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THE DISTRIBUTION OF HYDROXYCINNAMOYLPUTRESCINES IN DIFFERENT ORGANS OF *SOLANUM TUBEROSUM* AND OTHER SOLANACEOUS SPECIES

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IN HONOUR OF PROFESSOR MEINHART ZENK'S SIXTIETH BIRTHDAY

Key Word Index—*Solanum tuberosum*; Solanaceae; potato; tuberization; flowering; hydroxycinnamoylputrescines; polyamine conjugates; polyamines.

Abstract—The distribution of hydroxycinnamoylputrescines in different organs and developmental stages of seven solanaceous species was compared. In flowers of *Nicotiana tabacum* and *Lycopersicon esculentum*, caffeoylputrescine accumulates in the pistils, whereas in *Nicotiana plumbaginifolia* and *Capsicum annuum* it accumulates in the stamens. The accumulation of hydroxycinnamoylputrescines in floral parts is, however, not a general feature of the Solanaceae, since in potato and *Solanum nigrum*, levels of these compounds remain low in all organs at all stages of development of the plants. In the underground shoot of field grown plants of *Solanum tuberosum* ssp. *tuberosum* neither caffeoylputrescine, nor feruloylputrescine, *p*-coumaroylputrescine or diferuloylputrescine were found to accumulate during tuber development. These results indicate that hydroxycinnamoylputrescines are not likely to be causally involved in the tuberization process of potato plants and in the flowering process of Solanaceae species.

INTRODUCTION

Hydroxycinnamic acid amides (HCAs), conjugates between hydroxycinnamic acids (e.g. caffeic acid, ferulic acid and *p*-coumaric acid) and amines (e.g. putrescine, spermidine and spermine) are widely distributed in the plant kingdom. They have been suggested to have possible functions in several plant developmental processes, including tuberization and flowering [1, 2]. Accumulation of HCAs in tobacco flowers has been shown to occur parallel to flower formation. Basic HCAs, such as caffeoylputrescine and caffeoylspermidine, were found to accumulate in the female floral parts, while neutral HCAs, such as di-*p*-coumaroylputrescine, are predominantly found in the male floral parts [3]. The occurrence of HCAs as phenolic constituents in the reproductive organs from at least 13 families of higher plants has been reported [4]. High levels of HCAs were found in the anthers of fertile maize, and their absence in the anthers of cytoplasmic male sterile plants is striking [5]. Likewise, basic HCAs accumulate in the female flowers of the inflorescences of some Araceae species, while neutral HCAs are predominant in the male flowers, and sterile flowers are devoid of HCAs [6]. In pollen of Fagales species, diconjugated hydroxycinnamoylspermidines have been used as taxonomic markers [7, 8]. The tuberization of potato plants is a complex physiological process [9]. Paynot *et al.* [10] investigated the hybrid SDA_6 which forms tubers only in short days. Basic HCAs (*p*-coumaroylputrescine, caffeoylputrescine, feruloylputrescine and traces of caffeoylspermidine) were shown to accumulate only in stolons of leaf cuttings in parallel to tuberization. In contrast, long days promote flowering, and HCAs accumulate in the leaves while no tuberization occurs. It was inferred that HCAs might be causally involved in the tuberization process of potato plants [10]. In this communication we present the results of a survey of the distribution of hydroxycinnamoylputrescines in different organs at different developmental stages of seven solanaceous species in order to find support for or against a role of these secondary plant products in plant developmental processes.

RESULTS AND DISCUSSION

To test whether there is a correlation between flower development and HCA accumulation in general, and whether, in particular, it is a rule that basic HCAs accumulate in female floral parts, while neutral HCAs accumulate in male floral parts, we investigated the HCA content of the flowers of seven species belonging to the Solanaceae family. Our results for the levels of caffeoylputrescine (CP), feruloylputrescine (FP), *p*-coumaroylputrescine (*p*CP), and di-feruloylputescine (DFP) in flowers of *Solanum tuberosum* ssp. *tuberosum*, *S. nigrum*, *Physalis*

Table 1. Comparison of the endogenous levels of caffeoylputrescine (CP), feruloylputrescine (FP), diferuloylputrescine (DFP) and *p*-coumaroylputrescine (*p*CP) in male floral organs (stamens) and female floral organs (pistils) of Solanaceae

	HCA levels (μmol g^{-1} dry wt)							
	Stamens				Pistils			
Plant species	CP	FP	*p*CP	DFP	CP	FP	*p*CP	DFP
S. tuberosum ssp. *tuberosum*	0.001	0.005	0.003	0.024	0.010	0.005	0.001	0.021
S. nigrum	only total content of flowers was measured: <0.03 μmol g^{-1} dry wt							
P. edulis	0.11	0.02	0.03	0.01	0.08	0.01	<0.01	0.01
N. tabacum	0.97	0.02	0.12	0.04	7.67	0.06	0.15	0.01
N. plumbaginifolia	0.48	0.00	0.01	0.01	0.09	0.02	0.01	0.00
L. esculentum	0.01	0.45	0.03	0.01	0.68	0.21	0.01	0.00
C. annuum	3.28	0.16	0.19	0.58	0.29	0.24	1.02	0.01

Solanum tuberosum ssp. *tuberosum* cv. Astrid (in cv. Aula, cv. Hansa and cv. Indira similar values were measured), *Solanum nigrum*, *Physalis edulis*, *Nicotiana tabacum* cv. SCR, *N. plumbaginifolia*, *Lycopersicon esculentum* cv. Goldene Königin (in cv. Moneymaker and cv. Montserrat similar values were measured) and *Capsicum annuum* cv. Sweet Banana. All flowers were harvested in the mature, fully opened stage.

edulis, *Nicotiana tabacum*, *N. plumbaginifolia*, *Lycopersicon esculentum* and *Capsicum annuum* are shown in Table 1.

For *Nicotiana tabacum* flowers, we confirmed the results of Cabanne *et al.* [3] that caffeoylputrescine (CP) accumulates in high amounts in floral parts. Up to 8 μmol CP g^{-1} dry wt [=0.2% (w/w)] was measured in pistils, 2 μmol CP g^{-1} dry wt in floral buds, 1 μmol CP g^{-1} dry wt in stamens and 0.3 μmol CP g^{-1} dry wt in the corolla of *Nicotiana tabacum* cv. SCR (Table 1). In contrast, all vegetative organs and immature fruits contained only about 0.01 μmol CP g^{-1} dry wt. Feruloylputrescine (FP) was detected in the flowering tobacco plants in levels below 0.1 μmol FP g^{-1} dry wt in all organs. In contrast, non-flowering plants of *Nicotiana tabacum* cv. SCR contain higher levels of about 0.2–0.6 μmol FP g^{-1} dry wt, whereas CP-levels were below 0.05 μmol g^{-1} dry wt in all organs. In both flowering and non-flowering plants, diferuloylputrescine (DFP) and *p*-coumaroylputrescine (*p*CP) were detected in some organs in levels below 0.1 μmol g^{-1} dry wt. This accumulation of CP in floral parts of *Nicotiana tabacum* cv. SCR agrees with the results previously obtained for *Nicotiana tabacum* cv. Xanthi n.c. [1, 3]. In the female floral parts of this tobacco cultivar, the basic HCAs CP (10 μmol g^{-1} fr. wt) and caffeoylspermidine (CS) (20 μmol g^{-1} fr. wt) accumulate, while in anthers neutral HCAs were dominant, and in young plants no HCAs were detectable.

In contrast to the situation in *N. tabacum*, no accumulation of HCAs was found in any organ of *Solanum nigrum* and of *S. tuberosum* ssp. *tuberosum*. Levels of CP, FP, *p*CP or DFP in flowers of *S. nigrum* were below 0.03 μmol g^{-1} dry wt (Table 1). In all parts of the flowers of the *S. tuberosum* ssp. *tuberosum* cultivars Astrid, Aula, Hansa, and Indira, levels of CP, FP, *p*CP or DFP were below 0.01 μmol g^{-1} dry wt (Table 1, Fig. 1). Levels of CP in the female parts of potato flowers are 800-fold lower than in those of tobacco. This lack of HCA accumulation in potato does not appear to be due to a reduced polyamine- and/or hydroxycinnamic acid-metabolism. The levels of free putrescine and spermidine were in the range of 2–5 μmol g^{-1} dry wt in potato flowers (Fig. 2), i.e. 200- to 500-fold higher than the HCA levels. Among other hydroxycinnamic acid derivatives, chlorogenic acid and caffeoylglucose are, for example, typical constituents of potato flowers [11, 12].

In agreement with the data obtained for *N. tabacum*, CP accumulates in the female floral parts of the *Lycopersicon esculentum* cultivars Moneymaker, Goldene Königin and Montserrat (Table 1). In contrast, CP accumulation in *Nicotiana plumbaginifolia* and *Capsicum annuum* occurs in the male floral parts (Table 1 and Fig. 3). Of the basic HCAs, FP accumulated in both female and male floral parts of *L. esculentum* (Table 1) and *p*CP in anthers, immature fruits and other organs of *C. annuum* (Table 1 and Fig. 3). DFP was found to accumulate only in the male floral parts of *C. annuum* (0.6 μmol g^{-1} dry wt) (Table 1).

These results obtained for seven related species show that the accumulation of hydroxycinnamoylputrescines in flowers, and the correlation 'basic HCAs—female floral parts; neutral HCAs—male floral parts' are not a common characteristic of the Solanaceae (Table 1). Our results rather support the conclusions of Wyss-Benz *et al.* [13], that these HCAs are unlikely to be directly related to the process of flower formation.

For the tuberization process in potato plants, the accumulation of basic HCAs (0.02 μmol g^{-1} fr. wt *p*CP, CP, FP and traces of caffeoylspermidine) in tuberizing stolons of cuttings under short day conditions has previously been shown. HCAs have therefore been suggested to be involved in the tuberization process of potato plants, possibly functioning as a new class of plant growth regulators [1, 10]. Fig. 1 shows the distribution of CP, FP and DFP in different organs of *Solanum tuberosum* ssp. *tuberosum*. cv. Astrid. The levels of all HCAs remain

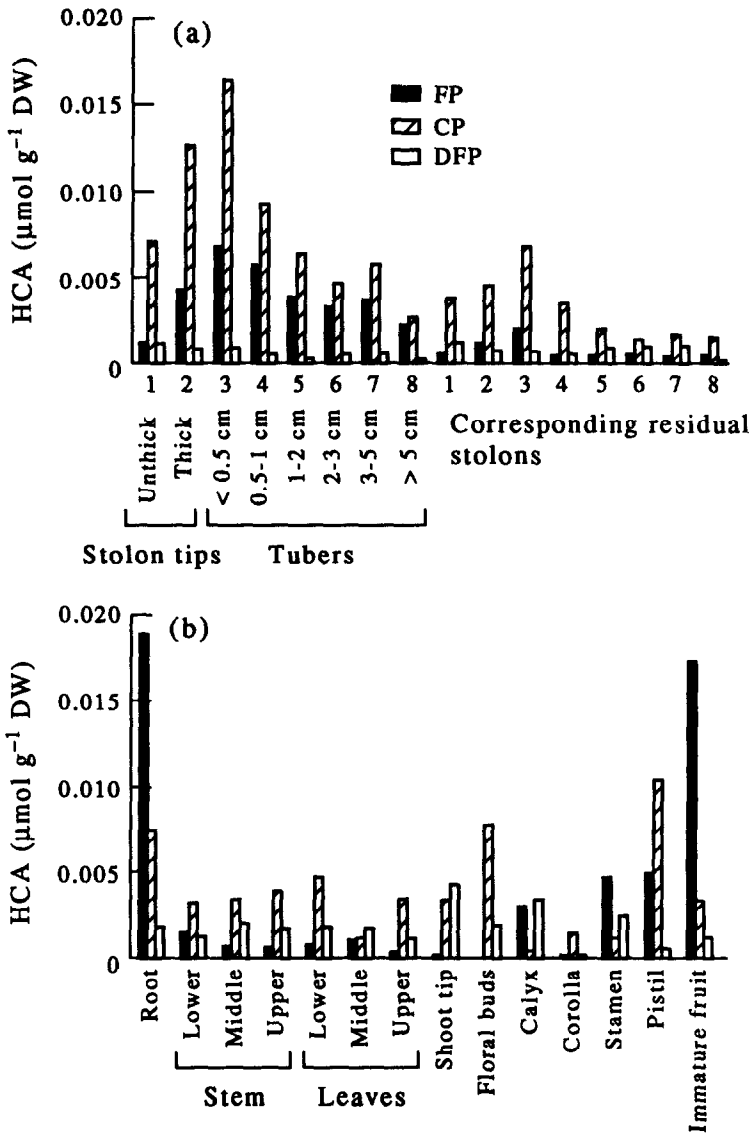

Fig. 1. Comparison of the endogenous distribution of caffeoylputrescine (CP), feruloylputrescine (FP) and diferuloylputrescine (DFP) in field grown *Solanum tuberosum* ssp. *tuberosum* cv. Astrid. (a) Distribution in the underground shoot. Unthick = unthickened, thick = thickened. (b) Distribution in the aboveground shoot and in the root. Results expressed as means of two independent measurements (extracts from organs taken from 230 and 14 plants, respectively), which did not differ by more than 50% (exceptions: unthickened stolon tips 60%, roots 120%). DW = dry weight.

below 0.02 μmol g^{-1} dry wt. Tuberized stolons were divided into different stages according to tuber development: unthickened stolons, thickened stolons, tubers of different size (<0.5 cm, 0.5–1 cm, 1–2 cm, 2–3 cm, 3–5 cm, >5 cm). The classification of the early tuber developmental stages of the stolon tips is the same as that used by Koda and Okazawa [14] in their study on endogenous phytohormone activities during tuber development. During tuberization, the levels of CP and FP in stolon tips increase to a maximum in small tubers (<0.5 cm) and decrease afterwards to the initial values (Table 2). Compared to the levels of CP and FP measured by Paynot *et al.* [10] no accumulation occurs. The maximal levels in the underground shoot of potato plants remain at least 10-fold lower, i.e. below 0.02 μmol g^{-1} dry wt. Compared to the endogenous phytohormone activities of tuberizing stolon tips [14], the time courses of CP and FP behave like the time course of the cytokinin activity (Table 2).

The clones 482W and 283W of the wild potato *S. tuberosum* L. ssp. *andigena* form tubers only in short days. No accumulation of HCAs was found to occur,

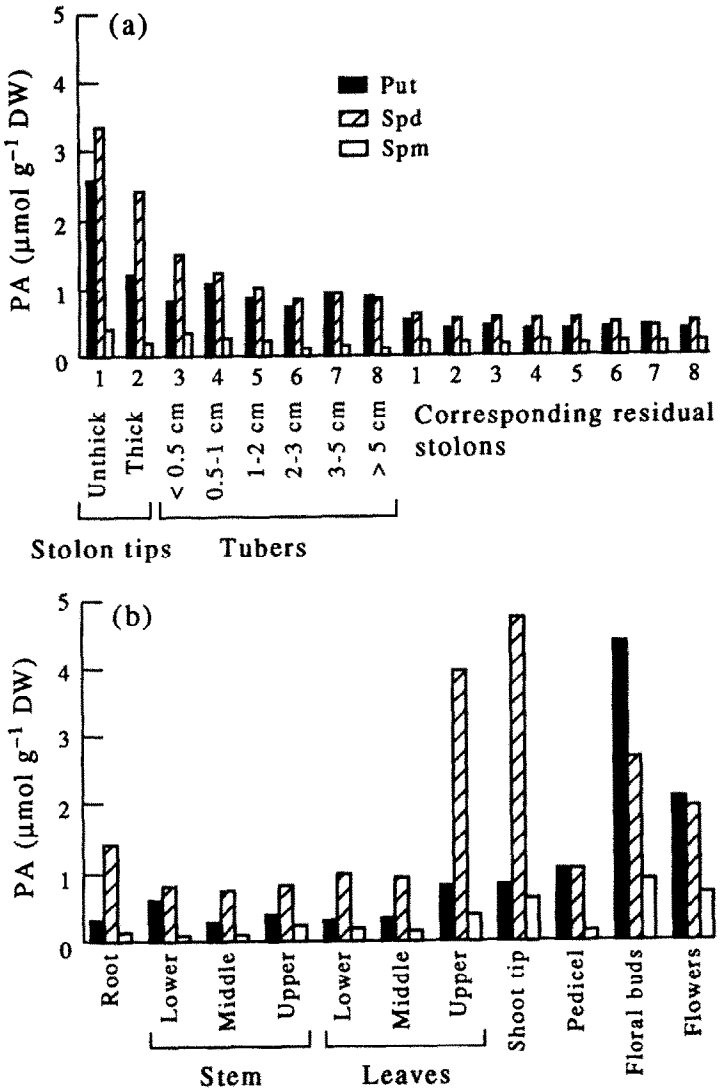

Fig. 2. Comparison of the endogenous distribution of free putrescine (Put), spermidine (Spd) and spermine (Spm) in field grown *Solanum tuberosum* ssp. *tuberosum* cv. Astrid. (a) Distribution in the underground shoot. Unthick = unthickened, thick = thickened. (b) Distribution in the aboveground shoot and in the root. Results expressed as means of two independent measurements (extracts from organs taken from 230 and 14 plants, respectively), which did not differ by more than 40% (exceptions: unthickened and thickened stolon tips 50%, roots 60%). DW = dry weight.

Table 2. Comparison of the endogenous levels of the hydroxycinnamoyl acid amides caffeoylputrescine (CP) and feruloylputrescine (FP) and the free polyamines (PA) putrescine (put) and spermidine (spd) with the endogenous phytohormone activities in equivalents of GA_3, zeatin, ABA und IAA as measured by Koda and Okazawa [14]

Developmental stage of tuber	HCA and PA (μmol g^{-1} dry wt) (this work)				Phytohormone activity ($\mu g\ kg^{-1}$ fr. wt) (ref. [14])			
	CP	FP	put	spd	GA_3	ZEATIN	ABA	IAA
Stage A	0.007	0.001	2.6	3.4	2.2	0.2	0.7	5.9
Stage B	0.013	0.004	1.2	2.4	0.9	0.3	2.1	8.6
Stage C	0.017	0.007	0 8	1.5	0.4	1.1	10.9	6.5
Stage D	0.008	0.005	0.9	1.1	0.6	0.9	25.5	3.7

Stage A = unthickened stolon tips; Stage B = thickened stolon tips; Stage C = young tubers < 0.5 cm; Stage D = young tubers 0.5–2 cm.

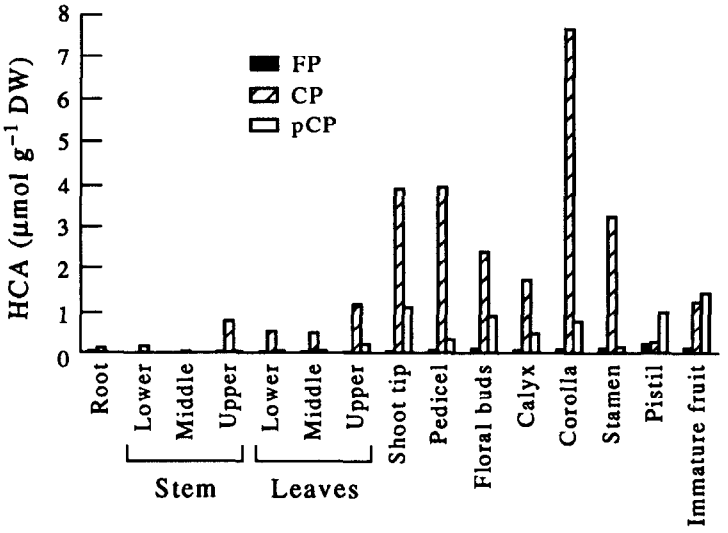

Fig. 3. Comparison of the endogenous distribution of caffeoylputrescine (CP), feruloylputrescine (FP) and *p*-coumaroylputrescine (*p*CP) in field grown *Capsicum annuum* cv. Sweet Banana. Results of a measurement of extracts from organs taken from seven plants. DW = dry weight.

either in long days or in short days. CP, FP, *p*CP and DFP levels remained low below 0.05 μmol g^{-1} dry wt (data not shown).

In comparison to other plants, young potato tuber shoots contain high levels of both free and total conjugated polyamines. The levels of total conjugated polyamines determined by Felix and Harr [15] in the cultivar Bintje are about 100-fold higher than the combined levels we found for CP or FP, but the fraction of HCAs in the pool of conjugated polyamines was not determined in the previous study [15].

On the other hand, Felix and Harr [15] measured levels of free polyamines, which are comparable with those measured by us. Figure 2 shows the distribution of free putrescine, spermidine and spermine in field grown potato plants. The highest levels of about 3 μmol g^{-1} dry wt in the underground shoot were found in unthickened stolon tips (Table 2). During tuberization, the levels of putrescine and spermidine in stolon tips decrease. The levels of free putrescine exceeded those of hydroxycinnamoylputrescines 30- to 300-fold in developing stolon tips. Compared to the time course of endogenous phytohormone activities of tuberizing stolon tips [14], putrescine and spermidine behave like the gibberellin activity (Table 2).

In contrast to tubers of *Helianthus tuberosus* [15] and *Cyperus rotundus* [16], cell division and growth of young tubers of *Solanum tuberosum* ssp. *tuberosum* are not correlated with high polyamine levels. This result is in agreement with the general finding of Felix and Harr [15] that cell division is not necessarily concomitant with the accumulation of polyamines in these tissues.

Our results reported here for HCA accumulation in field grown plants are in full agreement with those obtained in *in vitro* tuberization systems [17]. Taken together, these results indicate that HCAs are not causally involved in the tuberization process of potato plants. As the involvement of HCAs in flower formation has likewise been questioned [13], their function in plant development should clearly be reconsidered.

EXPERIMENTAL

Plant material. The *Solanum tuberosum* L. ssp. *tuberosum* cultivars Astrid, Aula, Indira and Hansa were grown in the experimental field of the Ruhr-University in Bochum (Germany). Sprouted tubers were planted in the soil in April. Plants were harvested between June and September. The clones 482W and 283W of the wild potato, *S. tuberosum* L. ssp. *andigena* (JUZ. et BUK.) HAWKES, were cultivated either under tuber-inducing short day conditions (6 hr photoperiod, 350 μmol sec^{-1} m^{-2}; temp.: day 25°, night 10 hr 13°) or non-inductive long day conditions (16 hr photoperiod, 6 hr 350 μmol sec^{-1} m^{-2}, 10 hr 40 μmol sec^{-1} m^{-2}; temp.: day and night 25°) in plant growth chambers.

All other Solanaceae species, *Nicotiana tabacum* L. cv. SCR, *N. sylvestris* SPEGAZZINI & COMES, *N. plumbaginifolia* VIV., *Lycopersicon esculentum* MILL. cv. Moneymaker, cv. Goldene Königin and cv. Montserrat, *Capsicum annuum* L. cv. Sweet Banana, *Physalis edulis* SIMS., and *Solanum nigrum* L. were grown in the field as well.

Plants were harvested, divided into different organs and freeze-dried prior to the extraction procedure. Tuberized or non-tuberized potato stolons were divided into tips and the corresponding residual stolons. Tuberized stolons were divided into different stages according to tuber development.

Chemicals. *p*-Coumaroylputrescine (*p*CP), feruloylputrescine (FP) and diferuloylputrescine (DFP) were a gift from Dr E. Ebert (Ciba-Geigy Ltd, Basel, Switzerland) and caffeoylputrescine (CP) was a gift from Merck, Darmstadt, Germany. All other chemicals were of analytical or HPLC grade.

Extraction and HPLC of HCAs. Extraction and HPLC analysis were performed according to Wyss-Benz *et al.* [18]. Freeze-dried plant tissue (50–200 mg) was extracted with H_2O–methanol (1 : 1) using an Ultra-Turrax homogenizer, followed by 2 hr on an overhead-shaker at room temp. After centrifugation for 10 min at 4000 *g* the clear supernatants were loaded on to a weakly acidic cation-exchange column [Fractogel TSK-CM 650 (M), Merck, Darmstadt, Germany], in its H^+-form. Bound HCAs were eluted with 8 M HOAc–MeOH (1 : 1), lyophilized, dissolved in H_2O–MeOH (1 : 1) and filtered prior to HPLC-injection.

HCAs were analysed by HPLC (System Gold, Beckman, San Ramon, California, U.S.A.) using a fluorescence detector (Spectroflow 980, Kratos Analytical, Ramsay, New Jersey, U.S.A.). Samples were injected on to a reversed-phase Nucleosil C-18 HPLC column (250 × 4.6 mm, particle size 5 μm, Stagroma, Wallisellen, Switzerland) through a 20 μl loop. Sepn was achieved by using a linear gradient of 25 mM NaOAc, pH 3.7 (solution A) to MeCN (solution B) at a flow rate of 1 ml min^{-1}. MeCN gradient: 0–13 min, 13%; 13–18 min, 13–35%; 18–28 min, 35%; 28–33 min, 35–100%; 33–38 min, 100%. Compounds were detected by fluorescence (excitation 320 nm, emission >389 nm) and absorption at 306 nm, and peaks were integrated using the computer software. CP, *p*CP, FP and DFP contents were calculated using standard curves.

Extraction and HPLC of free polyamines. Free polyamines were extracted from 50–200 mg lyophilized plant tissue with 5% perchloric acid and derivatized with either benzoyl chloride or dansyl chloride [19]. HPLC analysis of benzoylated putrescine, spermidine and spermine followed the method described by Flores and Galston [19], while analysis of the dansylated polyamines was according to Smith and Davies [20, 21]; the column used for the polyamines was the same as used for HPLC of HCAs.

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