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Plant Hormones Isolated from "Katahdin" Potato Plant Tissues and the Influence of Photoperiod and Temperature on Their Levels in Relation to Tuber Induction

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Abstract Qualitative and quantitative analyses were carried out on vegetative tissues of potato (*Solanum tuberosum* cv. "Katahdin") in search of natural products thought to play a role in tuber induction. Tissues were obtained from plants initially grown in a growth chamber under noninducing conditions (30°C day and 28°C night with an 18-h photoperiod), and then half of the plants were moved to inducing chambers (28°C day and 13°C night with a 10-h photoperiod) for 10 days prior to tissue harvest. Plants from each chamber were then harvested at 2-day intervals for 10 days, separated into above- and below-ground portions, and the lyophilized tissues were extracted and subjected to rigorous purification and separation using high-performance liquid chromatography. This was followed by identification and quantification using combined gas chromatography-mass spectrometry. Compounds isolated and identified included gibberellic acid; cytokinins *cis*-zeatin riboside, *trans*-zeatin, *trans*-zeatin riboside, and isopentenyladenine; and jasmonates jasmonic acid, tuberonic acid and its methyl ester, methyl 7-isocucurbate, and 9,10-dihydromethyljasmonate. Methyl 7-isocucurbate and 9,10-dihydromethyljasmonate were detected for the first time in potato tissue as endogenous compounds. Cytokinin and jasmonate levels generally increased under inducing conditions, whereas gibberellic acid levels declined progressively during the 10-day sampling period. Only gibberellic acid, jasmonic acid, and *cis*-zeatin riboside levels were significantly influenced by induction.

Keywords *Solanum tuberosum* · Potato tuberization · Phytohormones · Jasmonates · Cytokinins · Gibberellic acid

Introduction

The mechanism(s) involved in formation of the potato tuber (*Solanum tuberosum*) have eluded scientists for more than a century. The tuberization process is thought to be under control of environmental factors, primarily temperature and photoperiod which regulate levels of endogenous growth substances (Melis and van Staden 1984; Vreugdenhill and Struik 1989). Short days with cool night temperatures (inducing conditions) have been reported to favor tuberization, whereas long days and high night temperatures (noninducing conditions) may delay or inhibit the process (Gregory 1956; Slater 1968; Ewing and Wareing 1978; Dam and others 1996; Jackson 1999).

Using grafting experiments, Gregory (1956) and Chapman (1958) postulated on the existence of a tuber-forming stimulus synthesized in leaves under inducing conditions, and translocated below ground to the rhizome region. This stimulus is thought to include positive and negative regulators of tuberization (Jackson 1999). Although the exact nature of this stimulus has yet to be elucidated, several studies suggested hormones such as cytokinins (Palmer and Smith 1969; Mauk and Langille 1978), jasmonates (Yoshihara and others 1989; van den Berg and Ewing 1991; Abdala and others 1996), and gibberellins (Kumar and Wareing 1974) appear involved.

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Many of the early methods used to detect and estimate plant hormones were based on bioassays that were flawed due to the interference of specific compounds present in plant extracts. Moreover, certain plant hormone metabolites could not be detected by bioassay because they elicited no response. With the advent of high-performance liquid chromatography (HPLC) and combined gas chromatography-mass spectrometry (GC-MS), the twofold objectives of the present study became attainable. These were, first, to isolate and identify cytokinins, jasmonates, and gibberellic acid present in induced and noninduced "Katahdin" potato tissues and, second, to quantify levels of these endogenous compounds present during the ontogeny of tuber induction to ascertain their possible roles. The results are linked to some reported morphologic changes that occur during the induction period. This is a continuation of our earlier research work on potato tuberization (Langille and Forsline 1974; Forsline and Langille 1975; Mauk and Langille 1978; Langille and Hepler 1992), and to the best of our knowledge a detailed systematic study of simultaneous changes in the levels of such hormones during the potato tuber induction stage has not been reported.

Materials and Methods

Standard Samples

Standard compounds were either synthesized or purchased from commercial sources. Methyl jasmonate [methyl (1R,2R)-3-oxo-2-(2Z-pentenyl)-cyclopentane acetate] was purchased from Bedoukian Research (Danbury, CT, USA). This sample was a racemic mixture containing 92% (1R,2R)-methyl jasmonate and 8% (1R, 2S)-methyl jasmonate, as determined by GC. Jasmonates were synthesized according to literature procedures. Methyl jasmonate was used to synthesize jasmonic acid [(1R, 2R)-3-oxo-2-(2Z-pentenyl)-cyclopentane-1-acetic acid] (Kramell and others 1988), 6-epi-7-isocucurbic acid [(1R, 2R, 3R)-3-hydroxy-2-(2Z-pentenyl)cyclopentane-1-acetic acid] (and its methyl ester) (Gemal and Luche 1981), 7-isocucurbic acid [(1R, 2R, 3S)-3-hydroxy-2-(2Z-pentenyl)-cyclopentane-1-acetic acid] (and its methyl ester) (Gemal and Luche 1981), tuberonic acid [(1R,2R)-3-oxo-2-(5-hydroxy-2Z-pentenyl)-cyclopentane-1-acetic acid] (and its methyl ester) (Kitahara and others 1984), and 9,10-dihydrojasmonic acid methyl ester [methyl (1R,2R)-3-oxo-2-pentylcyclopentane acetate] (Miersch and others 1987). The following chemicals were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA): N^6-[2-isopentenyl]adenine, *trans*-zeatin [6-(4-hydroxy-3-methylbut-2-enylamino)purine], *trans*-zeatin riboside (t-ZR) [9-(β-D-ribofuranosyl)-*trans*-zeatin], *cis*-zeatin riboside [9-β-D-ribofuranosyl)-*cis*-zeatin], and gibberellic acid. All solvents used were also purchased from Sigma-Aldrich Chemical Company.

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS was completed with a Hewlett-Packard 5890 gas chromatograph interfaced with a mass selective detector (Hewlett-Packard 5970 series) working in the electron-impact ionization mode at 70 eV. The column was a DB-5MS fused-silica capillary column crosslinked and surface bonded with 5% phenyl/95% dimethylpolysiloxane stationary phase (60 m × 0.25 mm; 0.25 µm film thickness; J & W Scientific, Folsom, CA, USA). Helium carrier gas was used with a flow rate of 2.5 ml/min. Data acquisition was controlled by Hewlett-Packard G1034C MS ChemStation computer software. Data acquisition was achieved by scanning all ions from 50 to 800 atomic mass units. The following temperature programs were used:

1. Jasmonates: injection port and detector temperatures were 260°C and 300°C, respectively. The column temperature was increased from 100°C at 10°C min^{-1} to 170°C, whereupon the rate was slowed to 0.5°C min^{-1} until the column temperature reached 185°C. This temperature was held for 10 min.
2. Gibberellic acid: injection port and detector temperatures were 260°C and 300°C, respectively. The column temperature was programmed from 100°C to 220°C at 17°C min^{-1} followed by a rate of 6°C min^{-1} to 270°C with isothermal hold at 270°C for 25 min.
3. Free cytokinins: injection port and detector temperatures were 270°C and 300°C, respectively. The column temperature was increased from 120°C at 25°C min^{-1} until 240°C was reached and then ramped to 270°C at 5°C min^{-1}. The column temperature was then held at 270°C for 13 min.
4. Conjugated cytokinins: injection port and detector temperatures were 270°C and 300°C, respectively. From 130°C to 250°C the column temperature was increased at the rate of 30°C min^{-1} then at 5°C min^{-1} to 280°C with a 10-min isothermal hold.

Prior to GC-MS analysis, carboxylic acid groups were derivatized to methyl esters with *N,N*-dimethylformamide dimethyl acetal (Thenot and others 1972), whereas free amino groups in cytokinins and hydroxyl groups in gibberellic acid were converted to the corresponding trimethylsilyl derivatives upon treatment with *N,O*-bis(trimethylsilyl)acetamide (Klebe and others 1966). Peaks were identified by computer search of a user-generated reference library incorporating GC retention times and mass spectra of reference compounds.

HPLC Analyses

A Hewlett-Packard HPLC System Model 1090 equipped with a photodiode array detector was used. Cytokinins were chromatographed using a μ-Bondapak C_{18} column (30 cm long × 3.9 mm i.d.; particle size = 10 μm; pore size = 10 Å; Waters Corp., Taunton, MA, USA) and a Waters μ-Bondapak C_{18} Guard-Pak (10 μm packing, pore size = 125 Å) precolumn insert. Jasmonates and gibberellic acid were chromatographed on a Spherisorb ODS-2 C_{18} column (25 cm long × 4.6 mm i.d.; 5 μm packing; pore size = 80 Å; Waters Corp.) and a Spherisorb ODS-2 C_{18} precolumn (7.5 mm × 4.6 mm; 5 μm packing; Sigma-Aldrich, Alltech Associates, Deerfield, IL, USA). Elution solvents were water (0.1% acetic acid), methanol, and acetonitrile. Data acquisition was controlled by Hewlett-Packard 79995A operating software.

Four solvent systems of acetonitrile and water (containing 0.1% acetic acid, pH 4.0) were used. Solvent system #1 was a linear gradient from 50% acetonitrile to 80% acetonitrile over 10 min. Solvent system #2 was an isocratic elution with 80% acetonitrile over 10 min. In solvent system #3 the column was eluted with 35% acetonitrile for 10 min and then with a linear gradient to 50% acetonitrile over 5 min. This was followed by an isocratic elution with 50% acetonitrile over 40 min then a linear gradient to 80% acetonitrile over 10 min. Solvent system #4 involved a linear 10-min gradient of 20-35% acetonitrile followed by isocratic elution with 35% acetonitrile over 10 min. Conjugated cytokinins were eluted with solvent system #4 at a flow rate of 0.8 ml/min. Free cytokinins were purified using solvent system #3 at a flow rate of 0.8 ml/min. Acidic and neutral compounds were chromatographed using solvent system #1 (flow rate 0.9 ml/min) and solvent system #2 (flow rate 0.9 ml/min), respectively. Cytokinins were detected by UV absorbance at 254 nm. Jasmonates and gibberellic acid were monitored at 230 nm.

Quantitative Analysis

A standard calibration graph for each compound was produced using a solution of 10-50 mg of the metabolite in 1.0 ml of dichloromethane. Aliquots of this standard stock solution were then prepared by serial dilution to give six different solutions. Each solution was prepared in triplicate over three different days to determine both intra-assay and interassay precision. Five replicate injections into the gas chromatograph were made. The mean peak area of the analyte was plotted versus its amount and the resulting graph was then used to determine the amount of each metabolite in the potato tissues. The correlation coefficients for the standard curves were between 0.9990 and 0.9998. The intraday repeatability and interday reproducibility of retention times and peak areas showed a mean coefficient of variation of less than 4% for all samples.

Quantitative analysis of endogenous compounds was performed as follows: (1) the extract from a potato tissue sample spiked before the extraction procedure with 500 ng of an internal standard which was a synthetic sample of the hormone being analyzed; (2) the extract of a blank tissue sample from the same tissue spiked after the extraction procedure and before the GC-MS analysis, with the same amount of the same internal standard; (3) the extract of a blank sample of the same potato tissue. Quantitative determinations conducted in these three extracts will give recoveries for the extraction procedure as well as the amount in the original sample of potato tissue. The percent mean recovery from extracts was determined in duplicates; they were 70% jasmonic acid (JA), 65% tuberonic acid (TA), 73% methyltuberonate (TAMe), 75% methylcucurbate (CAMe), 70% dihydromethyljasmonate (DHJAMe), 82% *cis*-zeatin riboside (c-ZR), 80% *trans*-zeatin riboside (t-ZR), 78% *trans*-zeatin (t-z), 79% isopentenyladenine (ipA), and 70% gibberellic acid (GA_3). All measured amounts reported in this article were corrected for the percent recovery.

Because natural isojasmonic and tuberonic acids tend to isomerize from *cis*-oriented side chains relative to the cyclopentanone ring into a *trans* form, both detected isomers were added together as total jasmonic and tuberonic acids.

Plant Tissue

Potato plants (*Solanum tuberosum* cv. Katahdin) were grown as described previously (Mauk and Langille 1978). All determinations are based on two biological replicate samples with two independent analyses per replicate.

Extraction and Processing Procedures of Plant Material

The solvent-solvent extraction procedure of Mauk and Langille (1978) was employed with modification, including the use of polyvinylpolypyrrolidine for removal of phenols and quinones (Glenn and others 1972) and Extra-Clean C_{18} column (Alltech Associates, Deerfield, IL, USA).

Results and Discussion

Levels of Gibberellic Acid

The bioactive gibberellins in higher plants are reported to be GA_1, GA_3, GA_4, and GA_7 (Hedden and Phillips 2000).

Several gibberellins (GA_1, GA_8, GA_{19}, GA_{20}, GA_{29}, and GA_{44}) have been found in shoots of developing potato plants (Martinez-Garcia and others 2002). Because gibberellic acid (GA_3) was not identified in that experiment and, at the same time, GA_3 is known to have an inhibitory effect on tuberization, we decided to investigate GA_3 content in terms of photoperiod and temperature. Furthermore, GA_3 is known to promote longitudinal cell expansion and thus stolon elongation (Xu and others 1998; Fujino and others 1995; Shiboaka 1993). When GA_3 biosynthesis is chemically inhibited, lateral cell expansion and division are observed (Shiboaka 1993; Fujino and others 1995; Xu and others 1998), which mark the beginning of tuber induction. These observations suggested that quantitative analysis of endogenous GA_3 in Katahdin potato tissues was warranted. Accordingly, effects of induction, its duration, and tissue location on levels of GA_3 were investigated. Levels of GA_3 were 2.7 times greater in noninduced tissues than in those grown under inducing conditions. As shown in Figure 1, following transfer to inducing conditions, levels of GA_3 progressively declined, whereas they continued to increase in noninduced tissues with each successive harvest day. An elevated GA_3 level in noninduced tissues is consistent with earlier reports in the literature. For example, gibberellin-like activity in shoots and rhizomes has been reported to be higher under conditions that delay tuberization (Woolley and Wareing 1972; Krauss and Marschner 1982; Menzel 1983).

Railton and Wareing (1973) reported that as few as two short days produced a decline in GA-like activity extracted from *Solanum andigena* leaves. It should be noted that in the present study GA_3 levels declined in induced tissues possibly to reach a minimum value to permit tuberization. In our experiment, evidence of tuber formation was

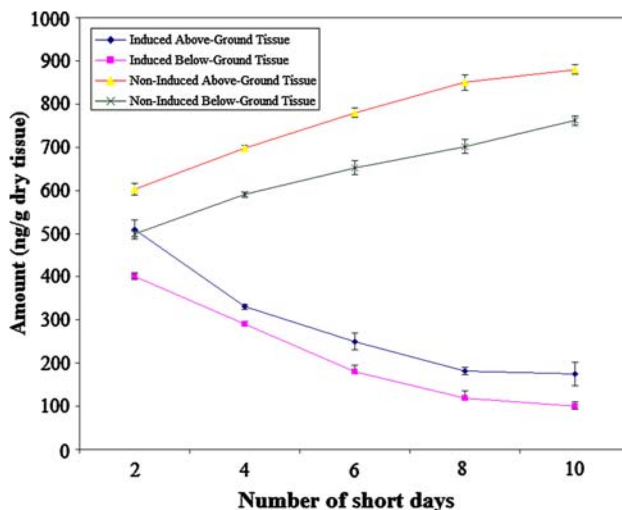

Fig. 1 Time course of changes in the levels of gibberellic acid after transfer to inducing conditions

consistently observed between 8 and 10 days after transfer to inducing conditions. Furthermore, studies by Langille and Hepler (1992) revealed that tuberization increased threefold in stem cuttings taken from noninduced donor plants previously treated with inhibitors of GA_3 biosynthesis. Foliar application of GA_3 to plants grown under identical conditions reduced tuberization 14-fold even though induced control potato cuttings exhibited almost complete tuberization (Langille and Hepler 1992). In accordance with results of previous studies (Jackson 1999; Fernie and Willmitzer 2001), our results confirm that GA_3 levels increased under noninducing conditions and that no tuber formation was observed. This striking correlation between increased level of GA_3 in noninducing conditions and absence of tuberization strongly suggests that regulation of GA_3 by light and temperature is at least one mechanism by which tuberization is inactivated in response to long days.

Levels of Jasmonates

Because jasmonates are a relatively new class of plant hormones, it was decided to screen for jasmonates present in our experimental potato tissues. Although other compounds cannot be excluded, the jasmonates discussed below were the only jasmonates that could be detected under our experimental conditions. Jasmonic acid levels (JA) were significantly higher in induced than in noninduced tissues (Figure 2). Overall, JA levels in the induced tissues increased 1.6-fold during the first two days of our studies and fourfold over the first four days when compared with noninduced tissues over the same time frame. On day 6 a slight decrease in JA levels was noticed, only to be followed by a rapid increase. By day 10 levels in induced aboveground and belowground tissues exceeded those observed during days 2–4. It should be reiterated that small tubers were noted only on the induced plants after 8–10 day inductive cycles. No dramatic changes were observed in JA levels of noninduced tissues.

Jasmonic acid is synthesized from linolenic acid in a lipoxygenase-dependent process (Vick and Zimmerman 1984; Creelman and Mullet 1997; Schaller and others 2005). It is possible that JA levels are under photoperiodic control via regulation of lipoxygenase activity, which increases during tuberization (Kolomiets and others 2001). Because JA was higher in leaf sheaths and roots of bulbing onion under long-day conditions (Nojiri and others 1992), it is of interest why the highest JA levels in potato were associated with short-day conditions. Thus, photoperiod may not be the sole factor controlling synthesis of jasmonic acid.

Several researchers (Koda and others 1991; Pelacho and Mingo-Castel 1991; Koda 1992; Koda and others 1992)

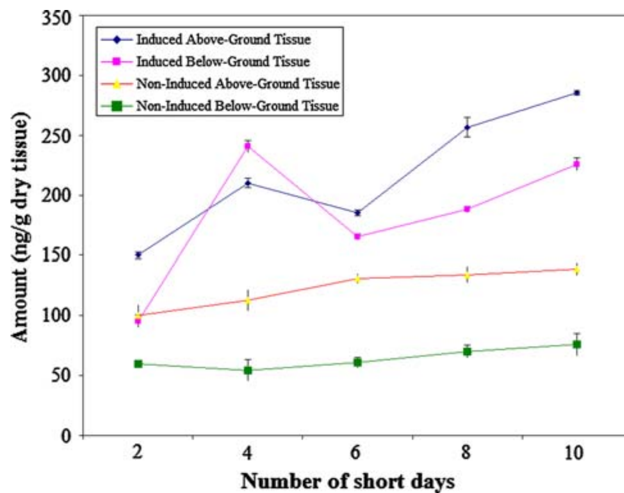

Fig. 2 Time course of changes in the levels of jasmonic acid after transfer to inducing conditions

have reported that JA has strong tuber-promoting activity *in vitro* and has perhaps the strongest tuber-inducing activity among jasmonates (Koda and others 1991). The increased JA levels observed in the present study under conditions favoring tuberization, along with its reported tuber-inducing activity, suggest that this compound has an important role in the tuberization process. This observation agrees with earlier suggestions that JA controls tuberization of yam and Jerusalem artichoke plants (Koda 1997). In both plants, which tuberize under short-day conditions, levels of endogenous JA increased progressively with growth of the storage organ.

Jasmonic acid has been reported to induce cell radial expansion at the subapical region of the rhizomes, a preliminary process in potato tuberization (Matsuki and others 1990; Shibaoka 1994; Fujino and others 1995; Cenzano and others 2003).

Effects of tissue type and induction on levels of tuberonic acid (TA) and its methyl ester (TAMe) are shown in Tables 1 and 2, respectively. TA and TAMe profiles during the induction period were similar. Their levels increased after transfer to inducing conditions and peaked at day 6, two days after the peak noted for JA, then declined. This pattern was observed in both belowground and aboveground tissues, with no such changes noted for noninduced tissues. Our data reinforce earlier reports by Koda and Okazawa (1988), who reported that under inducing conditions bioactivity of tuber-inducing substances in a potato leaf extract (later found to contain TA) increased but remained constant under noninducing conditions. In a related study, Helder and others (1993) isolated TA from *Solanum demissum* leaflets (a qualitative short-day species) grown under short-day conditions, whereas under long-day conditions TA was not detected.

Tuberonic acid and its methyl ester showed strong and equivalent tuber-inducing activities between 10^{-7} M and 10^{-5} M (Koda and others 1991). The decreased levels of JA observed at day 6 with a concomitant increase of tuberonic acid are consistent with the suggestion that JA may be converted to tuberonic acid. This conclusion is further supported by three reports in the literature. Yoshihara and others (1996) observed that ^{14}C-labeled JA was metabolized to tuberonic acid by *Solanum tuberosum* under inducing conditions. Kiyota and others (1996) also demonstrated that a hydroxyl group at the end of the pentenyl side chain of JA is not necessary for potato tuber-inducing activity. Moreover, Helder and others (1993) observed that enzyme(s) responsible for hydroxylation of JA in potato leaves are effective under short-day conditions.

After transfer to inducing conditions, methyl 7-isocucurbate (CAMe) increased dramatically only in belowground tissues (Table 3). Levels in the other tissues were virtually unchanged. Koda and others (1991) reported that the potato tuber-inducing activity of methyl cucurbate was somewhat lower than that of JA. It is likely that CAMe is a metabolite of JA.

Dihydromethyl jasmonate levels in induced and noninduced tissues were virtually indistinguishable and therefore appeared not to be influenced by induction (Table 4). It should also be noted, however, that dihydromethyl

Table 1 Levels of tuberonic acid in potato tissue

Harvest days	Amount (ng tuberonic acid/g dry tissue)[a,b]			
	Induced tissue		Noninduced tissue	
	Aboveground	Belowground	Aboveground	Belowground
2	200.0 ± 8.19 b	110.8 ± 1.93 c	170.0 ± 7.21 b	100.0 ± 8.00 c
4	235.0 ± 4.58 b	140.2 ± 2.03 c	180.8 ± 1.59 b	120.6 ± 2.62 c
6	290.8 ± 2.71 a	199.4 ± 2.95 b	186.0 ± 4.00 b	134.0 ± 3.61 c
8	230.4 ± 9.03 b	140.0 ± 9.17 c	197.6 ± 3.66 b	123.6 ± 2.12 c
10	220.7 ± 3.76 b	145.8 ± 6.36 c	205.3 ± 4.07 b	110.2 ± 5.43 c

[a] The data are presented as the mean ± standard deviation of two replicates (two independent determinations per replicate)

[b] Mean values followed by the same letter within a column are not significantly different by Duncan's multiple-range test at $P < 0.05$

Table 2 Levels of methyl tuberonate in potato tissue

	Amount (ng methyl tuberonate/g dry tissue)[a,b]			
	Induced tissue		Noninduced tissue	
Harvest days	Aboveground	Belowground	Aboveground	Belowground
2	301.0 ± 9.5 ab	251.0 ± 9.0 b	265.4 ± 12.9 b	220.2 ± 5.4 c
4	320.3 ± 5.0 ab	274.4 ± 14.1 b	279.0 ± 6.6 b	230.0 ± 6.2 c
6	362.8 ± 5.4 a	300.5 ± 8.8 b	290.2 ± 10.9 b	240.8 ± 6.7 c
8	339.9 ± 10.3 ab	287.0 ± 7.5 b	295.8 ± 5.2 b	235.9 ± 7.2 c
10	350.2 ± 11.3 ab	295.7 ± 2.12 b	282.4 ± 7.1 b	256.2 ± 7.1 bc

[a] The data are presented as the mean ± standard deviation of two replicates (two independent determinations per replicate)

[b] Mean values followed by the same letter within a column are not significantly different by Duncan's multiple-range test at $P < 0.05$

Table 3 Levels of methyl 7-isocucurbate in potato tissue

	Amount (ng methyl 7-isocucrubate/g dry tissue)[a,b]			
	Induced tissue		Noninduced tissue	
Harvest days	Aboveground	Belowground	Aboveground	Belowground
2	155.0 ± 7.0 c	271.6 ± 8.6 b	130.0 ± 9.5 c	101.6 ± 8.0 c
4	185.2 ± 8.0 bc	330.6 ± 6.1 ab	142.5 ± 6.9 c	115.0 ± 7.2 c
6	200.0 ± 10.6 bc	364.3 ± 5.1 a	161.0 ± 6.6 c	130.0 ± 9.8 c
8	215.1 ± 5.0 bc	395.1 ± 16.8 a	155.1 ± 5.0 c	140.4 ± 4.1 c
10	225.0 ± 7.2 bc	415.0 ± 10.8 a	167.5 ± 6.6 c	150.3 ± 5.5 c

[a] The data are presented as the mean ± standard deviation of two replicates (two independent determinations per replicate)

[b] Mean values followed by the same letter within a column are not significantly different by Duncan's multiple-range test at $P < 0.05$

jasmonate did not stimulate tuberization *in vitro* at concentrations below 10^{-5} M (Koda and others 1991), but it did show a synergistic promoting action in combination with JA. When applied simultaneously, the tuber-inducing activity was stronger than that of JA alone. Trewavas (1983) postulated that hormonal control in plant development might be due to changes in the sensitivity of target cells to hormones rather than to changes in their concentrations.

Levels of Cytokinins

Isopententyladenine and zeatin are reported to be predominant cytokinins found in tissues of higher plants (Kuroha and others 2002). *cis*-Zeatin and its riboside have been isolated from a number of plants, including potato (Mauk and Langille 1978). There is, however, a controversy over their abundance, biological activity, and function in whole plants (Veach and others 2003). Therefore, we decided to analyze levels of zeatin and its riboside as well as isopentenyladenine in *Solanum tuberosum* to shed more light on these compounds and their potential roles.

cis-Zeatin riboside (*cis*-ZR) was identified as the major cytokinin present, being significantly higher in induced aboveground tissues (Figure 3). In addition, induced tissue had a greater quantity of *cis*-ZR, *trans*-zeatin (Table 6), and isopentenyladenine (Table 7) than did noninduced tissue. In contrast, *trans*-ZR (Table 5) was more abundant under noninducing conditions. Langille and Forsline (1974) showed a twofold increase in induced versus noninduced tissue in total cytokinin-like activity. Subsequently, Mauk and Langille (1978) reported that the principal cytokinin present in "Katahdin" potato tissues was *cis*-ZR, whose levels were significantly higher in induced tissues. They showed the highest levels of *cis*-ZR in belowground tissue 4 days after transfer of plants to inducing conditions. This finding was further supported by the present study. The observation that *cis*-ZR levels, more than twice that of noninduced belowground tissues, were observed in induced tissues 4 days before the first evidence of tuber formation might suggest that Gregory's (1956) tuber induction state had been achieved. The *cis*-ZR trends in the present study mirror those reported by Mauk and Langille (1978), although values reported here are about 1.5 times higher than those for the earlier study. This

Table 4 Levels of dihydromethyljasmonate in potato tissue

Harvest days	Amount (ng dihydromethyljasmonate/g dry tissue)[a,b]			
	Induced tissue		Noninduced tissue	
	Aboveground	Belowground	Aboveground	Belowground
2	200.3 ± 5.5 a	149.7 ± 3.7 ab	180.0 ± 3.5 b	143.4 ± 4.4 b
4	219.6 ± 2.6 a	170.0 ± 7.2 ab	192.7 ± 3.8 b	147.6 ± 5.9 b
6	235.2 ± 4.7 a	185.3 ± 6.2 ab	200.6 ± 4.8 b	150.4 ± 3.1 b
8	230.2 ± 5.9 a	179.4 ± 6.4 ab	215.0 ± 4.6 b	144.5 ± 3.3 b
10	241.5 ± 5.7 a	186.1 ± 6.2 ab	224.4 ± 4.5 b	155.2 ± 6.7 b

[a] The data are presented as the mean ± standard deviation of two replicates (two independent determinations per replicate)

[b] Mean values followed by the same letter within a column are not significantly different by Duncan's multiple-range test at $P < 0.05$

difference may be the result of a modified extraction procedure and improved detection methods.

The pattern of accumulation of *cis*-ZR is similar to that observed for JA (Figure 2). Between days 2 and 4 under inducing conditions, levels of *cis*-ZR and JA increased 2.0-fold and 2.5-fold, respectively. It could be speculated that control of tuberization in *Solanum tuberosum* is influenced by the presence of increased quantities of both JA and *cis*-ZR. Because both compounds have been shown to stimulate tuberization *in vitro* (Langille and Forsline 1974; Mauk and Langille 1978; Koda and others 1991), one is led to speculate on which of the two is most important. Dermastia and others (1994) observed that treatment with JA of potato plantlets cultured *in vitro* increased their endogenous *cis*-ZR levels by 50%. It is possible that JA triggers some reaction leading to the biosynthesis of *cis*-ZR; the reverse is also possible. The fact remains that four days after *cis*-zeatin riboside reached its maximum level, signs of tuberization were observed. Similarly, Palmer and Smith (1969) demonstrated the requirement of cytokinins for *in vitro* tuberization of excised rhizomes. The present findings, along with those by Langille and coworkers (1974, 1978), suggest that *cis*-ZR may play a role in *in vivo* tuberization of the potato plant. Further support for this theory comes from the observation that doubling of the *cis*-ZR in transgenic potato plants was associated with increased tuber production (Ivana and others 1997).

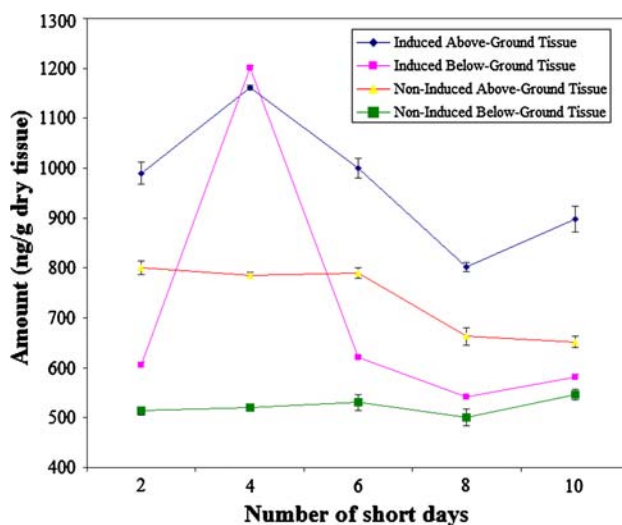

Fig. 3 Time course of changes in the levels of *cis*-zeatin riboside after transfer to inducing conditions

trans-Zeatin riboside did not appear to be influenced by induction because its levels increased slowly over the sampling period in both induced and noninduced tissues. Aboveground tissues, regardless of induction status, contained somewhat higher levels of this compound than belowground tissues (Table 5). *N*-Ribosylation may be an important process in regulating levels of active cytokinins by inactivation of certain forms (Letham and Palni 1983; Auer 1997). This hypothesis may be supported by comparing the levels of *trans*-ZR to those of *trans*-zeatin. The former compound was observed to increase in noninduced tissues (Table 5), whereas levels of the latter declined (Table 6). Under inducing conditions, *trans*-zeatin levels progressively increased. Therefore, *trans*-ZR is thought to be a precursor of *trans*-zeatin.

Recently, a *cis-trans*-isomerase of zeatin was isolated from *Phaseolus* roots (Bassil and others 1993). This enzyme preferentially converts *cis*-zeatin to *tran*-zeatin. Assuming that *cis-trans*-isomerase is present in the potato tissue and *cis*-zeatin riboside is biotransformed to *cis*-zeatin, the latter will be isomerized to *trans*-zeatin by the action of *cis-trans*-isomerase. Because *cis*-zeatin could not be detected in the present study, it is suggested that this enzyme might be present in the potato tissue. Nicander and others (1995) cited that *cis*-zeatin and its riboside have 5-100 times lower activity than the corresponding *trans*-zeatin compounds in cytokinin bioassays.

It has been reported that *cis*-zeatin riboside is the major cytokinin in potato leaves which, according to Ewing

Table 5 Levels of *trans*-zeatin riboside in potato tissue

	Amount (ng *trans*-zeatin riboside/g dry tissue)[a,b]			
	Induced tissue		Noninduced tissue	
Harvest days	Aboveground	Belowground	Aboveground	Belowground
2	320.3 ± 5.0 ab	216.2 ± 4.8 c	400.4 ± 9.3 a	270.0 ± 6.2 bc
4	360.4 ± 4.6 ab	232.0 ± 4.4 bc	448.5 ± 2.2 a	290.1 ± 2.6 bc
6	396.0 ± 4.4 ab	260.1 ± 7.7 bc	490.6 ± 4.8 a	310.2 ± 4.2 bc
8	400.8 ± 5.3 ab	242.5 ± 6.1 bc	500.8 ± 2.4 a	331.5 ± 3.5 bc
10	445.5 ± 3.9 ab	270.2 ± 4.2 bc	550.7 ± 5.5 a	350.6 ± 4.7 abc

[a] The data are presented as the mean ± standard deviation of two replicates (two independent determinations per replicate)

[b] Mean values followed by the same letter within a column are not significantly different by Duncan's multiple-range test at $P < 0.05$

Table 6 Levels of *trans*-zeatin in potato tissue

	Amount (ng *trans*-zeatin/g dry tissue)[a,b]			
	Induced tissue		Noninduced tissue	
Harvest days	Aboveground	Belowground	Aboveground	Belowground
2	450.0 ± 4.4 a	302.1 ± 3.4 b	410.2 ± 3.0 ab	280.4 ± 3.4 b
4	500.9 ± 4.3 a	325.2 ± 3.0 b	380.4 ± 2.1 ab	258.3 ± 2.1 b
6	550.0 ± 7.2 a	362.5 ± 5.2 b	350.8 ± 4.5 b	240.8 ± 3.0 bc
8	560.4 ± 4.0 a	340.0 ± 3.6 b	337.0 ± 3.0 b	210.5 ± 3.1 bc
10	572.9 ± 6.6 a	380.0 ± 5.6 b	325.0 ± 3.5 b	220.3 ± 2.5 bc

[a] The data are presented as the mean ± standard deviation of two replicates (two independent determinations per replicate)

[b] Mean values followed by the same letter within a column are not significantly different by Duncan's multiple-range test at $P < 0.05$

Table 7 Levels of isopentenyladenine in potato tissue

	Amount (ng isopentenyladenine/g dry tissue)[a,b]			
	Induced tissue		Noninduced tissue	
Harvest days	Aboveground	Belowground	Aboveground	Belowground
2	170.0 ± 3.5 bc	258.8 ± 7.1 b	162.1 ± 3.4 bc	210.0 ± 5.6 bc
4	221.3 ± 3.5 bc	345.2 ± 4.2 a	185.0 ± 4.6 bc	240.3 ± 5.1 bc
6	232.1 ± 3.6 bc	325.2 ± 4.5 a	241.8 ± 5.3 bc	270.5 ± 2.8 b
8	201.8 ± 4.6 bc	280.4 ± 4.1 b	182.0 ± 2.6 bc	220.3 ± 2.5 bc
10	176.2 ± 3.3 bc	240.0 ± 6.2 bc	156.1 ± 2.5 bc	188.0 ± 5.3 bc

[a] The data are presented as the mean ± standard deviation of two replicates (two independent determinations per replicate)

[b] Mean values followed by the same letter within a column are not significantly different by Duncan's multiple-range test at $P < 0.05$

(1995), seems to be unlikely. His reasoning was that the *trans*- rather than the *cis*- form of cytokinins is thought to predominate in higher plants. The present investigation seems to put an end to this disagreement by showing that *cis*-ZR was isolated in higher amounts than was *trans*-ZR.

Induction influenced only belowground levels of isopentenyladenine, suggesting that this compound is synthesized in belowground tissues and is not translocated. Maximum levels of this compound were attained at the fourth day following initiation of inducing conditions. Levels of isopentenyladenine did not differ in the other tissues, regardless of induction conditions (Table 7).

Conclusions

The tuberization process in *Solanum tuberosum* L. is under the control of photoperiod and temperature. Short days and

relatively low temperature stimulate the process, whereas long days and higher temperature are inhibitory.

The present study suggests that the levels of various endogenous phytohormones controls the tuber-inducing mechanism. A decrease in gibberellic acid levels is a prerequisite for tuberization with a concomitant increase in both *cis*-ZR and JA. It is proposed that effects of these compounds depend on their critical levels as well as on the "sensitivity" of the organs to them. Tissue sensitivity itself is likely to be partly a reflection of the endogenous hormone balance within these tissues. The rapid accumulation of cytokinins and jasmonates prior to evidence of tuber formation suggests that these compounds are critical to the tuber induction process. However, the relationship between endogenous jasmonates, cytokinins, and development of tubers after the induction stage (during the development of tubers) remains to be determined. It is important to note that our measurements of hormonal levels were made during the induction period (which was 10 days after the initiation of inducing conditions). We cannot exclude further changes in hormonal levels later in the development stage.

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