Noninvasive Expressions of *ipt* in Whole Plants or Roots through pOp/LhG4 Indicate a Role of Plant Aerial Parts and Light in Cytokinin Synthesis and Root Inhibition

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Abstract To study plant roots in response to *ipt* gene activation, the transcriptional fusions of ipt-GUS and GUS-ipt were expressed in roots or in whole plants of *Arabidopsis* under the control of a root-specific promoter TobRT7 or a CaMV35S promoter through the pOp/LhG4 system in noninvasive conditions. The transgenic plants with constitutive expression of ipt-GUS or GUS-ipt showed 15–25-fold or 1–2-fold increased cytokinin levels, respectively. ipt-GUS-expressing *Arabidopsis* had severe root inhibition, enlarged shoot apical parts, serrated leaves, and no or few sterile flowers, whereas GUS-ipt-expressing *Arabidopsis* grew faster, flowered early, and had more lateral shoots. However, when ipt-GUS and GUS-ipt were specially expressed in roots under the control of TobRT7, neither cytokinin content in roots or shoots nor phenotypes were altered. In cytokinin-overproducing, ipt-GUS-expressing *Arabidopsis*, the light and aerial parts of plants played an important role for cytokinin synthesis and root inhibition, and the *ipt* gene was vigorously expressed at the shoot apical parts. Meanwhile, calli were induced at the shoot apical parts of some cytokinin-overproducing, ipt-GUS-expressing *Arabidopsis*.

Keywords Noninvasive expression · Cytokinin synthesis · Constitutive expression · Root-specific expression · *ipt* Gene · pOp/LhG4

Abbreviations

ipt	Isopentenyl transferase gene
GUS	β-1,3-glucuronidase gene
cZ	*cis*-zeatin
cZR	*cis*-zeatin riboside
cZRP	*cis*-zeatin riboside phosphate
DHZ	Dihydrozeatin
DH Z7G	Dihydrozeatin-7-glucoside
DHZR	Dihydrozeatin riboside
DHZRP	Dihydrozeatin riboside phosphate
DHZROG	Dihydrozeatin riboside O-glucoside
DH Z9G	Dihydrozeatin-9-glucoside
iP	N^6-Isopentenyladenine
iP7G	Isopentenyl adenine-7-glucoside
iPR	Isopentenyl riboside
iPRP	Isopentenyl riboside phosphate
Z	Zeatin
Z7G	Zeatin-7-glucoside
Z9G	Zeatin-9-glucoside
ZOG	Zeatin-O-glucoside
ZOX	Zeatin oxidase
ZR	Zeatin riboside
ZROG	Zeatin riboside O-glucoside
ZRP	Zeatin riboside phosphate

Introduction

Cytokinins, N^6-substituted adenine derivatives, are a class of plant hormones that have been implicated in many aspects of plant growth and development, including promotion of cell division, initiation of shoot growth, release of axillary bud, delay in leaf senescence, and induction of photomorphogenic development. The *ipt* gene isolated from the plant pathogenic bacterium *Agrobacterium tumefaciens* encodes

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the key enzyme isopentenyl transferase in the cytokinin biosynthesis pathway (Akiyoshi and others 1984; Barry and others 1984). *ipt* has been used to construct transgenic plants with elevated levels of endogenous cytokinins. In those transgenic plants the *ipt* gene was driven by various promoters, including constitutive and native promoters (Smigocki and Owens 1989; Geng and others 2001; van der Graaff and others 2001), heat shock promoters (Smigocki 1991; van Loven and others 1993; Faiss and others 1997; Schmülling and others 1998; Rupp and others 1999), a light-inducible promoter (Beisberger and others 1991), a wounding-inducible promoter (Smigocki and others 1993; Smigocki 1995), tetracycline-inducible promoters (Redig and others 1996; Faiss and others 1997), a hormone-specific promoter (Li and others 1992), a senescence-specific promoter (Gan and Amasino 1995), and organ- or tissue-specific promoter (Martineau and others 1994; Geng and others 2002). Although a number of studies have been done on the role of cytokinins in cell development, how the altered hormonal concentrations in vivo trigger developmental events is still not completely understood. The question of whether the exogenous addition of cytokinins adequately duplicates the endogenous hormone action remains. Expression of *ipt* driven by a constitutive promoter usually increases endogenous cytokinins to an extreme amount, which results in drastic changes in morphology and sterility. Thus, many studies on this problem observe the effects of *ipt* gene expression under inducible conditions or the effects of tissue-specific expression. Inducible *ipt* expression requires exposing the plants to abnormal conditions for short periods, which transiently produce high and unphysiologic cytokinin levels. Thus, continuous elevated cytokinin levels in the physiologic range cannot be maintained this way (van Loven and others 1993; Wang and others 1997b).

Here we use a novel method to regulate *ipt* expression under the pOp/LhG4 transcription activation system (Moore and others 1998), and cytokinins are constantly produced under noninvasive conditions. In this study *ipt* is transcriptionally fused with the *GUS* gene in the manner of ipt-GUS and GUS-ipt, then placed under the control of a chimeric pOp promoter, which results in pOp-ipt-GUS and pOp-GUS-ipt reporters (Fig. 1A). The pOp promoter consists of a CaMV35S minimal promoter (TATA box) and two bacterial *lac* operators cloned upstream of the TATA box, which lacks intrinsic transcription activation because the bacterial *lac* operator sequences upstream of the TATA box are not sufficient for recognition by endogenous plant transcription factors. Thus, the transgenic plants normally generate and propagate without any interference from the transgenes (Moore and others 1998; Guo and others 2005). In parallel, lines express a novel chimeric transcription activation factor (LhG4) under the control of a 35S promoter and a root-specific promoter,

which results in 35S-LhG4 and Tob-LhG4 activators (Fig. 1A). LhG4 consists of the DNA-binding domain of a *lacI^his* repressor and the transcription activation domain of a yeast Gal4 transcription activator. The *lacI^his* DNA-binding domain can specifically recognize the pOp promoter that is integrated in the plant genomes (Moore and others 1998; Guo and others 2005). Upon LhG4 binding to the pOp promoter, the Gal4-binding transcription activation domain initiates the transcription of ipt-GUS and GUS-ipt fused genes behind the pOp promoter. The expression of ipt-GUS and GUS-ipt fused genes is achieved only upon crossing pOp-ipt-GUS and pOp-GUS-ipt reporter lines with activator lines (35S-LhG4 or Tob-LhG4). Moreover, the expression of ipt-GUS and GUS-ipt fused genes is restricted only in those cells in which the transcription factor LhG4 is expressed (Fig. 1A).

In previous work, ipt-GUS fused genes were constitutively expressed in pOp-ipt-GUS transgenic *Arabidopsis* modulated through the pOp/LhG4 system, in which the pOp promoter was activated by a constitutively expressed transcription factor LhG4, and cytokinins were highly increased in vivo. A number of visibly altered phenotypes, including enlarged and retarded-growth phenotypes, were observed. The rate of retarded-growth phenotypes and enlarged phenotypes was affected by light (Guo and others 2005). Anthocyanins and lignins accumulated in the shoots, especially in the shoot apical meristems, and light increased the rate of accumulation (Guo and others 2005). Thus, the shoots or the shoot apical parts seemed more sensitive to the increased cytokinins, and light seemed to be a signal for phenotype alteration in cytokinin-overproducing, ipt-GUS-expressing *Arabidopsis* (Guo and others 2005).

Roots were postulated to be the main site of cytokinin synthesis (Chibnall 1939; Mothes and Engelbrech 1962; Richmond and Lang 1957). However, subsequent evidence showed that other tissues, including leaves, shoot apical meristems, and immature seeds, could produce cytokinins (summarized by Letham 1994). To advance our understanding in these areas, in this article we allocated *ipt* gene expression to whole plants or to the roots through the pOp/LhG4 system, which was obtained by crossing pOp-GUS-ipt and pOp-ipt-GUS transgenic reporter lines with a constitutively expressing or a root-expressing transcription factor LhG4 (35S-LhG4, Tob-LhG4) in the pOp/LhG4 system (Fig. 1A). The objective of this research was to study plant root responses to *ipt* gene activation through the controlled expression of the *ipt* gene in roots or in whole plants of *Arabidopsis thaliana* under noninvasive conditions. Because no good anti-ipt antibodies are currently available, the fusion of *ipt* with the *GUS* gene is one of the convenient ways to confirm the localization of *ipt* expression. Thus, ipt-GUS
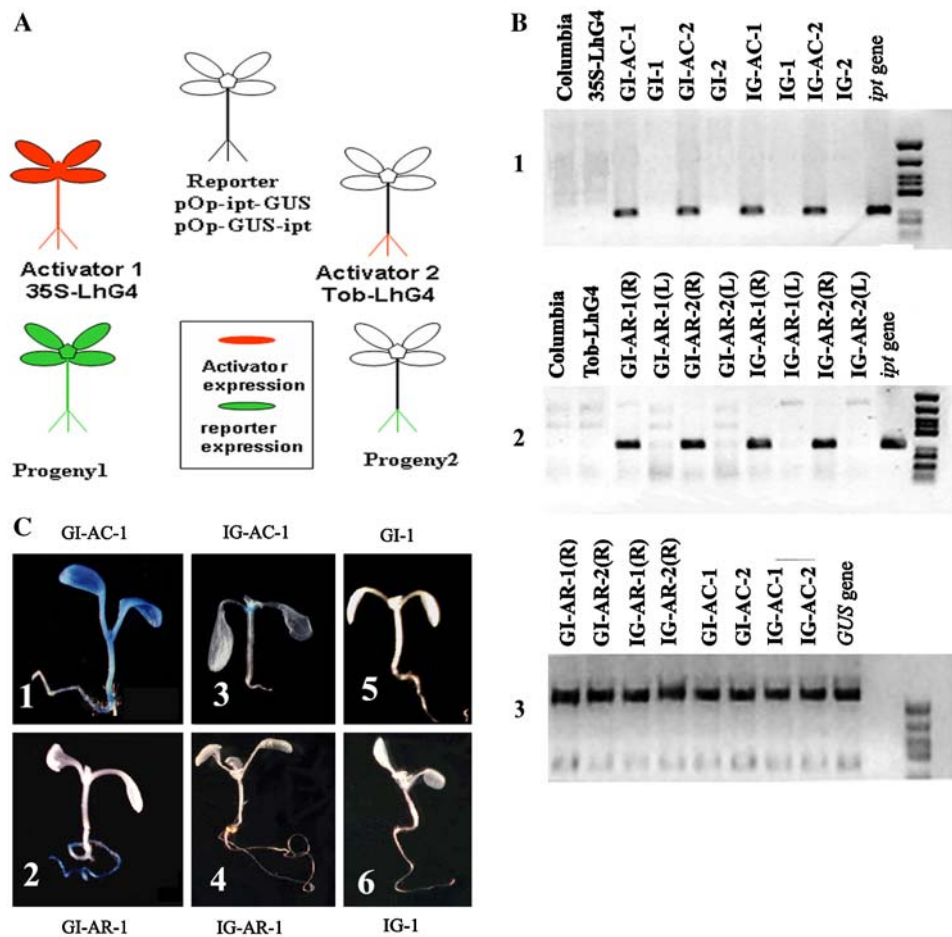
Fig. 1 ipt/GUS activation by the pOp/LhG4 system. (**A**) pOp/LhG4 transcription system. (**B**) RT-PCR of *ipt* and *GUS* genes in ipt-GUS- and GUS-ipt-expressing *Arabidopsis* and inactive reporter isolines. B-1: RT-PCR of *ipt* gene, which was activated by 35S-LhG4; B-2: RT-PCR of *ipt* gene, which was activated by Tob-LhG4; B-3: RT-PCR of *GUS* gene, which was activated by 35S-LhG4 and Tob-LhG4. (**C**) GUS staining in GUS-ipt- and ipt-GUS-expressing *Arabidopsis* and their inactive reporter isolines. (1) GUS-ipt expression was activated by 35S-LhG4; (2) GUS-ipt expression was activated by Tob-LhG4; (3) ipt-GUS expression was activated by 35S-LhG4; (4) ipt-GUS expression was activated by Tob-LhG4; (5) GUS staining in the GUS-

ipt reporter line; (6) GUS staining in the ipt-GUS reporter line. The common label interpretations are as follows: IG-1, IG-2 and GI-1, GI-2: pOp-ipt-GUS and pOp-GUS-ipt independent reporter lines, respectively; IG-AC-1, IG-AC-2 and GI-AC-1, GI-AC-2: the progenies of IG-1, IG-2 and GI-1,GI-2 were in crosses with activator line 35S-LhG4; IG-AR-1, IG-AR-2 and GI-AR-1, GI-AR-2: the progenies of IG-1, IG-2 and GI-1, GI-2 were in crosses with Tob-LhG4; 35S-LhG4: activator line driven by CaMV35S promoter; Tob-LhG4: activator line driven by TobRB7 promoter; Columbia: wild-type *Arabidopsis*. In (**B**), R and L behind IG-AR-1, IG-AR-2 are the roots and upper parts of seedlings

and GUS-ipt transcriptional fusions were used in this research.

Materials and Methods

Reporter *Arabidopsis*

The coding sequence of the *isopentenyl transferase* (*ipt*) gene (AF242881 from 7865 to 8627 bp) from the plant pathogenic bacterium *Agrobacterium tumefaciens* was fused in-frame with the coding sequence of *GUS*, which resulted in ipt-GUS and GUS-ipt transcriptional fusions. The resulting two-gene combinations were placed

downstream of the chimeric promoter pOp. The constructs pOp-ipt-GUS and pOp-GUS-ipt were inserted into a pVKH18 plant binary vector backbone with the *HPT* gene as a selectable marker and transformed into *Arabidopsis* Columbia through *Agrobacterium tumefaciens* GV3101 by vacuum infiltration. After three generations, 20 independent single-locus lines of pOp-GUS-ipt and 25 independent single-locus lines of pOp-ipt-GUS were selected and designated as reporters. IG-1, IG-2, IG-3 and GI-1, GI-2 were the representatives of single-locus and homozygous reporter lines of pOp-ipt-GUS and pOp-GUS-ipt, respectively. The transgenic reporter *Arabidopsis* grew normally without any interruption from the transgenes because the pOp promoter is silent.

Activator *Arabidopsis*

A CaMV35S promoter or a root-specific promoter from tobacco, TobRB7, was placed upstream of LhG4 and inserted into a pBINPLUS plant binary vector backbone with the *NPTII* gene for selection. The resulting combinations were transformed into *Arabidopsis* Columbia through *A. tumefaciens* GV3101 by vacuum infiltration. After three generations, 15 independent single-locus lines of CaMV35S-LhG4 and 20 independent single-locus lines of Tob-LhG4 were selected. AC-8-LhG4 was driven by CaMV35S and AR-6 (LhG4 was driven by TobRB7); single-locus and homozygous activator lines were used in this research.

Plant Cultivation

Seeds were sown on the surface of MS ($1\times$) medium with 1% sucrose or in a mixture of organic soil and sand (1:1). The seeds on MS medium were kept at 4–8°C for 2 days, then transferred to tissue culture conditions in 16-h light/8-h dark photoperiod at 22°C and light intensity of 45–60 μmol m^{-2} s^{-1}, or dark conditions at 22°C. The seeds planted in soil were cultured under the above tissue culture conditions (22°C, 16-h light/8-h dark).

Cytokinin Analysis

For root collection the seeds derived from the progenies of pOp-ipt-GUS and pOp-GUS-ipt in crosses with Tob-LhG4 (IG-AR and GI-AR) and their inactive isolines (IG and GI) were sown on the surface of a wet paper submerged by MS ($1\times$) liquid medium with 1% sucrose for 12 days. The seeds derived from the progenies of pOp-ipt-GUS and pOp-GUS-ipt in crosses with 35-LhG4 (IG-AC and GI-AC) and their inactive isolines (IG and GI) were sown on the surface of MS ($1\times$) solid medium with 1% sucrose for 30 days or 12 days. Around 50–100 mg of seedlings, shoots, or roots were used for cytokinin analysis. The methods for cytokinin extraction and purification were the same as previously described by Guo and others (2005). Twenty-two kinds of cytokinins were analyzed as they are listed here: Z, cZ, DHZ, ip, ZR, cZR, DHZR, iPR, ZRP, cZRP, DHZRP, iPRP, ZOG, ZROG, DHZROG, ZOX, Z7G, DHZ7G-1, DHZ7G-2, iP7G, Z9G, and DHZ9G.

GUS Histochemical Assays

After 1 week of seed germination on MS medium with 1% sucrose under tissue culture conditions, IG-AC, GI-AC and IG-AR, GI-AR or their inactive isoline seedlings were incubated in a solution of 0.3% formaldehyde (prepared with 10 mM MES and 0.3 M mannitol, pH 5.6) for 45 min at room temperature, and then washed three times with 50 mM NaH$_2$PO$_4$ (pH 7.0). The above seedlings were completely submerged in X-gluc solution, which contained 8 ml of 50 mM sodium pH 7.2 phosphate buffer containing 9 mg of 5-bromo-4-chloro-3-indolyl-D-glucuronide, 8 μl of triton X-100, and 8 μl of mercaptoethanol, for 8–12 h at 37°C. The stained seedlings were bleached with 70–80% ethanol until the background (chlorophylls) disappeared.

RT-PCR Analysis

After 12 days of seed germination on the surface of a wet paper submerged by MS ($1\times$) liquid medium with 1% sucrose under tissue culture conditions, approximately 50–100 mg of seedlings, shoots, or roots were harvested for isolating total RNA by TRIZOL reagent. Reverse transcription was performed immediately after RNA isolation, and each reaction was in 40-μl volume containing 5 μg of total RNA in 5 μl, 2 μl (5 pmol/μl) of oligo-dT, 8 μl of $5\times$ RT buffer, 4 μl of DTT (10 mM), 2 μl of dNTP (10 mM), and 17 μl of ddH$_2$O. The reaction was incubated at 42°C for 2 min and then 2 μl of superscript II RT (200 U/μl) were added. The reaction was incubated at 42°C for 50 min and stopped at 70°C for 15 min.

The PCR reactions were in a 50-μl mixture containing 2 μl of the above RT reaction buffer, 6 μl (5 pmol/μl) of *ipt* gene-specific primers (primer 1: TTT CGCTTGATCGG GTCC; primer 2: GGTCTCTTGGTCGGGTAA), or 6 μl of *GUS* gene-specific primers (primer 1: TAATCCGCACC TCTGGCAACCG; primer 2: TGTTTGCCTCCCTGCTG CGGTT), 3 μl of dNTPs (5 mM), 5 μl of $10\times$ PCR buffer, and 34 μl of ddH$_2$O. The mixture was preheated at 94°C for 5 min before 3 U of *Taq* DNA polymerase in 3 μl were added. PCR cycle conditions were 94°C for 45 s, 52°C for 45 s, 72°C for 90 s for *ipt* gene, and 94°C for 45 s, 54°C for 45 s, 72°C for 90 s for *GUS* gene.

Results

ipt Gene Activation by 35S-LhG4 and Tob-LhG4

In the pOp/LhG4 transcription system, the pOp promoter is activated by the LhG4 transcription factor, and the expression of *ipt* and *GUS* downstream of pOp is restricted to those cells in which the transcription factor LhG4 is expressed. Thus, ipt-GUS and GUS-ipt fusion genes were expressed in whole plants when the reporter *Arabidopsis* (IG-1, IG-2 and GI-1, GI-2) was crossed with the activator line 35S-LhG4 (AC-8), or in the roots when the reporter was crossed with the activator line Tob-LhG4 (AR-6) (Fig. 1A). The seedlings that developed from the seeds of the crosses were designated IG-AC and GI-AC when pOp-

ipt-GUS and pOp-GUS-ipt were activated by 35S-LhG4, or IG-AR and GI-AR when they were activated by Tob-LhG4. RT-PCR showed that the transcription of *ipt* was observed only in IG-AC and GI-AC or only in the roots of IG-AR and GI-AR; the transcription rate of *ipt* was similar in IG-AC, GI-AC and IG-AR, GI-AR (Fig. 1B).

GUS Gene Activation in ipt-GUS- and GUS-ipt-Expressing *Arabidopsis*

Reporter lines of pOp-ipt-GUS and pOp-GUS-ipt transgenic *Arabidodpsis* were in crosses with the 35S-LhG4 activator line (AC-8). Recognizable GUS staining could be detected in 19 of 20 single-locus lines of GI-AC (Fig. 1C-1), but only in some plant apical parts of IG-AC (Fig. 1C-3). No GUS staining was found in their inactive reporter lines (Fig. 1C-5 and 1C-6). Ninety-five percent of GI-AC and 48% of IG-AC had detectable GUS staining. Subsequently, we chose reporter lines of IG-1, IG-2 and GI-1, GI-2 that showed clear GUS staining when they were activated by 35S-LhG4 crossed with the activator line Tob-LhG4 (AR-6). Visible GUS staining was observed in the roots of GI-AR (Fig. 1C-2) but not in IG-AR (Fig. 1C-4). RT-PCR showed that *GUS* transcription was detected, and the transcription rate of *GUS* was similar in IG-AC, GI-AC, IG-AR, and GI-AR (Fig. 1B-3).

Cytokinin Levels in ipt-GUS- and GUS-ipt-Expressing *Arabidopsis*

When the seedlings were 12 or 30 days old on MS medium, the seedlings from reporter lines IG-1, IG-2 and GI-1, GI-2 and F_1 progenies of their crosses with AC-8 and AR-6 (IG-AC, GI-AC and IG-AR, GI-AR) were selected for cytokinin analysis. In response to *ipt* constitutive expression, the content of total cytokinins was increased 15–25-fold in IG-AC but only 1–2-fold in GI-AC (Table 1). In IG-AC, the general pattern of cytokinin metabolites in 12-day-old seedlings was very similar to that in 30-day-old seedlings (Table 1). In the cases of increased cytokinins, Z was below detection in all IG-inactive transgenic *Arabidopsis* and wild-type Columbia, whereas values ranging from 12 to 47 pmol g^{-1} were detected in IG-AC. Surprisingly, no apparent correlation was found between iP levels and *ipt* activation. Similarly, very little change upon *ipt* expression was found in cZR, DHZR, iPR, cZRP, iPRP, and DHZRP, but ZRP was highly increased. Regarding cytokinin glucosides, ZOG, ZROG, Z7G, and Z9G were highly increased, and the markedly increased cytokinin metabolites were Z9G, Z7G, and ZOG, whereas DHZROG was less increased. Clearly increased levels of iP7G and iP9G were also found in IG-AC. Interestingly, concerning cytokinin conjugates, no increase was found between ZOX level and *ipt* activation (Table 1).

In GI-AC, the increased cytokinins upon *ipt* expression were Z, ZR, IPR, and ZRP; and the markedly increased cytokinin metabolite was ZR (Table 1).

In parallel, when ipt-GUS and GUS-ipt fusion genes were activated byTob-LhG4 in their progenies of crosses (IG-AR and GI-AR), cytokinin levels were analyzed in roots (R), shoots (S+L) of IG-AR and GI-AR, and their inactive isolines (IG and GI). No clear or consistent increases in cytokinin levels were observed in roots or in shoots of IG-AR and GI-AR (Table 2), which was consistent with the absence of any apparent phenotype alteration in the seedlings of IG-AR and GI-AR.

Phenotypes of ipt-GUS- and GUS-ipt-Expressing *Arabidopsis*

When ipt-GUS was activated by 35S-LhG4, the ipt-GUS-expressing *Arabidopsis* (IG-AC) showed a number of visibly altered phenotypes, including serrated leaves (Fig. 2C, D), severe root inhibition (Fig. 3A, Table 3), enlarged shoot apical parts, and no or few sterile flowers (Fig. 2A).

When GUS-ipt was activated by 35S-LhG4, the GUS-ipt-expressing *Arabidopsis* (GI-AC) grew faster, flowered early, and had more lateral shoots (Fig. 2F–H). The fresh weight (FW) of GI-AC was one- to twofold that of their inactive reporter isolines when the plants were 21 days old on MS medium. Inflorescence initiation of GI-AC was 5–7 or 10–14 days earlier when the plants were cultured in Petri dishes on MS or in soil, respectively. The roots of GI-AC grew normally. GI-AC developed shortened siliques, which contained half or a quarter the amount of seed per centimeter of silique of their inactive reporter isolines.

No visibly changed phenotypes were observed in ipt-GUS- and GUS-ipt-expressing *Arabidopsis* of IG-AR and GI-AR activated by Tob-LhG4 when they grew either on MS medium (Figs. 2E and 3A), or in soil (Fig. 2B).

pOp-ipt-GUS and pOp-GUS-ipt inactive reporter lines grew normally on MS medium and in soil (Fig. 2).

Root Growth of ipt-GUS-Expressing *Arabidopsis* Under Light Conditions

Under the tissue culture conditions, inhibition of root development was generally severe in response to the extent of the increased cytokinin content in cytokinin-overproducing, ipt-GUS-expressing *Arabidopsis* (IG-AC) (Tables 1 and 3). Root growth of IG-AC was almost completely stopped after 12 days on MS medium (Table 3), whereas it was more vigorous than in inactive reporter lines and wild-type Columbia in the first 2 days before cotyledon expansion on MS medium under tissue culture conditions (Fig. 3C). The primary root blockage was accompanied by more hairy root formation (Fig. 3A,

Table 1 Cytokinin levels in progeny of pOp-ipt-GUS and pOp-GUS-ipt in crosses with 35S-LhG4 (pmol/g FW)

Cytokinin type	Columbia		AC-8		IG-1		IG-AC-1		IG-2		IG-AC-2		GI-1	GI-AC-1	GI-2	GI-AC-2
	12 Day	30 Day	12 Day	30 Day	12 Day	30 Day	12 Day	30 Day	12 Day	30 Day	12 Day	30 Day	12 Day	12 Day	12 Day	12 Day
Z	nd	nd	nd	nd	nd	nd	46.7	18	nd	nd	19.4	12.3	nd	20.4	nd	12.8
cZ	nd	nd	1.7	3.1	nd	nd	nd	nd	nd	5.9	nd	4.9	19.9	22	nd	nd
DHZ	nd	nd	nd	nd	nd	nd	2.3	nd	nd	nd	nd	nd	nd	nd	nd	nd
iP	12.2	nd	9.6	nd	nd	nd	20.2	1.2	9.3	0.7	11.2	nd	nd	1.7	0.4	5.4
ZR	2.9	nd	0.3	33.9	9.2	nd	398.2	77.8	1.4	nd	103.3	42.7	nd	273.1	nd	132.8
cZR	13.9	nd	3.2	nd	4.2	nd	5.5	nd	nd	nd	5.6	nd	nd	nd	nd	nd
DHZR	7	nd	5	0.4	5.6	nd	23.2	nd	7.4	nd	6	0.3	nd	nd	nd	nd
iPR	2.5	nd	1.6	nd	1.4	nd	32.7	nd	1	nd	3.1	nd	1.2	26.6	0.8	37.1
ZRP	126.9	nd	27	3.7	117.1	96.9	189.8	1968.4	64.1	5.3	1677	970.9	4.6	70.3	5.8	48.5
cZRP	18.7	nd	2.8	nd	6.1	nd	2.3	2.8	7.9	nd	31.9	nd	nd	0.1	nd	nd
DHZRP	15.5	62.7	4.2	9.9	17.2	54.3	21.3	29.5	3.1	28.9	27.3	40.3	nd	nd	nd	nd
iPRP	28.9	nd	27	nd	11.6	1.7	16.1	nd	27	nd	73.8	nd	5.9	11	8.1	9
ZOG	9.2	11.1	8	2.2	14.1	9.5	1110	590.9	15.6	3.6	289.7	292.3	17.5	19.9	17.3	16.9
ZROG	2.9	nd	2	nd	3.2	nd	329.2	406.6	2.3	nd	83.1	98.7	nd	nd	nd	nd
DHZROG	3.6	nd	2.3	nd	1.6	nd	79.9	7	3.7	nd	7.8	nd	nd	5.1	nd	1.7
ZOX	nd	nd	2.3	2.5	nd	nd	nd	nd	nd	nd	1.3	nd	1.1	nd	2	1.3
Z7G	22.3	34.5	25.4	31.7	38.1	31.4	4087	2719.9	43.9	37.8	1965	1617	10.5	16.6	14.8	15.7
DHZ7G	0.1	1.3	5.6	0.4	2.8	0.9	414.1	335.1	0.8	0.6	49.3	185	nd	4.8	6	nd
iP7G	104.6	234.6	168	223.3	98.1	161.6	555.5	432.7	123.9	162.1	900.8	424.5	143.3	113.1	148.1	126.7
Z9G	9.7	7	9.7	8.2	13.2	11	1540	672.7	17.5	6.7	758	332.2	8.2	10.1	nd	6.4
DHZ9G	1	12	2.1	11.6	0.9	11.4	73	17.9	3	10.1	11	11.6	nd	0.8	nd	0.4
iP9G	5.5	7.4	6.6	6.2	5.8	5.2	46.6	10.4	9	5.9	35.3	16.1	14.7	14.2	7.5	14.5
Total content	387.4	370.6	314.4	337.1	350.2	383.9	8993	7290.9	340.9	267.6	6060	4049	226.9	609.8	210.8	429.2

IG-1, IG-2 and GI-1, GI-2 = pOp-ipt-GUS and pOp-GUS-ipt independent reporter lines; IG-AC-1, IG-AC-2 and GI-AC-1, GI-AC-2 = activated isolines of IG-1, IG-2 and GI-1, GI-2 driven by 35S-LhG4; AC-8 = 35S-LhG4 activator line; Columbia = wild-type *Arabidopsis*. Seedlings were grown for 12 days or 30 days on MS medium. nd = not detectable (levels were lower than 0.1 pmol/g FW)

Table 2 Cytokinin levels in progeny of pOp-ipt-GUS and pOp-GUS-ipt in crosses with Tob-LhG4 (pmol/g FW)

Cytokinin type	Columbia		AR-6		IG-1		IG-AR-1		IG-2		IG-AR-2		GI-1		GI-AR-1		GI-2		GI-AR-2	
	R	S+L	R	S+L	R	S+L	R	S+L	R	S+L	R	S+L	R	S+L	R	S+L	R	S+L	R	S+L
Z	5.9	nd	nd	nd	7.6	nd	nd	nd	nd	nd	nd	nd	9.6	nd	nd	nd	nd	6.8	nd	nd
cZ	nd	nd	nd	nd	26.7	20.2	nd	nd	27.9	nd	nd	nd	nd	nd	25.2	nd	nd	nd	19.7	nd
DHZ	nd	nd	nd	nd	nd	0.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
iP	17.8	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	5.6	nd	nd	nd	nd	nd	nd	nd	nd
ZR	nd	nd	nd	nd	nd	nd	nd	nd	0.6	nd	nd	nd	17.5	nd	nd	nd	nd	nd	nd	nd
cZR	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
DHZR	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
iPR	6.1	5.4	2.8	0.4	2	3.7	2.5	1	2.3	1.6	1.6	5.6	12	5.8	4.3	2.4	1.4	1.3	0.8	1
ZRP	12.5	nd	nd	10.2	4.6	3.6	5.8	nd	9.7	11.9	nd	0.2	52.9	9.2	10.8	25.8	69.6	54.3	nd	nd
cZRP	10.5	13.1	13.4	39.6	nd	nd	nd	2.2	27.1	4.3	nd	5.9	3.5	nd	nd	24	nd	8.2	nd	nd
DHZRP	75.3	19.9	36.8	37.6	2.3	nd	nd	4.3	27	nd	nd	2.7	16.4	17.9	18.9	8.5	73.5	nd	2	nd
iPRP	2.3	7.9	13.8	10.8	0.7	3.8	nd	4.7	3.6	5.6	nd	1.3	3.8	8.2	1.9	3	nd	7.2	nd	5.5
ZOG	17.7	5.5	nd	11.9	21.3	11.2	46.4	5.8	22.7	7.1	20	nd	17.8	1.8	13.5	11.4	25.6	7.8	11.9	10.2
ZROG	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
DHZROG	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.4	nd	nd	nd	nd	nd	nd	nd
Z7G	28.1	15	13	10.4	37.8	24	63.4	14.5	25.4	5.8	50.8	14	26.7	14	16.9	20.9	25.1	13.8	28.8	20
DHZ7G	nd	nd	6.3	1.5	17.7	4.1	19.7	6.6	8.9	7.1	19.5	nd	13.4	4.1	6.4	2.4	4.1	3.8	4.8	2.4
iP7G	46.7	180	36	136	93.3	26.7	61	143	46.3	192	195	162	66.4	215.4	181.6	69.2	64.2	219	77.4	222
Z9G	7.1	4.4	4	1.3	16	11.3	29.6	8.2	9.5	11.8	7.8	nd	21.7	6.5	7.2	6.3	15.8	2.3	16.5	4.4
DHZ9G	4	2.7	2.5	2.7	10.9	nd	2.4	2.4	4.5	nd	1.8	0.9	2.7	1.4	0.3	2.8	4.9	0.4	6.7	0.5
iP9G	7	8.6	8.4	9.6	11.7	4.9	6.4	9	8.4	8	19.4	11.4	5.3	13	8.8	7.5	7.7	13.2	12.7	11.6
Total cytokinins	241	263	137	272	253	114	237	201	224	255	316	210	271	297.3	295.8	184	292	338	181.3	278

IG-1, IG-2 and GI-1, GI-2 = pOp-ipt-GUS and pOp-GUS-ipt independent reporter lines; IG-AR-1, IG-AR-2 and GI-AR-1 ,GI-AR-2 = activated isolines of IG-1, IG-2 and GI-1, GI-2 driven by Tob-LhG4; AR-6 = Tob-LhG4 activator line; Columbia = wild-type *Arabidopsis*. Seedlings were grown for 12 days on MS medium. nd = not detectable (levels were lower than 0.1 pmol/g FW)
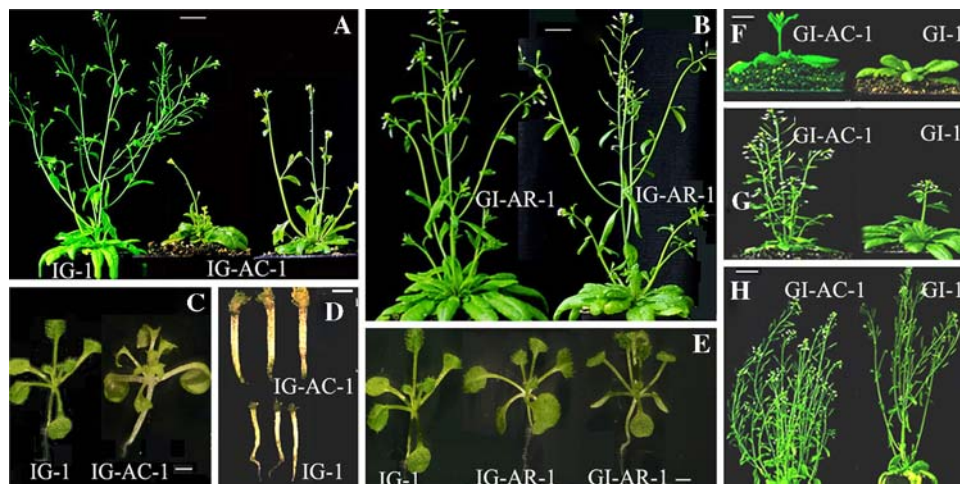

Fig. 2 Phenotypes of ipt-GUS- and GUS-ipt-expressing *Arabidopsis* driven by 35S-LhG4 or Tob-LhG4. See Fig. 1 for the plant labels. (**A**, **C**, **D**) Phenotypes of ipt-GUS-expressing *Arabidopsis* driven by 35S-LhG4 and their inactive reporter lines grown on MS medium + 1% sucrose for 3 weeks (**C**, **D**) or in soil for 60 days (**A**). (**B**, **E**) Phenotypes of ipt-GUS- and GUS-ipt-expressing *Arabidopsis* driven by Tob-LhG4 and their inactive reporter isolines on MS medium + 1% sucrose for 3 weeks (**E**) or in soil for 60 days (**B**). (**F**, **G**, **H**) Phenotypes of GUS-ipt-expressing *Arabidopsis* driven by 35S-LhG4 and their inactive reporter lines grown in soil for 30 days (**F**), or 45 days (**G**), or 60 days (**H**). (**A**, **B**, **F**, **G**, and **H**) Scale bar = 2 cm; (**C**, **D**, and **E**) scale bar = 2 mm. The labels are the same as in Fig. 1

Fig. 3 Root growth of ipt-GUS-expressing *Arabidopsis* driven by 35S-LhG4. See Fig. 1 for plant labels; Columbia is wild-type *Arabidopsis*. (**A**) Seedlings of ipt-GUS-expressing *Arabidopsis* driven by 35S-LhG4 and Tob-LhG4 on MS medium under tissue culture conditions for 1 week. (**B**) The complete or sectioned dark-growth seedlings of ipt-GUS-expressing *Arabidopsis* driven by 35S-LhG4 were transferred to tissue culture conditions. a = root parts; b = shoot parts; c = the complete seedlings. The first two plants of the left-hand photo were inactive reporter *Arabidopsis*; the third and fourth plants were ipt-GUS-expressing *Arabidopsis*. (**C**) Seeds from the progenies of pOp-ipt-GUS reporter line crossed with 35S-LhG4 germinated on MS medium under tissue culture conditions for 2 days. (**D**) Root tips of ipt-GUS-expressing *Arabidopsis* driven by 35S-LhG4 on MS medium under tissue culture conditions for 3 weeks
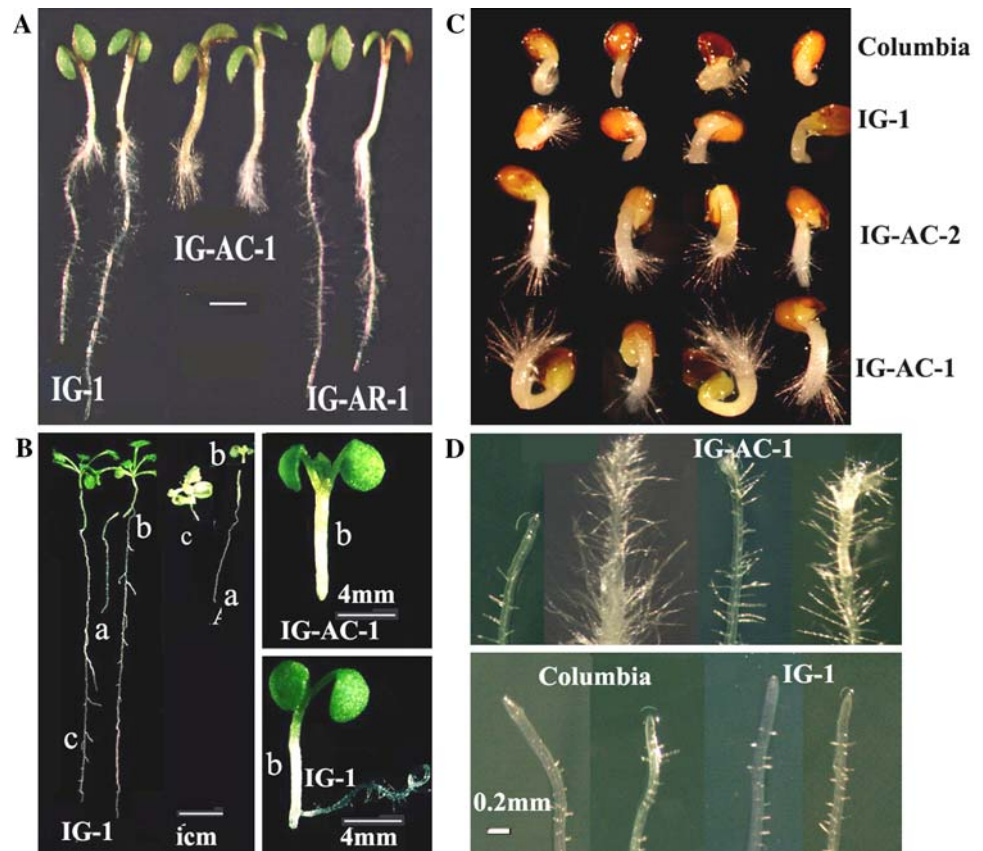
D). The development of root tips in IG-AC was retarded (Fig. 3D).

Root growth of ipt-GUS-expressing *Arabidopsis* driven by Tob-LhG4 (IG-AR) was as normal as all reporter lines (IG) (Table 3, Fig. 3A).

Root Growth of ipt-GUS-Expressing *Arabidopsis* (IG-AC) Under Dark Conditions

The seeds derived from the progeny of pOp-ipt-GUS reporter lines (IG-1, IG-2, IG-3) in crosses with a 35S-LhG4 activator line were sown on MS medium with 1% sucrose and cultivated under dark conditions for 8 days. Root growth of IG-AC was almost at the same rate of their inactive reporter lines (IG) under dark conditions (Table 4), which was much slower than their inactive reporter lines (IG) under light conditions (Table 3).

On the fourth day after IG-AC and their reporter isolines (IG) were cultured under dark conditions, the seedlings were sectioned at the base of shoots and divided into root parts (a) and aerial parts (b). Afterward, the complete seedlings and the sectioned seedlings were transferred to tissue culture conditions (22°C, 16-h light/8-h dark). The root parts (a) almost grew at the same rate in both ipt-GUS-expressing *Arabidopsis* (IG-AC) and their inactive reporter

lines (IG) (Fig. 3C-a; IG-AC-a and IG-a in Table 5). The shoot parts (b) grew well and adventitious roots were grown from the shoot base of IG inactive reporter lines; however, *Arabidopsis* cytokinin-overproducing phenotypes developed and no adventitious roots grew from the shoot base of ipt-GUS-expressing *Arabidopsis* (IG-AC) (Fig. 3C-b; IG-AC-b and IG-b in Table 5). When the complete seedlings from dark conditions were transferred to tissue culture conditions, the root growth of IG-AC was completely stopped around 12 days, again after transferring to light, whereas the roots of their inactive reporter isolines (IG) grew normally (Fig. 3C-c; IG-AC and IG in Table 5).

Callus Formation in ipt-GUS-Expressing *Arabidopsis* (IG-AC)

The seeds derived from the progeny of pOp-ipt-GUS reporter lines (IG-1, IG-2) in crosses with an activator line (AC-8) were sown on MS medium and cultivated under tissue culture conditions for more than 30 days. Ten percent of seedlings occasionally induced calli from their shoots of IG-AC, which were located at the shoot apical parts (8%) (Fig. 4A–C, E–G) or at the bases of shoots (2%) (Fig. 4D, H). Some of the callus cells synthesized chlorophylls (Fig. 4D, H), or formed roots (Fig. 4C).

Table 3 Root length of pOp-ipt-GUS *Arabidopsis* grown under the tissue culture conditions

Plants	4 Days			8 Days			12 Days			21 Days		
	IG (cm)	IG-AC (cm)	IG-AR (cm)	IG (cm)	IG-AC (cm)	IG-AR (cm)	IG (cm)	IG-AC (cm)	IG-AR (cm)	IG (cm)	IG-AC (cm)	IG-AR (cm)
IG-1	0.82 ± 0.20	0.2 ± 0.10	0.9 ± 0.25	1.78 ± 0.46	0.22 ± 0.10	1.90 ± 0.51	4.12 ± 0.80	0.3 ± 0.10	4.50 ± 0.83	4.12 ± 0.80	0.32 ± 0.10	4.89 ± 0.90
IG-2	0.7 ± 0.28	0.25 ± 0.12	0.88 ± 0.2	1.63 ± 0.40	0.38 ± 0.14	1.83 ± 0.49	3.67 ± 0.75	0.4 ± 0.12	4.0 ± 0.88	3.67 ± 0.75	0.40 ± 0.12	4.10 ± 0.79
IG-3	0.85 ± 0.22	0.25 ± 0.13	0.93 ± 0.28	1.58 ± 0.40	0.4 ± 0.14	1.69 ± 0.46	3.35 ± 0.80	0.55 ± 0.14	3.98 ± 0.89	3.35 ± 0.80	0.60 ± 0.16	3.98 ± 0.89

IG-1, IG-2, IG-3 = three pOp-ipt-GUS independent reporter lines; IG = root length of pOp-ipt-GUS inactive reporter lines; IG-AC = root growth of pOp-ipt-GUS activated lines driven by 35S-LhG4; IG-AR = root growth of pOp-ipt-GUS activated lines driven by Tob-LhG4. Data are the mean of three repeat measurements. The variety was SE.

Table 4 Root length of pOp-ipt-GUS plants grown under dark conditions

Plants	4 Days		8 Days	
	IG (cm)	IG-AC (cm)	IG (cm)	IG-AC (cm)
IG-1	0.50 ± 0.088	0.50 ± 0.13	1.2 ± 0.23	1.05 ± 0.25
IG-2	0.51 ± 0.12	0.52 ± 0.18	1.4 ± 0.33	0.9 ± 0.20
IG-3	0.50 ± 0.16	0.53 ± 0.12	1.2 ± 0.28	1.1 ± 0.33

Labels are as in Table 1

Discussion

Plant Aerial Parts are More Sensitive than Roots to Synthesize Cytokinins in Response to *ipt* Activation

Roots have been postulated to be the main site for cytokinin synthesis (Chibnall 1939; Richmond and Lang 1957; Mothes and Engelbrech 1962). The basis of this hypothesis was that a root hormone was necessary for balancing protein synthesis in leaves (Chibnall 1939), and it was subsequently demonstrated by Mothes and Engelbrech (1962) that the senescence of detached leaves was retarded when adventitious roots formed. Leaf senescence was delayed when cytokinins were exogenously fed to detached leaves (Richmond and Lang 1957). Thus, it was hypothesized that the root hormone could be cytokinins, and these cytokinins were synthesized in roots. Tobacco leaf senescence was correlated with cytokinin content change in leaves, and cytokinins had antisenescence activity. However, where these cytokinins were synthesized in plants remained unclear (Singh and others 1992a). Active cytokinins could be synthesized in leaves or be transported from elsewhere, potentially from roots (Letham 1994). Singh and others (1992b) showed that there was no difference in the amount of xylem-fed labeled cytokinins transported to old and young leaves, and there was no difference in the metabolism of the labeled cytokinins. However, in contrast to old leaves, young leaves were able to synthesize cytokinins *in situ*, which raised the question of whether roots are a source of antisenescence. When the *ipt* gene was expressed in tobacco plants by the CHS promoter, the *ipt* gene was strongly expressed in stems and weakly expressed in roots. The cytokinin level was markedly elevated (Wang and others 1997a, b). In this study, the *ipt* gene was specifically expressed in *Arabidopsis* roots through pOp-ipt-GUS and pOp-GUS-ipt reporter lines in crosses with a Tob-LhG4 activator line. However, ipt-GUS and GUS-ipt expression did not show any significant changes in either phenotypes or cytokinin content. When pOp-ipt-GUS and pOp-GUS-ipt were activated by 35S-LhG4, cytokinins were highly increased in ipt-GUS-expressing transgenic *Arabidopsis* (IG-AC) and slightly increased in GUS-ipt-expressing

Table 5 Root length of pOp-ipt-GUS dark-growth seedlings grown under light conditions

Plants	Root length of inactivated plants (cm)			Root length of activated plants (cm)		
	IG	IG-a	IG-b	IG-AC	IG-AC-a	IG-AC-b
IG-1[a]	2.0 ± 0.53	1.5 ± 0.38	0.7 ± 0.15	0.7 ± 0.17	0.6 ± 0.18	0
IG-2[a]	1.9 ± 0.55	1.3 ± 0.30	0.8 ± 0.23	0.8 ± 0.36	1.35 ± 0.45	0
IG-3[a]	2.0 ± 0.68	1.3 ± 0.25	1.0 ± 0.35	0.7 ± 0.25	1.3 ± 0.43	0
IG-1[b]	6.0 ± 0.89	1.5 ± 0.38	4.5 ± 0.67	0.8 ± 0.16	0.8 ± 0.21	0
IG-2[b]	5.5 ± 0.90	1.3 ± 0.30	4.0 ± 0.58	1.0 ± 0.24	1.5 ± 0.38	0
IG-3[b]	5.5 ± 0.78	1.4 ± 0.25	3.6 ± 0.54	0.9 ± 0.22	1.4 ± 0.36	0
IG-1[c]	9.8 ± 0.8	1.6 ± 0.38	9.0 ± 1.5	0.8 ± 0.16	0.8 ± 0.21	0
IG-2[c]	9.8 ± 1.0	1.3 ± 0.30	8.7 ± 1.2	1.0 ± 24	1.5 ± 0.38	0
IG-3[c]	10.0 ± 0.8	1.4 ± 0.25	8.9 ± 1.05	0.9 ± 0.22	1.4 ± 0.36	0

IG-1, IG-2, IG-3, IG, IG-AC are the same as in Table 1. IG-a = root length of the shootless root of IG inactive reporter lines; IG-b = adventitious root length growing from the rootless seedlings of IG inactive reporter lines; IG-AC-a = root length of the shootless root of IG-AC activated lines; IG-AC-b = adventitious root length growing from the rootless seedlings of IG-AC activated lines. Data are mean of three repeat measurements. The variety was SE

[a] Seedlings were transferred 7 days from dark conditions

[b] Seedlings were transferred 14 days from dark conditions

[c] Seedlings were transferred 21 days from dark conditions

transgenic *Arabidopsis* (GI-AC) (Table 1); however, *GUS* was translated more in GI-AC than in IG-AC (Fig. 1C). Thus, pOp-ipt-GUS and pOp-GUS-ipt functionally worked well in a transcriptional fusion manner when they were activated by 35S-LhG4. When the same reporter lines of pOp-ipt-GUS and pOp-GUS-ipt were activated by Tob-LhG4, *ipt* transcription was detected in the roots of ipt-GUS- and GUS-ipt-expressing *Arabidopsis* (IG-AR; GI-AR) (Fig. 1B-2), but GUS staining was detected only in the roots of GUS-ipt-expressing *Arabidopsis* (GI-AR), not in the roots of ipt-GUS-expressing *Arabidopsis* (IG-AR) (Fig. 1C-2, IC-4). The invisible GUS staining in IG-AR possibly resulted from reduced *GUS* translation, which was similar in IG-AC. Thus, pOp-ipt-GUS and pOp-GUS-ipt functionally worked well in a transcriptional fusion manner when they were activated by Tob-LhG4. The above results showed that roots were not sensitive to synthesizing cytokinins in response to *ipt* activation in IG-AR and GI-AR. In contrast to roots, the markedly enhanced cytokinins in IG-AC were detected in seedlings at early and late development stages, whereas those seedlings had severe root inhibition. These results do not support previous studies of *ipt*-gene transformed plants, in which the *ipt* gene was expressed by tetracycline and Cu^{2+}-inducible factors, and some cytokinin metabolites were increased in roots or shoots (Faiss and others 1997; Mckenzie and others 1998). In their experiments, the tetracycline- and Cu^{2+}-inducible promoters were modified from the 35S promoter; thus, *ipt* gene expression depended on inducible factors, and cytokinins could be synthesized in whole plants after induction. In another case, when the roots of *ipt*-gene transformed plants were first exposed to unphysiologic conditions (such as high levels of

tetracycline and high levels of Cu^{2+}), greatly elevated transient levels of cytokinins could result. Thus, it was difficult to distinguish between cause and effect. In this study, *ipt* has been constantly expressed in plant roots through the pOp/LhG4 system under noninvasive conditions. Our results showed that the aerial parts of plants were more sensitive to synthesizing cytokinins in response to *ipt* activation.

Plant Aerial Parts and Light Play Important Roles in Root Inhibition in Cytokinin-Overproducing, ipt-GUS-Expressing *Arabidopsis*

In shoot meristems, cytokinins and light cause similar effects such as cotyledon expansion, leaf development, and induction of light-inducible gene expression (Flores and Tobin 1986). Light was a sensitive stimulus for anthocyanin accumulation in cytokinin-overproducing *Arabidopsis* (Mustilli and others 1999; Guo and others 2005). In this study we used cytokinin-overproducing, ipt-GUS-expressing *Arabidopsis* (IG-AC) and their inactive reporter isoline (IG) seedlings to examine their response to light and dark conditions. Under light conditions, root growth of cytokinin-overproducing *Arabidopsis* IG-AC seemed to be more vigorous than their inactive reporter isolines (IG) and wild-type Columbia when their seeds were germinated on MS medium the first 2 days before cotyledon expansion (Fig. 3C), and was almost completely stopped around 12 days. Under dark conditions, root growth of cytokinin-overproducing *Arabisopsis* IG-AC was almost at the same rate as their inactive reporter isolines (IG), and the roots stopped growing around 12 days, again when the dark-
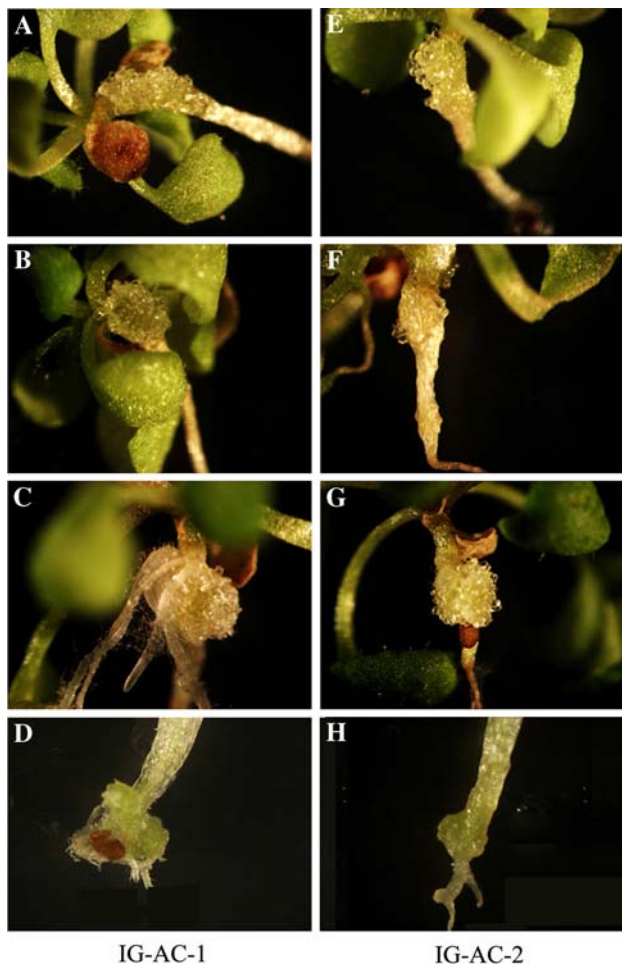
Fig. 4 Callus formation in ipt-GUS-expressing *Arabidopsis* driven by 35S-LhG4. See Fig. 1 for the plant labels. Seedlings were cultivated on MS medium + 1% sucrose under tissue culture conditions for more than 30 days. (**A–C**, **E–G**) Calli induced on the upper parts of seedlings near the apical parts of shoots; meanwhile, some calli formed roots (C) or synthesized chlorophylls in the callus cells (**A**, **B**, **E**). (**D**, **H**) Calli induced on the base of shoots, and the chlorophylls were synthesized in the callus cells. IG-AC-1, IG-AC-2: activated lines of reporter lines IG-1, IG-2 in crosses with 35S-LhG4. A-D: four individual plants of IG-AC-1; E-H: four individual plants of IG-AC-2

grown IG-AC seedlings were exposed to the light conditions (Table 5). Several experiments have shown that high cytokinin content is correlated with severe root inhibition (Schmulling and others 1989; Smigocki and others 1989; Li and others 1992; Hewelt and others 1994; Wang and others 1997a). In our study, the root inhibition in cytokinin-overproducing, ipt-GUS-expressing *Arabidopsis* correlated with light conditions. Currently, there is no direct evidence to explain why the roots of cytokinin-overproducing *Arabidopsis* completely stopped growing after 12 days when the seedlings were directly exposed to light or when transferred from dark to light. Perhaps it is the period in which the synthesized cytokinins are transported to roots and accumulate a high enough level for root inhibition.

We studied how aerial parts and root parts of plants respond to *ipt* activation under light and dark conditions. First, the complete seedlings of cytokinin-overproducing, ipt-GUS-expressing *Arabidopsis* (IG-AC) and their inactive reporter isolines (IG) were cultured on MS medium under dark conditions for 4 days. IG-AC and IG seedlings developed similar hypocotyl (1 cm) and root (0.5 cm) lengths, but cotyledons were open in IG-AC and showed a hook in IG. Then, the dark-growth seedlings were sectioned into aerial parts and root parts which were separately exposed to light conditions. The results showed that root parts grew at nearly the same rate in IG-AC and their inactive IG, aerial parts grew well, and adventitious roots grew from the inactive reporter lines IG, *Arabidopsis* cytokinin-overproducing phenotypes developed, and no adventitious roots grew from IG-AC (Fig. 3C; Tables 4 and 5). Evidence showed that high levels of cytokinins caused root inhibition; thus, we deduced that the inhibition of adventitious root formation was possibly caused by high cytokinin levels in the aerial parts of IG-AC. If this is true, the aerial parts of plants are sensitive in response to *ipt* activation to synthesizing cytokinins under light conditions.

In ipt-GUS transcriptional fusion, according to Kozak's ribosome screening model, the mRNAs consist of *ipt* and *GUS* genes; and *GUS* expression is supposed to be weaker than *ipt*, but should be evenly distributed in whole seedlings when they are activated by 35S-LhG4. However, GUS staining located only at the shoot apical parts of ipt-GUS-expressing *Arabidopsis* (Fig. 1C-3) (Kozak and others 1978; Bevan 1984; Rogers and others 1985; Peabody and others 1986; van Duijn and others 1988). The reasonable explanation is that the *GUS* gene was translated more strongly at the shoot apical parts than at other parts in ipt-GUS-expressing *Arabidopsis*. Thus, the *ipt* gene in the same transcriptional frame of ipt-GUS mRNA should be translated much more strongly than the *GUS* gene, which indirectly identified that *ipt* was also vigorously translated at the shoot apical parts. Thus, light and aerial parts would be important for cytokinin synthesis (or assembly) and root inhibition in cytokinin-overproducing *Arabidopsis*.

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