M Tris-HCl + 5 mM $MgCl_2$, 1,800 μL of water, and 2 units of alkaline phosphatase and held at 37°C for 3 h. The sample was prepared for HPLC and injected onto a reverse phase HPLC column. Radioactivity and UV absorbance were recorded as described above. Acid hydrolysis was performed on radioactive HPLC fractions coeluting with BAR, BA-7-G, and BA-9-G. An HPLC fraction was dried in vacuo and combined with 1 mL of 1 N HCl and held at 100°C for 3 h. The samples were analyzed using reverse phase HPLC as described above.

Results

Cytokinin Activity in the Arabidopsis Root Growth Inhibition Assay

Seeds of *Arabidopsis* genotypes Ler-0, Col-2, and Col were germinated and grown on medium containing BA, iP, or DHZ. Without exogenous cytokinin, the mean primary root length at 6 days varied among genotypes; the Ler-0 root length was 15.9 ± 0.85 mm, Col-2 root length was 8.3 ± 0.46 mm, and Col root length was 20.7 mm ± 0.59. These differences could not be attributed to delays in seed germination. All three cytokinins tested reduced primary root growth (Fig. 1). However, the cytokinin

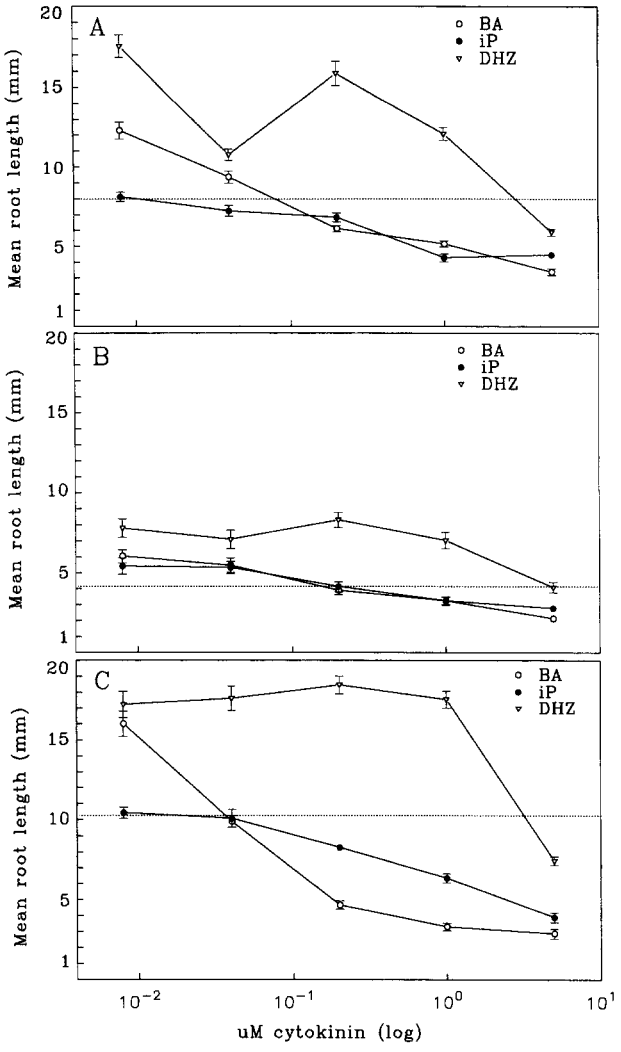

Fig. 1. Root growth response of *Arabidopsis* seedlings from three genotypes to the exogenous cytokinins BA, iP, and DHZ. Seedlings were grown on media containing, 0.008, 0.04, 0.2, 1, or 5 μM BA, iP, or DHZ. The mean primary root length (mm) of 6-day-old seedlings ± S.E. is shown. The *dotted line* represents 50% inhibition of root growth for the genotype. **A,** *Arabidopsis* Ler-0 genotype. The mean root length of Ler-0 seedlings grown on media without cytokinin was 15.9 ± 0.85 mm. **B,** *Arabidopsis* Col-2 genotype. The mean root length of Col-2 seedlings grown on media without cytokinin was 8.3 ± 0.46 mm. **C,** *Arabidopsis* Col genotype. The mean root length of Col seedlings grown on media without cytokinin was 20.7 mm ± 0.59.

concentration at which root growth was inhibited by 50% differed among genotypes. For example, iP produced 50% root inhibition at 0.008 μM in Ler-0, 0.04 μM in Col, and 0.2 μM in Col-2. BA produced 50% root inhibition between 0.04 and 0.2 μM. DHZ had the weakest inhibitory activity, requiring between 1 and 5 μM for 50% inhibition. The decrease in Ler-0 root length at 0.4 μM DHZ is surprising because Col-2 and Col seedlings grown on the same medium did not exhibit this pattern.

Transfer experiments were conducted by moving Col

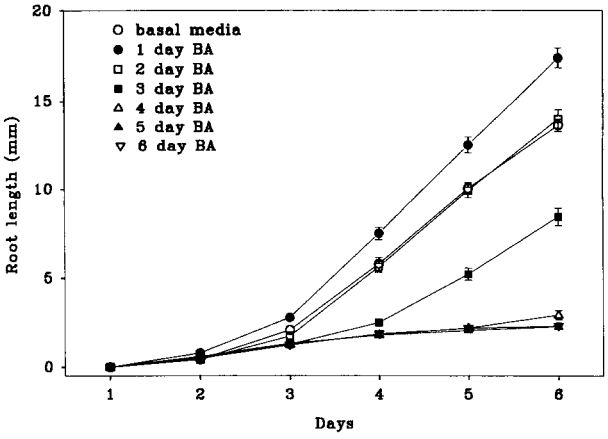

Fig. 2. Root growth of *Arabidopsis* seedlings on media containing 5 μM BA for 1, 2, 3, 4, or 5 days prior to transfer to basal media and continued growth to 6 days of age. Additional treatments consisted of basal media or 5 μM BA for 6 days without transfer. Primary root length was measured daily on each seedling in the seven treatments. Mean root length (mm) ± S.E. is shown.

seeds or young seedlings from media with 5 μM BA to basal media after 1, 2, 3, 4, or 5 days of exposure to the cytokinin. In addition, seedlings were left on basal media or media with BA for 6 days. Daily measurement of primary root length showed a recovery of root growth for those seedlings removed from BA on days 1, 2, or 3 (Fig. 2). Root length for seedlings treated with BA for 1 day was greater than seedlings on basal media. In effect, these plants received BA only during seed imbibition because roots could not be detected visually until the 2nd or 3rd day in culture. Seedlings without BA and those exposed to BA for 2 days had the same pattern of root growth throughout the experiment. Seedlings on BA for 4, 5, or 6 days showed small increases in root length up to day 4. Seedlings on BA for 4 days showed some root growth at day 6, whereas seedlings on BA for 5 or 6 days had almost no root growth.

Aromatic Cytokinins: Uptake, Metabolism, and Activity

Arabidopsis seedlings (14-day-old Col) were exposed to [^{3}H]BA for 6 h or 1, 3, or 6 days. Expressed as a percentage of the [^{3}H]BA available, seedlings had a mean uptake of 11.6% at 6 h, 27.5% at 1 day, 39.7% at 3 days, and 44.4% at 6 days.

The cytokinin compounds recovered from *Arabidopsis* seedlings were BA, BAR, BAMP, BA-7-G, BA-9-G, in agreement with the results of Moffatt et al. (1991). Identification of the compounds was based on their HPLC retention times and supported by the results of the enzymatic or acid hydrolysis treatments performed on the BA conjugates. Recovery of radioactive label from plant tis-

Table 1. N^6-Benzyladenine metabolism in *Arabidopsis* seedlings. *Arabidopsis* Col seedlings (14 days old) were floated on 1 mL of MS medium containing 43 MBq of [^{3}H]BA (58 μM [^{3}H]BA) for 6 h or 1, 3, or 6 days. the cytokinin compounds recovered are given as mean nmol/gFW ± S.E. The percentage of the total pool of radioactive cytokinins is given in parentheses.

Time	BA	BAR	BAMP	BA-7-G	BA-9-G
6 h	30.8 ± 18.4 (24.0%)	3.9 ± 2.0 (3.0)	18.8 ± 11.5 (14.7)	13.0 ± 1.9 (10.1)	61.8 ± 11.4 (48.2)
1 day	6.9 ± 1.3 (4.4)	5.3 ± 0.7 (3.4)	11.0 ± 9.5 (7.0)	19.9 ± 2.3 (12.6)	114.3 ± 10.3 (72.6)
3 days	1.1 ± 1.1 (0.4)	0 (0)	2.4 ± 1.3 (0.9)	37.5 ± 4.1 (13.8)	231.3 ± 23.8 (84.9)
6 days	0 (0)	0 (0)	0.4 ± 0.1 (0.3)	21.9 ± 1.5 (15.1)	123.0 ± 10.5 (84.6)

Table 2. *A. thaliana* Col-3 root length *in vitro* with BA and BA conjugates. *Arabidopsis* Col-3 seedlings were grown in the presence or absence of cytokinins (basal MS media). Cytokinin treatments consisted of 5 MM BA, BAR, BA-9-G, BA-7-G, *m*OH BA, *o*-OH BA, or *p*-OH BA. Data shown represent mean primary root length (mm) of 6-day-old seedlings ± S.E.

Cytokinin compound	Root length (mm)
No hormone	16.9 ± 0.65
BA	3.0 ± 0.20
BAR	3.0 ± 0.25
BA-7-G	18.6 ± 0.62
BA-9-G	18.6 ± 0.62
m-OH BA	3.1 ± 0.28
o-OH BA	4.1 ± 0.30
p-OH BA	15.0 ± 0.85

sue was greater than 95%, and the identification of radioactive cytokinins was greater than 86% of the total radioactive label in the extracted samples.

Over 6 days the concentration of BA, BAR, and BAMP within the seedlings decreased, representing a continuous reduction in the interconvertible pool of BA compounds (Table 1). For example, BA decreased from 30.8 to 1.1 nmol/gFW from 6 h to 3 days and could not be detected at 6 days. After 6 days, neither BA or BAR could be detected, and BAMP was only 0.4 nmol/gFW.

N-Glucoside conjugates accumulated in the seedlings during the experiment (Table 1). BA-9-G was the predominant compound throughout the study and increased from 61.8 nmol/gFW after 6 h to 231.3 nmol/gFW at 3 days. An apparent decease from days 3 to 6 was due to the continued increase in seedling fresh weight while the percentage of BA-9-G and total pool of radioactive cytokinins remained constant. BA-7-G followed a similar pattern.

The activities of BA and six related conjugates were compared in Col-3 seedlings (Table 2). BA, BAR, *m*-OH BA, and *o*-OH BA were highly effective in inhibiting root growth at 5 μM. *p*-OH BA inhibited root growth slightly compared with seedlings grown without cytokinin, as determined by a *t*-test comparison of root lengths. BA-7-G and BA-9-G did not affect root growth.

Discussion

Roots are quite sensitive to applied cytokinins, and endogenous cytokinins are undoubtedly involved in root growth and development (Schiefelbein and Benfey 1991, Feldman 1984, Torrey 1976). These experiments demonstrated that the effects of exogenous cytokinins on the growth of the roots of *Arabidopsis* seedlings were dependent upon the structure of the cytokinin compound, the cytokinin concentration, the *Arabidopsis* genotype, and the duration of exposure to cytokinin. It is difficult to determine why exogenous cytokinins exhibited different activity levels in the root growth bioassays (Fig. 1). Besides the interaction of the chemical structure with a receptor, differences in uptake, metabolism, tissue sensitivity, and/or other factors may explain differences in compound activity (Evans et al. 1994, Jameson 1994, Letham and Palni 1983, Matsubara 1980). Duration of exposure also affected root growth response. Seeds given a brief 1-day exposure to inhibitory concentrations of BA had longer roots than control seeds without cytokinin exposure (Fig. 2). Therefore, a short exposure to cytokinin during seed imbibition promoted root growth. Although emerging roots were first observed between days 2 and 3 in all treatments, it is possible that the cytokinin caused slightly earlier seed germination followed by vigorous root growth.

Three hydroxylated BA compounds had different effects on root growth depending on the position of the hydroxyl group on the benzyl ring (Table 2). Cytokinins with an aromatic ring are not common in plant tissues, although *Populus* leaves have been identified as a source of hydroxylated BA compounds (Strnad et al. 1992, Kaminek et al. 1987). In four different bioassays, hydroxylation of BAR in the *ortho* or *para* position decreased activity, whereas the *meta* position generally increased activity (Kaminek et al. 1987). In the *Arabidopsis* root growth inhibition assay, hydroxylation of BA in the *meta* or *ortho* position produced a high level of activity similar to BA. In contrast, hydroxylation in the *para* position produced a cytokinin with very low activity.

Studies using the *Arabidopsis* mutants *ckr1* (cytokinin resistant) and *ein* (ethylene resistant) suggest that the effects of exogenous BA on dark-grown *Arabidopsis* seedling roots are mediated by the increased production of ethylene (Cary et al 1995). In this study, the ethylene

triple response was not observed in light-grown seedlings. Undoubtedly, an interaction can exist among cytokinins, ethylene, and auxin in some developmental events (Bertell and Eliasson 1992, Yoshii and Imaseki 1981, Corriveau and Krul 1986, Svensson 1972). It is not known if all active exogenous cytokinins affect root growth through the production of ethylene.

The metabolism of BA has been studied in a variety of plant species during different types of developmental events (Binns 1994, Jameson 1994, Auer et al. 1992a, 1992b, Moffatt et al. 1991, Laloue and Pethe 1982). In general, BA can be metabolized to riboside or ribotide conjugates that can be converted back to the putative active free base form. The interconvertible cytokinins found in *Arabidopsis* were BA, BAR, and BAMP, which decreased continuously for 6 days (Table 1). In contrast, BA was metabolized quickly to *N*-glucoside conjugates that accumulated in the seedlings. *Arabidopsis* seedlings grown in the presence of BA-9-G or BA-7-G did not show any root growth inhibition (Table 2), consistent with other reports that *N*-glucoside conjugates are inactivation products (Jameson 1994, Laloue and Pethe 1982). Experiments by Moffatt et al. (1991) with 4-day-old *Arabidopsis* seedlings exposed to [^{3}H]BA for up to 23 h showed similar trends in BA metabolism. Together, BA metabolism and root growth experiments suggested that the interconvertible BA pool decreased quickly, but root growth remained greatly inhibited during the 6 days in culture (Table 1 and Fig. 2). How does BA produce a strong growth response in plants when the putative active compound (BA) is rapidly removed from the plant tissue? First, it is possible that active BA molecules are present but below the limits of detection. Second, BA may trigger important changes in other hormones such as ethylene or endogenous cytokinins, resulting in root growth inhibition. Although *Arabidopsis* mutants are a powerful tool to study plant hormones, better information about cytokinin biochemistry and activity could lead to more effective selection screens for cytokinin mutants.

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References

Auer CA, Cohen JD, Laloue M, Cooke TJ (1992a) Comparison of benzyladenine metabolism in two *Petunia hybrida* lines differing in shoot organogenesis. Plant Physiol 98:1035–1041

Auer CA, Laloue M, Cohen JD, Cooke TJ (1992b) Uptake and metabolism of benzyladenine during shoot organogenesis in *Petunia* leaf explants. Plant Growth Regul 11:105–114

Bertell G, Eliasson L (1992) Cytokinin effects on root growth and possible interactions with ethylene and indole-3-acetic acid. Physiol Plant 84:255–261

Binns AN (1994) Cytokinin accumulation and action: Biochemical, genetic, and molecular approaches. Annu Rev Plant Physiol 45:173–196

Butcher DN, Street HE (1960) Effects of kinetin on the growth of excised tomato roots. Physiol Plant 13:46–55

Cary AJ, Liu W, Howell SH (1995) Cytokinin action is coupled to ethylene in its effects on the inhibition of root and hypocotyl elongation in *Arabidopsis thaliana* seedlings. Plant Physiol 107:1075–1082

Corriveau JL, Krul WR (1986) Inhibition of benzyladenine-induced radial expansion in soybean hypocotyls by silver ion. J Plant Physiol 126:297–300

Deikman J, Ulrich M (1994) A novel cytokinin-resistant mutant of *Arabidopsis* with abbreviated shoot development. Planta 195: 440–449

Evans ML, Ishikawa H, Estelle MA (1994) Responses of *Arabidopsis* roots to auxin studied with high temporal resolution: Comparison of wild type and auxin-response mutants. Planta 194:215–222

Feldman LJ (1984) Regulation of root development. Annu Rev Plant Physiol 35:223–242

Groot SPC, Bouwer R, Busscher M, Lindhout P, Dons HJ (1995) Increases of endogenous zeatin riboside by introduction of the *ipt* gene in wild type and the lateral suppressor mutant of tomato. Plant Growth Regul 16:27–36

Holding DR, McKenzie RJ, Coomber SA (1994) Genetic and structural analysis of five *Arabidopsis* mutants with abnormal root morphology generated by the seed transformation method. Ann Bot 74:193–204

Jameson PE (1994) Cytokinin metabolism and compartmentation. In: Mok DWS, Mok MC (eds) Cytokinins: Chemistry, activity, and function. CRC Press, Boca Raton, pp 113–128

Kaminek M, Vanek T, Motyka V (1987) Cytokinin activities of N^6-benzyladenosine derivatives hydroxylated on the side-chain phenyl ring. J Plant Growth Regul 6:113–120

Laloue M, Pethe C (1982) Dynamics of cytokinin metabolism in tobacco cells. In: Wareing PF (ed) Plant growth substances. Academic Press, New York, pp 185–195

Letham DS, Palni LMS (1983) The biosynthesis and metabolism of cytokinins. Annu Rev Plant Physiol 34:163–197

Li Y, Hagen G, Guilfoyle TJ (1992) Altered morphology in transgenic tobacco plants that overproduce cytokinins in specific tissues and organs. Dev Biol 153:386–395

Matsubara S (1980) Structure-activity relationships of cytokinins. Phytochemistry 19:2239–2253

Moffatt B, Pethe C, Laloue M (1991) Metabolism of N^6-benzyladenine is impaired in a mutant of *Arabidopsis thaliana* lacking adenine phosphoribosyltransferase activity. Plant Physiol 95:900–908

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Schiefelbein JW, Benfey PN (1991) The development of plant roots: New approaches to underground problems. Plant Cell 3:1147–1154

Strnad M, Peters W, Beck E, and Kaminek M (1992) Immunodetection and identification of N^6-(*o*-hydroxybenzylamino) purine as a naturally occurring cytokinin in *Populus* × *canadensis* Moench cv. Robusta leaves. Plant Physiol 99:74–80

Su W, Howell SH (1992) A single genetic locus, *Ckr1*, defines *Arabidopsis* mutants in which root growth is resistant to low concentrations of cytokinin. Plant Physiol 99:1569–1574

Svensson SB (1972) A comparative study of the changes in root growth, induced by coumarin, auxin, kinetin, and gibberelic acid. Physiol Plant 26:115–135

Torrey JG (1976) Root hormones and plant growth. Annu Rev Plant Physiol 27:435–459

Yoshii H, Imaseki H (1981) Biosynthesis of auxin-induced ethylene: Effects of indole-3-acetic acid, N^6-benzyladenine, and abscisic acid on endogenous levels of 1-aminocyclopropane-1-carboxylic acid (ACC) and ACC synthase. Plant Cell Physiol 22:369–379