PHYSIOLOGIA PLANTARUM 86: 495–501. Copenhagen 1992

Hydroxycinnamoylputrescines are not causally involved in the tuberization process in potato plants

Gerhard Leubner-Metzger and Nikolaus Amrhein

Leubner-Metzger, G. and Amrhein, N. 1992. Hydroxycinnamoylputrescines are not causally involved in the tuberization process in potato plants. – Physiol. Plant. 86: 495–501.

The possible role of hydroxycinnamoylputrescines in the tuberization process of potato plants was studied using in vitro tuberization systems. Minitubers in shoot cultures of *Solanum tuberosum* ssp. *andigena* and *S. tuberosum* ssp. *tuberosum* were obtained in vitro within 3 weeks of dark incubation after increasing the sucrose concentration in the Murashige-Skoog (T. Murashige and F. Skoog. 1962. Physiol. Plant. 15: 473–497.) medium (without hormones) from 60 to 240 m*M*, both in the presence and absence of benzylaminopurine (BAP). Feruloylputrescine (FP) and caffeoylputrescine (CP) increased with tuberization, with a sharp maximum at day 9 in the shoot, but only when the medium contained BAP. When inhibitors of phenylalanine ammonia-lyase (PAL) and of polyamine biosynthesis were added to the medium containing BAP, the levels of FP and CP were reduced to values lower than those observed in the absence of BAP, but there was no significant effect on the number and dry weight of tubers formed. Addition of BAP without increasing the sucrose content also resulted in CP and FP accumulation, but failed to induce tuberization of the cultures. Experiments with in vitro stolon cultures and leaf cuttings also supported the conclusion that CP and FP accumulated as a response to the application of BAP, without having any effect on optimal tuberization. These results indicate that the increase of hydroxycinnamoylputrescines during tuber formation is unlikely to be causally involved in the tuberization process in potato plants.

Key words – Benzylaminopurine, caffeoylputrescine, feruloylputrescine, hydroxycinnamic acid amides, in vitro minitubers, polyamines, potato, Solanaceae, tuberization.

G. Leubner-Metzger and N. Amrhein (corresponding author): Institute of Plant Sciences, Plant Biochemistry and Physiology, Swiss Federal Institute of Technology, Sonneggstr. 5, CH-8092 Zürich, Switzerland.

Introduction

Tuberization of potato plants is a complex physiological process, in which tubers are formed on underground shoots, the stolons (Ewing 1987, Vreugdenhil and Struik 1989). The first sign of tuberization after tuber induction and tuber initiation is a swelling of the subapical region of the stolon tip. In vitro tuberization systems have proven to be useful tools in the analysis of this process (Ewing 1985, Tovar et al. 1985).

Hydroxycinnamic acid amides (HCAs) are conjugates of hydroxycinnamic acids (such as caffeic acid, ferulic acid, *p*-coumaric acid) and amines (such as putrescine, spermidine, spermine) and are widely distributed in the plant kingdom. They have been suggested to have possible functions in several plant developmental processes (Martin-Tanguy 1985, Flores et al. 1989, Burtin et al. 1990).

It was inferred that HCAs might be causally involved in the tuberization process of potato plants (Paynot et al. 1983, Martin-Tanguy 1985). In their experiments these authors used the potato hybrid SDA_6, which forms tubers only in short days. In stolons of leaf cuttings, basic HCAs (*p*-coumaroylputrescine, caffeoylputrescine, feruloylputrescine and traces of caffeoylspermidine) were shown to accumulate parallel to the formation of tubers. Under short day conditions, levels of basic HCAs in the tuberizing stolons were about 0.02

Received 28 July, 1992

μmol (g fresh weight)$^{-1}$, whereas in the leaves of these cuttings and in nontuberizing stolons of cuttings cultured under long day conditions levels remained low, at about 0.002 μmol (g fresh weight)$^{-1}$ (data also compiled in Tab. 1).

In field grown plants of *Solanum tuberosum* ssp. *tuberosum* cv. Astrid we have shown (Leubner-Metzger and Amrhein 1992); (data also in Tab. 1) that levels of CP, FP, *p*CP and DFP were below 0.02 μmol (g dry weight)$^{-1}$ in all organs and that no accumulation of these compounds in tuberizing stolon tips was observed as described by Paynot et al. (1983).

In the present paper we have examined hydroxycinnamoylputrescines in in vitro tuberization systems of potato plants. We have tested the hypothesis of Paynot et al. (1983) using in vitro minituber cultures, in vitro stolon cultures and leaf cuttings in combination with inhibitors of PAL (AOPP, APEP, AIP) and of polyamine biosynthesis (DFMA, DFMO), which were expected to block HCA accumulation. AOPP and APEP have been introduced as potent inhibitors of PAL, and especially AOPP has been widely used to block phenylpropanoid synthesis in vivo (Amrhein 1986, Laber et al. 1986). Recently AIP has been shown to be a potent inhibitor of PAL from buckwheat and other sources, both in vitro and in vivo (Zoń and Amrhein 1992).

Abbreviations – AIP, 2-aminoindan 2-phosphonic acid; AOPP, α-aminooxy-β-phenylpropionic acid; APEP, (1-amino-2-phenylethyl)-phosphonic acid; BAP, benzylaminopurine; CP, caffeoylputrescine; *p*CP, *p*-coumaroylputrescine; DFMA, α-difluoromethylarginine; DFMO, α-difluoromethylornithine; DFP, diferuloylputrescine; FP, feruloylputrescine; HCAs, hydroxycinnamic acid amides; MS, Murashige and Skoog (1962); NAA, 1-naphthyl acetic acid; PAL, phenylalanine ammonia-lyase.

Materials and methods

Chemicals

p-Coumaroylputrescine (*p*CP), feruloylputrescine (FP) and diferuloylputrescine (DFP) were a gift from Dr E. Ebert (Ciba-Geigy Ltd, Basel, Switzerland). Caffeoylputrescine (CP) was a gift from Merck, Darmstadt, Germany. The patatin-cDNA-clone pcT58 was kindly provided by Prof. L. Willmitzer (Institut für Genbiologische Forschung, Berlin, Germany), and AIP by Dr J. Zoń (Technical University, Wroclaw, Poland). All other chemicals were of analytical or HPLC grade.

Plant material

Solanum tuberosum L. ssp. *tuberosum* cv. Astrid, Carola and Indira, were grown in the experimental field of the Ruhr-University Bochum (Germany). Sprouted tubers were planted in soil in April. Plants were harvested between June and September. Sterile plantlets of these cultivars and of the two clones 482W and 283W of the wild potato *S. tuberosum* L. ssp. *andigena* (Juz. et Buk.) Hawkes were cultivated as described below.

In vitro minituber culture

Using a 3-step organ culture system (Tovar et al. 1985), minitubers of *S. tuberosum* ssp. *tuberosum* cultivars and of *S. tuberosum* ssp. *andigena* clones were obtained in vitro.

Step 1: Sterile plantlets were cultivated in 0.5-l glass vessels containing 80 ml MS-medium with 1% (w/v) Bacto-agar, 60 m*M* sucrose and 5.4 n*M* NAA. Long day culture conditions were: 16 h photoperiod, 80 μmol m^{-2} s^{-1}, 20°C. After 4 weeks, single-node cuttings were taken for vegetative propagation.

Step 2: Three single-node cuttings from each plantlet were cultivated in 100-ml Erlenmeyer flasks containing 10 ml liquid MS-medium with 60 m*M* sucrose and without hormones. Long day culture condition were: 16 h photoperiod, 200 μmol m^{-2} s^{-1}, 20°C, shaking at 60 rpm. After 4 weeks, a green shoot culture had developed.

Step 3: For induction of in vitro minitubers the shoot culture medium was replaced by a hormone-free MS-medium with the sucrose concentration increased to 240 m*M*. After 3–4 weeks incubation in the dark with shaking at 20°C, minitubers had been formed. Cultures were harvested at intervals of a few days, divided into shoot, minitubers, and root, and lyophilized.

Leaf cuttings

Cuttings (Ewing 1985) were taken from tuberized potato plants *S. tuberosum* ssp. *tuberosum* cv. Astrid grown in the field. Only the 5th and 6th leaves from the top were used. Cuttings consisted of a single leaf, its node with the axillary bud and some of the internode below. For experiments, internodes of the leaf cuttings were placed into small, non-transparent vessels containing 15 ml 1/10 strength Hoagland-solution (Wilson et al. 1978). In addition, either 22 μ*M* BAP or 10 μ*M* methyl jasmonate were applied, but not in the control. The axillary bud was covered with aluminum foil, whereas the leaf was illuminated. Culture conditions were 8 h photoperiod, 350 μmol m^{-2} s^{-1}, temperature: day 25°C, night 13°C. Axillary buds developed into tubers within two weeks of incubation.

In vitro stolon culture

Isolated stolons from lateral shoots of tubers of *S. tuberosum* ssp. *tuberosum* cv. Indira were incubated in the dark in MS-medium containing 180 m*M* sucrose and either 22 μ*M* BAP or 170 μ*M* coumarin, or no further addition, according to the method of Palmer and Smith (1969, 1970).

Extraction and HPLC of HCAs and free polyamines

Extraction and HPLC analysis of HCAs and free polyamines were performed as described elsewhere

(Leubner-Metzger and Amrhein 1992), HCAs according to the procedure of Wyss-Benz et al. (1988) and free polyamines according to Flores and Galston (1982) and Smith and Davies (1985, 1987).

Northern blot analysis of patatin-specific mRNA

Total RNA from shoots of in vitro minituber cultures was extracted according to Eckes et al. (1986) at various times during the dark incubation. A radioactively labelled 700 bp-patatin-cDNA-fragment was obtained by restriction of the patatin-cDNA-clone pcT58 (Rosahl et al. 1986a,b) with EcoRI and HindIII. RNA-agarose-gel electrophoresis and Northern-blot analysis were carried out as described by Sambrook et al. (1989).

Results

Total levels of HCAs and free polyamines in in vitro minituber cultures

The clone 482W of the wild potato *S. tuberosum* ssp. *andigena* was chosen for the experiments, because it forms tubers only in short days. Because of the long day incubation conditions in step 1 and 2 of the organ culture system, plantlets developed in a non-induced state at the beginning of step 3.

Under the inductive conditions of step 3, minitubers of *S. tuberosum* ssp. *andigena* 482W were obtained in vitro. The following series were performed, with different supplements to the culture medium in step 3: MS-medium with 240 m*M* sucrose (control); addition of either 10 µ*M* AIP (+AIP); or 22 µ*M* BAP (+BAP); or a combination of both (+BAP+AIP). Addition of BAP to the medium increased the number of obtained minitubers to up to 130% (7.7 per culture) compared to the 5.9 tubers per culture in the control (100%), but BAP was, in principle, not required for tuber formation. In the series +BAP+AIP this enhancing effect of BAP was partially reduced to 6.5 per culture (110%). Neither BAP nor AIP had a significant effect on the average dry weight of a minituber.

In all series, first swellings of axillary buds were visible at about day 9 after transfer to 240 m*M* sucrose. Additionally, patatin-mRNA became detectable at this day in the series +BAP. The expression of patatin, the main potato storage protein, is normally restricted to tubers (Park et al. 1985, Rosahl et al. 1986a,b). As patatin-mRNA was not detectable *before* visual swelling occurred, its determination did not provide an advantage in the recognition of the early steps of tuberization.

Figure 1 shows the changes in the total levels of CP and FP in the shoot cultures during minituber formation in the dark incubation period. In the +BAP series, the levels of CP and FP began to rise 2 to 3 days after transfer of the cultures to 240 m*M* sucrose and reached a sharp maximum on day 9. At this maximum, coincident with the time of tuber initiation, CP and FP had increased 30-fold and 4-fold, respectively. The levels of CP and FP then decreased, but rose again at the end of the dark incubation period (Fig. 1, Tab. 1). In all other series, minituber formation occurred without significant changes in the levels of CP. In the control series, minitubers were formed in parallel to a slow, continuous increase in the FP-level (Fig. 1, Tab. 1). This increase was totally blocked by the addition of 10 µ*M* AIP, without any influence on tuber formation. In the series +BAP+AIP, the inhibitor blocked the biosynthesis of FP partially, and that of CP totally, without any influence on tuber formation (Fig. 1).

DFP was detected only at low levels. The pattern of its changes was the same as that found for CP and FP. There was also a maximum at day 9, but only in the series with 22 µ*M* BAP (Tab. 1). *p*CP and *p*-coumaroylspermidine were detected only in the series +BAP. Their levels remained constant during the entire period of dark incubation, *p*CP at 0.01 µmol (g dry weight)$^{-1}$ only in shoots and *p*-coumaroylspermidine at 0.1 µmol (g dry weight)$^{-1}$ in all organs.

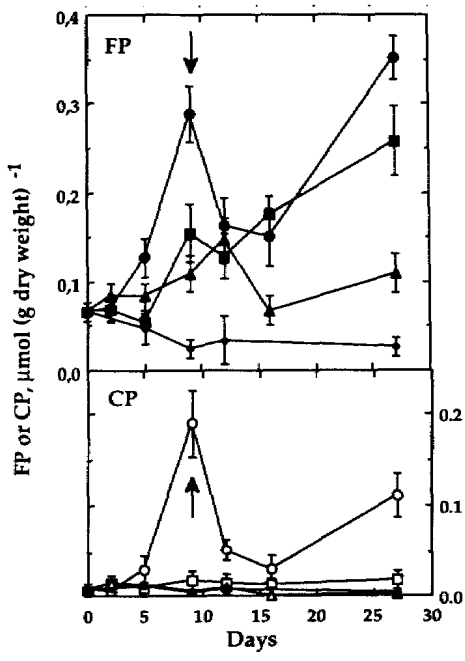

Fig. 1. Total endogenous levels of feruloylputrescine (FP; upper panel) and caffeoylputrescine (CP; lower panel) in µmol (g dry weight)$^{-1}$ during the dark incubation period of the in vitro minituber cultures of *S. tuberosum* ssp. *andigena* 482W. Series with 240 m*M* sucrose in the MS-medium: no addition (control) (■, FP; □, CP), addition of 22 µ*M* BAP (+BAP) (●, FP; ○, CP), addition of 22 µ*M* BAP and 10 µ*M* AIP (+BAP+AIP) (▲, FP; △, CP), addition of 10 µ*M* AIP (+AIP) (◆, FP; ◇, CP). The arrow at day 9 marks the time when first swellings of axillary buds were visible in all series and patatin-mRNA became detectable in the series +BAP. Results are expressed as means ±SD (n = 5).

Similar results were obtained with organ cultures of the *S. tuberosum* ssp. *tuberosum* cultivars Carola (Tab. 1) and Astrid. Other inhibitors of PAL (*R*-APEP, *S*-AOPP) as well as of polyamine biosynthesis (DFMA, DFMO), applied in 10 μ*M* concentration either alone or in combination, produced a suppression of HCA levels comparable to AIP but had, likewise, no influence on the formation of in vitro minitubers.

These results demonstrate that the increase in the sucrose content of the MS-medium from 60 to 240 m*M* was fully sufficient for optimal formation of in vitro minitubers. The application of exogenous hormones such as BAP was clearly not required. Accumulation of CP and FP during early tuberization was observed exclusively when BAP was present in the medium and therefore represents a response to the hormone independent of tuber formation. Suppression of the accumulation of FP and CP by application of suitable inhibitors of their biosynthesis did not affect the formation of in vitro minitubers. These results therefore indicate that hydroxycinnamoylputrescines are not causally involved in the tuberization process in potato plants.

Shoot cultures of *S. tuberosum* ssp. *andigena* 482W contained total levels of free polyamines of 1.7 μmol (g dry weight)$^{-1}$ putrescine and about 0.7 μmol (g dry weight)$^{-1}$ of both spermidine and spermine at the beginning of the dark incubation period. Within 1 to 2 weeks these levels decreased to values below 0.1 μmol (g dry weight)$^{-1}$ in all series (Tab. 1).

HCA accumulation as a response to BAP in in vitro minituber cultures

Figure 2 shows the tissue-specific accumulation of HCA as a response to the application of BAP. The hormone caused the accumulation of CP and FP only in the shoot. Only the shoot was responsible for the maximum at day 9, and therefore for the pattern of the changes. In roots, there was only a slow and continuous increase of FP and a very slight increase in CP levels up to the end

Tab. 1. Comparison of the endogenous levels of caffeoylputrescine (CP), feruloylputrescine (FP), diferuloylputrescine (DFP), free putrescine (Put) and free spermidine (Spd) in field grown potato plants (data of G. Leubner-Metzger and N. Amrhein 1992) and in leaf cuttings of *S. tuberosum* ssp. *tuberosum* cv. Astrid, in in vitro stolon cultures of *S. tuberosum* ssp. *tuberosum* cv. Indira, and in in vitro minituber cultures of *S. tuberosum* ssp. *tuberosum* cv. Carola and of *S. tuberosum* ssp. *andigena* 482W (this work). Data on the levels of basic HCAs (CP, FP, *p*CP and traces of caffeoylspermidine) in leaf cuttings of the F_1-potato hybrid SDA_6 are from Paynot et al. (1983). ND, Not determined.

Experimental system	Levels of HCAs and free polyamines, μmol (g dry weight)$^{-1}$				
	CP	FP	DFP	Put	Spd
Field grown plants cv. Astrid					
unthickened stolon tips	0.007	0.001	0.001	2.6	3.4
thickened stolon tips	0.013	0.004	0.001	1.2	2.4
young tubers <0.5 cm	0.017	0.007	0.001	0.8	1.5
young tubers 0.5–2 cm	0.008	0.005	0.000	0.9	1.1
Leaf cuttings cv. Astrid (in nutrient solution)					
tuberizing axillary buds (without BAP)	0.000	0.020	0.001	ND	ND
tuberizing axillary buds (with 22 μ*M* BAP)	0.130	0.570	0.090	ND	ND
In vitro stolon culture cv. Indira					
untuberized culture (without BAP)	0.017	0.195	ND	ND	ND
tuberized culture (with 22 μ*M* BAP)	0.072	0.477	ND	ND	ND
In vitro minituber culture cv. Carola					
culture day 32 (without BAP)	0.100	0.264	ND	ND	ND
culture day 32 (with 22 μ*M* BAP)	1.926	1.432	ND	ND	ND
In vitro minituber culture ssp. *andigena* 482W					
culture day 0	0.007	0.067	0.006	1.7	0.7
culture day 9 (without BAP)	0.018	0.154	0.013	0.4	0.1
culture day 9 (with 22 μ*M* BAP)	0.190	0.288	0.029	0.8	0.3
culture day 27 (without BAP)	0.018	0.257	0.006	0.1	0.0
culture day 27 (with 22 μ*M* BAP)	0.111	0.351	0.010	0.1	0.0

	Total levels of basic HCAs (CP, FP, *p*CP, traces of caffeoylspermidine), μmol (g fresh weight)$^{-1}$
Leaf cuttings of the F_1-potato hybrid SDA_6	
tuberized stolons (short day)	0.020
untuberized stolons (long day)	0.002

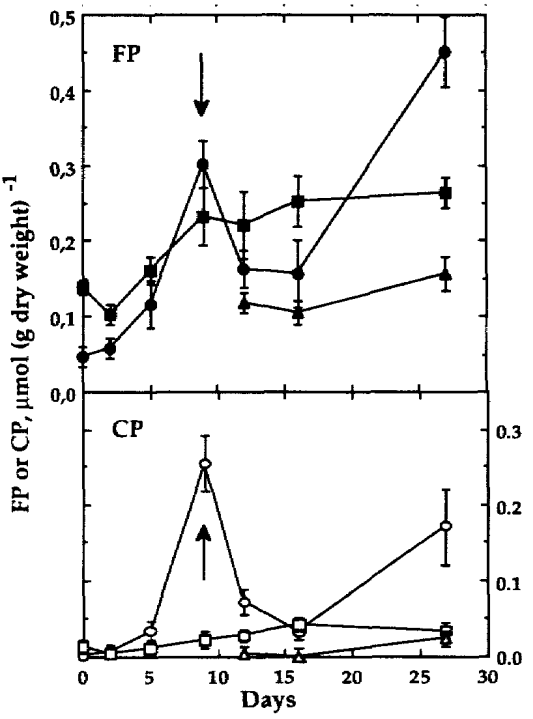

Fig. 2. Tissue-specific accumulation of HCAs as a response to the application of BAP. Endogenous levels of feruloylputrescine (FP; upper panel) and caffeoylputrescine (CP; lower panel) in µmol (g dry weight)$^{-1}$ during the dark incubation period of the in vitro minituber cultures of *S. tuberosum* ssp. *andigena* 482W. Series with 240 m*M* sucrose and 22 µ*M* BAP added to the MS-medium (+BAP). Levels in shoot (●, FP; ○, CP), minitubers (▲, FP; △, CP) and root (■, FP; □, CP) are shown. Means ± SD (n = 5). Arrow as in Fig. 1.

of the dark incubation period. This FP accumulation in roots is independent of the application of BAP, and was also found in the roots of the control series. Application of 10 µ*M* AIP inhibited the increase of CP levels in all cases, and abolished the FP increase in the roots. The accumulation of FP in the shoot as a response to the application of BAP was partially inhibited by 10 µ*M* AIP. The maximum at day 9 was reduced and shifted to day 12. In all cases, no accumulation of CP, FP, *p*CP or DFP was measured in in vitro minitubers.

Figure 3 shows the effect of 9 days preincubation of shoot cultures with 10 µ*M* AIP in the light. This treatment resulted in low levels of CP and FP during the entire following dark incubation period in the presence of BAP. The formation of in vitro minitubers was not affected by this procedure.

Incubation of shoot cultures in the dark without increasing the sucrose concentration of the MS-medium, never resulted in tuberization, regardless of the application of BAP. Addition of 22 µ*M* BAP to a series with 60 m*M* sucrose caused a 12-fold increase in CP and a 7-fold increase in FP at the end of the dark incubation as compared to the control without BAP. In this case, CP- and FP-accumulation clearly occurred as a response to the application of BAP, without the formation of in vitro minitubers.

HCA accumulation as a response to BAP in cuttings and in in vitro stolon cultures

In control experiments with leaf cuttings from *S. tuberosum* ssp. *tuberosum* cv. Astrid, tubers at the end of stolons were formed from the axillary buds. While this work was in progress, jasmonates (for review, see Parthier 1990) were reported to induce tuberization (Yoshihara et al. 1989.) Addition of either 22 µ*M* BAP or 10 µ*M* methyl jasmonate to the Hoagland nutrient solution caused the formation of sessile tubers, which represent the strongest degree of tuberization (Ewing 1985).

When BAP was added, a 290-fold FP, 30-fold CP- and 20-fold DFP-accumulation was observed after 6 days in the young axillary buds during tuberization (Tab. 1). Levels of the 3 HCAs were at least 10-fold lower in the axillary buds of the control, in the tubers which had developed after two weeks, and in leaves and stems at any time. In both, in vitro minituber cultures and leaf cuttings, BAP increased the levels of CP, FP and DFP, but this increase was not a prerequisite for tuberization.

For in vitro stolon cultures (Palmer and Smith 1969,

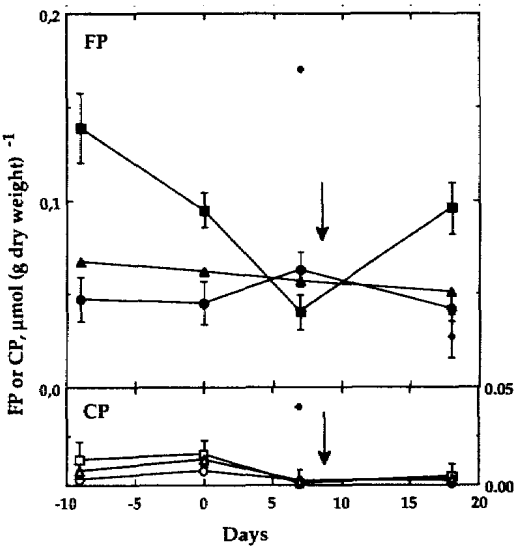

Fig. 3. Effect of 9 days preincubation of shoot cultures in the light with MS-medium containing 60 m*M* sucrose and 10 µ*M* AIP. Endogenous levels of feruloylputrescine (FP; upper panel) and caffeoylputrescine (CP; lower panel) in µmol (g dry weight)$^{-1}$ of the in vitro minituber cultures of *S. tuberosum* ssp. *andigena* 482W. Dark incubation with 240 m*M* sucrose, 22 µ*M* BAP and 10 µ*M* AIP applied to the MS-medium starting at day 0. Total levels (▲, FP; △, CP) and levels in shoot (●, FP; ○, CP), minitubers (◆, FP; ◇, CP) and root (■, FP; □, CP) are shown. Means ± SD (n = 5). Arrow as in Fig. 1.

1970) of *S. tuberosum* ssp. *tuberosum* cv. Indira, increasing the sucrose concentration of the MS-medium was not sufficient for tuberization. In this system, application of either BAP or coumarin (Stallknecht and Farnsworth 1982) was necessary. After 5 weeks of dark incubation, CP had accumulated 4-fold and FP 15-fold in tuberized cultures, but only when BAP was added (Tab. 1).

In all other cases CP-levels remained low, whereas FP-levels increased 5-fold, regardless of whether the culture was tuberized (addition of coumarin) or not (no addition). Comparable to our results with in vitro minituber cultures and leaf cuttings, CP accumulated in in vitro stolon cultures solely as a response to BAP application, while FP accumulation was promoted by the addition of BAP.

Discussion

Table 1 compares the endogenous levels of the hydroxycinnamoylputrescines CP, FP and DFP, and of the free polyamines putrescine and spermidine, in field grown potato plants (Leubner-Metzger and Amrhein 1992), with corresponding values in leaf cuttings, in in vitro stolon cultures and in in vitro minituber cultures (this communication) as well as with the levels of basic HCAs in leaf cuttings of the F_1-potato hybrid SDA_6 (Paynot et al. 1983). In contrast to the hypothesis of Paynot et al. (1983), our results clearly indicate that HCAs are unlikely to be causally involved in the tuberization process of potato plants.

We have shown that in field grown potato plants (Leubner-Metzger and Amrhein 1992), HCAs do not accumulate in tuberizing stolon tips. Levels of CP, FP, *p*CP and DFP remain at least 5-fold below the values found in tuberizing stolons of SDA cuttings by Paynot et al. (1983) (Tab. 1). In contrast to our dry weight based values, Paynot et al. (1983) based their HCA levels on fresh weight. For comparing HCA levels, we therefore considered a water content of about 85% for leaves and of 80 to 85% for stolons and tubers.

In our experiments with leaf cuttings and tuberization systems, HCA accumulation occurred solely in response to the application of BAP (Tab. 1). In in vitro minituber cultures, it was exclusively the sucrose concentration that determined tuberization. Application of BAP caused the accumulation of CP and FP in the shoot, regardless of tuberization. At the high sucrose concentration, addition of BAP had no further effect on tuber formation.

As in stolon tips of field grown potato plants, levels of free polyamines decreased in in vitro minituber cultures during the formation of tubers (Tab. 1).

Our observation that hydroxycinnamoylputrescines accumulate in response to hormone application in the in vitro tuberization systems is in agreement with results obtained in other systems. Martin-Tanguy et al. (1988) have described the accumulation of FP, CP, free putrescine and free spermidine in in vitro cultivated tobacco leaf explants as a response to the application of 2,4-D and/or BAP. Likewise, root formation in response to auxin application was accompanied by the accumulation of hydroxycinnamoylputrescines (Burtin et al. 1990). As a reaction to auxin application, free polyamines accumulate in tuber explants of *Helianthus tuberosus* (Evans and Malmberg 1989), and the content of free putrescine of tobacco callus cultures was found to depend on the concentration of NAA in the medium (Palazon et al. 1987). Free putrescine accumulates in cotyledons of *Cucumis sativus* upon cytokinin application (Suresh et al. 1978, Walker et al. 1988). Wyss-Benz et al. (1989, 1990) measured FP- and CP-accumulation in stem explants of tobacco species. They observed a correlation between FP-accumulation and the formation of cortical callus, and between CP-accumulation and in vitro flower formation, which they explained as a reaction to the distribution of endogenous auxin and cytokinin during the development of the explants. Using the same inhibitors that we have used, they showed that CP and FP are not involved in growth and floral bud formation of tobacco stem explants.

The inhibitors of PAL and of polyamine biosynthesis effectively suppressed the CP and FP accumulation caused by BAP in the in vitro minituber cultures, while they did not affect tuberization. These results indicate that the correlation between HCA-accumulation and tuber formation in some tuberization systems, or after application of BAP, does not reflect a causal relationship in this developmental process.

Recently, Yoshihara et al. (1989) isolated the jasmonate derivative tuberonic acid and its glycoside as natural tuber inducing substances from potato leaves. Several other jasmonate derivatives, such as methyl jasmonate, were shown to induce tuberization in a bioassay at concentrations of 10 μM (Koda and Okazawa 1988, Koda et al. 1988, Yoshihara et al. 1989). In our work, 10 μM methyl jasmonate, added to the Hoagland nutrient solution in the potato leaf cutting system, increased the tuberization of the axillary buds by causing sessile tubers, but without concomitant HCA accumulation. This again rules out HCAs as endogenous regulators of the tuberization of potato plants.

Acknowledgements – We thank Drs E. Ebert and M. Wyss-Benz (Ciba-Geigy Ltd, Basel, Switzerland), Dr L. Schilde-Rentschler (Universität Tübingen, Germany) and Dr H. Uhrig (Max-Planck-Institut für Züchtungsforschung, Köln, Germany) for discussions and suggestions, Prof. L. Willmitzer (Institut für Genbiologische Forschung, Berlin, Germany) for the patatin cDNA clone, Dr J. Zoń (Technical University, Wroclaw, Poland) for AIP, D. Halter for technical assistance, and Dr M. Kertesz for critical reading of the manuscript. Support by a grant from Ciba-Geigy Ltd, Basel, Switzerland, is gratefully acknowledged.

References

Amrhein, N. 1986. Specific inhibitors as probes into the biosynthesis and metabolism of aromatic amino acids. – Rec. Adv. Phytochem. 20: 83–117.

Burtin, D., Martin-Tanguy, J., Paynot, M., Carré, M. & Rossin, N. 1990. Polyamines, hydroxycinnamoylputrescines, and root formation in leaf explants of tobacco cultivated in vitro. – Plant Physiol. 93: 1398–1404.

Eckes, P., Rosahl, S., Schell, J. & Willmitzer, L. 1986. Isolation and characterization of a light-inducible, organ-specific gene from potato and analysis of its expression after tagging and transfer into tobacco and potato shoots. – Mol. Gen. Genet. 205: 14–22.

Evans, P. T. & Malmberg, R. L. 1989. Do polyamines have roles in plant development? – Annu. Rev. Plant Physiol. Plant Mol. Biol. 40: 235–269.

Ewing, E. E. 1985. Cuttings as simplified models of the potato plant. – *In* Potato Physiology (P. H. Li, ed.), pp. 154–199. Academic Press, London. ISBN 0-12-447660-0.

– 1987. The role of hormones in potato (*Solanum tuberosum* L.) tuberization. – *In* Plant Hormones and their Role in Plant Growth and Development (P. J. Davies, ed.), pp. 515–538. Kluwer Academic Publishers, Dordrecht. ISBN 90-247-3498-3.

Flores, H. E. & Galston, A. W. 1982. Analysis of polyamines in higher plants by high performance liquid chromatography. – Plant Physiol. 69: 701–706.

– , Protacio, C. M. & Signs, M. W. 1989. Primary and secondary metabolism of polyamines in plants. – Rec. Adv. Phytochem. 23: 329–393.

Koda, Y. & Okazawa, Y. 1988. Detection of potato tuber-inducing activity in potato leaves and old tubers. – Plant Cell Physiol. 29: 969–974.

– , Omer, E. A., Yoshihara, T., Shibata, H., Sakamura, S. & Okazawa, Y. 1988. Isolation of a specific potato tuber-inducing substance from potato leaves. – Plant Cell Physiol. 29: 1047–1051.

Laber, B., Kiltz, H. H. & Amrhein, N. 1986. Inhibition of phenylalanine ammonia-lyase in vitro and in vivo by (1-amino-2-phenylethyl) phosphonic acid, the phosphonic analogue of phenylalanine. – Z. Naturforsch. 41c: 49–55.

Leubner-Metzger, G. & Amrhein, N. 1992. The distribution of hydroxycinnamoylputrescines in different organs of *Solanum tuberosum* and other solanaceous species. – Phytochemistry (In press).

Martin-Tanguy, J. 1985. The occurrence and possible function of hydroxycinnamoyl acid amides in plants. – Plant Growth Regul. 3: 381399.

– , Martin, C., Paynot, M. & Rossin, N. 1988. Effect of hormone treatment on growth, bud formation and free amine and hydroxycinnamoyl putrescine levels in leaf explant of *Nicotiana tabacum* cultivated in vitro. – Plant Physiol. 88: 600–604.

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

Palazon, J., Pinol, M. T., Altabella, T., Cusido, R. & Serrano, M. 1987. Auxin-induced regulation of amino acid and putrescine in the free state and nicotine content in cultured tobacco callus. – J. Plant. Physiol. 128: 153–159.

Palmer, C. E. & Smith, O. E. 1969. Cytokinins and tuber initiation in the potato *Solanum tuberosum* L. – Nature 221: 279–280.

– & Smith, O. E. 1970. Effect of kinetin on tuber formation on isolated stolons of *Solanum tuberosum* L. cultured in vitro. – Plant Cell Physiol. 11: 303–314.

Park, W. D., Hannapel, D. J., Mignery, G. A. & Pikaard, C. S. 1985. Molecular approaches to the study of the major tuber proteins. – *In* Potato Physiology (P. H. Li, ed.), pp. 262–279. Academic Press, London. ISBN 0-12-447660-0.

Parthier, B. 1990. Jasmonates: hormonal regulators or stress factors in leaf senescence? – J. Plant Growth Regul. 9: 57–63.

Paynot, M., Perennec, P., Martin, C., Martin-Tanguy, J., Vernoy, R., Javelle, F. & Gautheret, R. 1983. Photopériodisme, tubérisation et phénolamides. – C. R. Acad. Sci. Paris Ser. III 297: 87–90.

Rosahl, S., Eckes, P., Schell, J. & Willmitzer, L. 1986a. Organ-specific gene expression in potato: isolation and characterization of tuber-specific cDNA sequences. – Mol. Gen. Genet. 202: 368–373.

– , Schmidt, R., Schell, J. & Willmitzer, L. 1986b. Isolation and characterization of a gene from *Solanum tuberosum* encoding patatin, the major storage protein of potato tubers. – Mol. Gen. Genet. 203: 214–220.

Sambrook, J., Fritsch, E. F. & Maniatis, T. 1989. Molecular Cloning – a Laboratory Manual, 2nd Ed. – Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. Vol 1, p. 7.43.

Smith, M. A. & Davies, P. J. 1985. Separation and quantitation of polyamines in plant tissue by high performance liquid chromatography of their dansyl derivatives. – Plant Physiol. 78: 89–91.

– & Davies, P. J. 1987. Monitoring polyamines in plant tissues by high performance liquid chromatography. – *In* HPLC in Plant Sciences (H. F. Linskens and J. F. Jackson, eds), pp. 209–277. Springer-Verlag, Berlin. ISBN 3-540-17243-2.

Stallknecht, G. F. & Farnsworth, S. 1982. General characteristics of coumarin-induced tuberization of axillary shoots of *Solanum tuberosum* L. cultured in vitro. – Am. Potato J. 59: 17–32.

Suresh, M. R., Ramakrishna, S. & Adiga, P. R. 1978. Regulation of arginine decarboxylase and putrescine levels in *Cucumis sativus* cotyledons. – Phytochemistry 17: 57–63.

Tovar, P., Estrada, R., Schilde-Rentschler, L. & Dodds, J. H. 1985. Induction and use of in vitro potato tubers. – International Potato Center, Lima, Peru, CIP-Circular 13: 1–5.

Vreugdenhil, D. & Struik, P. C. 1989. An integrated view of the hormonal regulation of tuber formation in potato (*Solanum tuberosum*). – Physiol. Plant. 75: 525–531.

Walker, M. A., Roberts, D. R. & Dumbroff, E. B. 1988. Effects of cytokinin and light on polyamines during the greening response of cucumber cotyledons. – Plant Cell Physiol. 29: 201–205.

Wilson, L. G., Bressan, R. A. & Filner, P. 1978. Light-dependent emission of hydrogen sulfide from plants. – Plant Physiol. 61: 184–189.

Wyss-Benz, M., Streit, L. & Ebert, E. 1988. Hydroxycinnamoyl amides in stem explants from flowering and non-flowering *Nicotiana tabacum*. – Physiol. Plant. 74: 294–298.

– , Streit, L. & Ebert, E. 1989. Feruloyl- and caffeoylputrescine in stem explants from photoperiodically determined *Nicotiana* species. – Physiol. Plant. 77: 593–598.

– , Streit, L. & Ebert, E. 1990. Feruloylputrescine and caffeoylputrescine are not involved in growth and floral bud formation of stem explants from *Nicotiana tabacum* L. var. Xanthi nc. – Plant Physiol. 92: 924–930.

Yoshihara, T., Omer, E. A., Koshino, H., Sakamura, S., Kikuta, Y. & Koda, Y. 1989. Structure of a tuber-inducing stimulus from potato leaves (*Solanum tuberosum* L.). – Agric. Biol. Chem. 53: 2835–2837.

Zoń, J. & Amrhein, N. 1992. Inhibitors of phenylalanine ammonia-lyase: 2-aminoindan-2-phosphonic acid and related compounds. – Justus Liebigs Ann. Chem. 1992: 625–628.

Edited by L. Eliasson

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.