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Involvement of phenolic acids in disease resistance of potato tubers from CEPA-treated plants

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Abstract

Treatment of vegetative parts of potato plants two weeks before the harvest with 0.2 % 2-chloroethylphosphonic acid (CEPA) delayed the sprouting of tubers and increased the resistance of tubers to infections caused by *Phytophthora infestans*, *Erwinia carotovora* and *Fusarium* spp. during the storage period. Levels of free, soluble ester- and glycoside-bound phenolic acids and cell wall-bound phenolics were determined in cortical parenchyma of tubers (periderm). The enhancement of phenolic acids in tubers from treated plants was caused primarily by the increase in the contents of free vanillic, caffeic and *p*-hydroxybenzoic acids and cell wall-bound ferulic, vanillic and *p*-coumaric acids.

Key words: 2-chloroethylphosphonic acid, resistance to diseases, *Solanum tuberosum*.

Introduction

Release of tuber dormancy by external agents is a desirable goal for rapid post-harvest disease testing procedures while, on the contrary, prolonged tuber dormancy is important for minimizing potato tuber losses caused by early tuber sprouting and diseases during longer storage (Coleman and Murphy 1990). The exogenous application of the ethylene-releasing compound (CEPA) significantly prolongs the period of potato dormancy (Rylski *et al.* 1974) and is associated with higher resistance to pathogens (Metlitskii *et al.* 1982). The increased resistance to *Phytophthora infestans* in CEPA-treated potato tubers was attributed to a much higher production of the potato phytoalexin rishitin after inoculation with this pathogen (Korableva *et al.* 1989).

In potato various phenylpropanoid pathways are stimulated by pathogen attack

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although the phytoalexins rishitin and lubimin (sesquiterpenoids) are synthesized via the acetate-mevalonate pathway (Shih and Kuc 1973). Toxins from the culture medium of *Phytophthora infestans* were shown to increase phenylalanine ammonia-lyase activity and induce lignification in addition to the increased accumulation of phytoalexins in tuber tissue (Henderson and Friend 1979). The increased levels of phytoalexins as well as phenolics were observed in the potato tubers infected by *Erwinia carotovora* or *Fusarium spp.* (Röber 1989). Defense responses include also increasing amount of phenolic acids esterically bound to the cell walls and wall-bound hydroxycinnamic amides (Clarke 1982). This, together with increased suberization and induced lignification in infected tuber tissue, appears to be involved in resistance reactions of potato (Hammerschmidt 1984, 1985).

The present study examined if the increased resistance to pathogens induced by application of an ethylene-releasing compound is associated with changes in phenolics contents. We have taken into consideration a possible causal relationship between the increased level of ethylene and metabolism of phenolics mediated through the induction of phenylalanine ammonia-lyase (Cvikrová *et al.* 1994). We describe changes in the levels of free, soluble ester- and glycoside-bound phenolic acids and cell wall-bound phenolics during storage of potato tubers from control and CEPA-treated plants.

Materials and methods

Plant material and treatment with 2-chloroethylphosphonic acid (CEPA): The vegetative parts of potato plants (*Solanum tuberosum* L.) cv. Nevskii were sprayed with 0.2 % aqueous solution of CEPA 15 d before harvest. Potato tubers were stored in darkness at 4 °C and high relative humidity (about 90 %) for 8 months. The contents of phenolic acids were determined in cortical parenchyma (periderm) of tubers from control and CEPA-treated plants during the storage period.

Determination of sprouting: The growth of buds of tubers from control and CEPA-treated plants was determined starting 30 d after treatment (*i.e.* 15 d after harvest) in 30-d intervals until day 240 (7.5 months of storage). Tubers were taken from the storage room (4 °C), transferred into the greenhouse and kept in darkness at 25 °C with high relative humidity (90 %). The length of sprouts was measured after 14 d (10 determinations in each measurement).

Resistance of potato tubers during storage period: The resistance of potato tubers to phytopathogenic microorganisms was determined after 8 months of storage. For the determination of the infections caused by *Phytophthora infestans*, soft rot bacteria *Erwinia carotovora* and dry rot fungi *Fusarium spp.* the method of tuberous analysis was used (Metlickii *et al.* 1982). The observation of tuber cross-sections was performed for three consecutive years using 200 tubers from control and treated plants. The resistance is expressed as the percentage of infected tubers and statistical significance evaluated with a *t*-test.

Phenolic acids analysis: Phenolic acids were extracted and analysed as described earlier (Cvikrová *et al.* 1991). Briefly, free (F_1), ester-bound (F_2; released after alkaline hydrolysis) and glycoside-bound (F_4; released after acid hydrolysis) phenolic acids were obtained from a methanol extract of tissue ground in liquid nitrogen. The fraction of cell wall-bound phenolic acids (F_3) was obtained after alkaline hydrolysis of the residual material after methanol extraction. The antioxidant 2,6-ditercbutyl-β-cresol, was used to minimize the oxidation of phenolic acids during alkaline hydrolysis and nitrogen was immediately bubbled through the sample after NaOH addition. Phenolic acids were analysed by means of HPLC using a *Pye Unicam PU 4002* - Video Liquid Chromatograph with a *Spherisorb 5 ODS* column. Column eluate was monitored at 260 and 300 nm using a multichannel detector *PU 4021*. Identity of phenolic acids was confirmed by co-chromatography on HPLC with authentic standards (*Serva*, Germany). Analyses were performed for two consecutive years using 40 tubers from control and treated plants in each time.

Results

Sprouting and resistance to diseases: Treatment of vegetative parts of potato plants two weeks before harvest with 0.2 % CEPA delayed sprouting of tubers (Table 1). The buds of control tubers started to sprout, after the transfer of tubers to the favourable conditions in the greenhouse, at the end of December. Sprouting of tubers from treated plants was shifted to the middle of March and was accompanied with significant increase in the resistance of tubers to infections caused by *Phytophthora infestans*, *Erwinia carotovora* and *Fusarium* spp. during storage (Table 2).

Table 1. The effect of CEPA treatment on the sprouting of potato tubers. The vegetative parts of potato plants were sprayed by 0.2 % aqueous solution of CEPA two weeks before the harvest. The values represent the mean ± SE (n = 10).

Time after treatment [d]	Length of sprouts [cm] 30	60	90	120	150	180	210	240
Control	0	0	0	0.1 ± 0.01	0.7 ± 0.07	1.2 ± 0.08	2.8 ± 0.10	6.8 ± 0.30
CEPA	0	0	0	0	0	0	0.5 ± 0.02	1.7 ± 0.06

Table 2. The effect of CEPA treatment on the resistance of potato tubers to diseases after 8 months of storage. Potato plants were treated with 0.2 % CEPA 14 d before the harvest of tubers.

	Infected potato tubers [%]			
	Phytophthora infestans	*Erwinia carotovora*	*Fusarium spp.*	Common losses
Control	5.2*	0.6 NS	17.3*	23.1*
CEPA	0	0	2.8	2.8

NS - not significant,* - significant at $P = 0.01$

Content of phenolic acids: CEPA treatment of potato plants led to a large increase in the total content of phenolic acids in the periderm of tubers (Fig. 1). The increase in the phenolics content started 1 d after the treatment, reached a maximum on day 70 and then gradually decreased until the buds started to sprout when it was still more than 50 % higher than the content determined in the control. The level of total phenolic acids in periderm of control did not change significantly during the storage

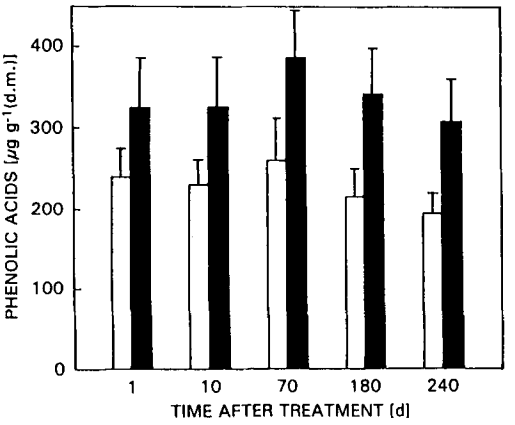

Fig. 1. Phenolic acids accumulation in the periderm of tubers from control and CEPA-treated plants during the storage period. Total contents of phenolic acids represent the sum of the contents of free, ester-, glycoside- and cell wall-bound phenolic acids. Bars represent the standard errors of 4 - 5 replicates in two independent experiments. Control - *empty columns*, CEPA-treated - *full columns*.

Table 3. Contents of phenolic acids in the periderm of tubers from control and CEPA-treated plants during the storage period. The values represent the mean of the sum of all four determined fractions (mean ± SE) (with the exception of ChA and CaA where the values of phenolic acids released after alkaline hydrolysis are missing). CaA - caffeic acid; ChA - chlorogenic acid; FA - ferulic acid; pCA - *p*-coumaric acid; pHBA - *p*-hydroxybenzoic acid; VA - vanillic acid;

Storage [d]		Phenolic acids [μg g^{-1}(d.m.)] pHBA	VA	ChA	CaA	pCA	FA
1	Control	8.2 ± 0.5	36.0 ± 5.3	2.1 ± 0.7	151.1 ± 21.4	13.1 ± 1.3	34.8 ± 6.3
	CEPA	8.7 ± 1.6	39.8 ± 5.2	3.3 ± 0.9	219.6 ± 40.7	20.0 ±2.5	41.9 ± 7.4
10	Control	7.6 ± 1.2	32.6 ± 4.8	2.2 ± 0.3	143.2 ± 18.0	11.3 ± 1.4	39.7 ± 6.6
	CEPA	9.3 ± 2.8	43.1 ± 8.0	2.6 ± 0.4	206.8 ± 31.2	18.2 ± 1.5	57.4 ± 9.8
70	Control	6.4 ± 0.9	40.9 ± 8.1	2.2 ± 0.4	156.1 ± 27.4	12.7 ± 1.8	40.2 ± 7.4
	CEPA	8.2 ± 1.2	55.4 ± 9.2	4.8 ± 0.7	237.0 ± 40.5	17.2 ± 3.1	60.4 ± 8.1
180	Control	6.4 ± 1.3	36.1 ± 6.0	4.2 ± 0.6	119.3 ± 20.0	13.1 ± 2.0	27.9 ± 4.4
	CEPA	7.2 ±1.1	58.6 ± 9.5	4.8 ± 0.6	204.4 ± 34.9	17.4 ± 2.9	52.8 ± 9.2
240	Control	4.0 ± 0.7	22.6 ± 3.9	14.2 ± 2.2	116.9 ± 17.3	7.6 ± 0.8	21.4 ± 3.8
	CEPA	8.0 ± 0.7	49.5 ± 8.1	26.4 ± 4.1	168.2 ± 29.8	13.4 ± 1.8	43.7 ± 6.4

period and was highest on day 70, *i.e.* in the middle of innate dormancy of this cultivar (Fig. 1).

Caffeic acid was the most abundant phenolic acid in the periderm of tubers (Table 3). In spite of adding the antioxidant to minimize the oxidation of phenolic acids during alkaline hydrolysis in a nitrogen atmosphere, the contents of caffeic acid in the fractions of ester-bound phenolics (F_2, F_3) were seriously lowered as indicated by the degradation of internal standard. The next highest in concentration were ferulic and vanillic acids. The content of chlorogenic acid (3-*O*-(caffeoyl) quinate)

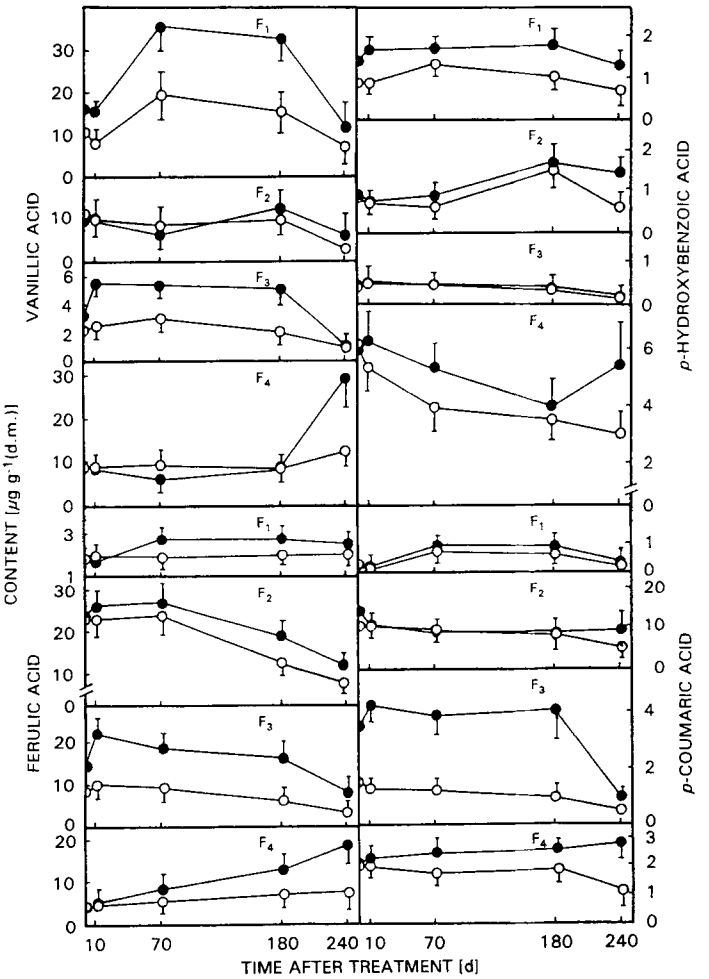

Fig. 2. Time courses of changes in the levels of *p*-hydroxybenzoic (pHBA), vanillic (VA), *p*-coumaric (pCA) and ferulic (FA) acids in the periderm of tubers from control (*open circles*) and CEPA-treated (*closed circles*) plants. F_1 - free phenolic acids, F_2 - ester-bound phenolic acids, F_3 - cell wall-bound phenolic acids, F_4 - glycoside bound phenolic acids. Note the differences in the y-axis scales. Bars represent the standard errors of 4 - 5 replicates in two independent experiments.

was influenced during alkaline hydrolysis in a similar manner to caffeic acid. For this reason the values of ester-bound fractions of these two acids are not shown in Table 3 and Fig. 3 although the values determined in the extracts from periderm of tubers from treated plants were higher than those determined in control tubers. While the levels of most of the phenolic acids decreased at the beginning of sprouting, the content of chlorogenic acid was the highest one during this period.

The proportions of prevailing binding forms of individual phenolic acids in the total content of phenolics changed during the study period (Figs. 2 and 3). Significant increase in the levels of free caffeic, vanillic and *p*-hydroxybenzoic acids (F_1) and in the contents of cell wall-bound ferulic, vanillic and *p*-coumaric acids (F_3) occurred in the periderm of tubers from CEPA-treated plants. Although the fractions of soluble esterically bound phenolic acids represented the dominant part of the phenolic pool in the periderm of control tubers (mainly esters of *p*-coumaric and ferulic acids) their amount was not influenced by CEPA treatment. Significant increase in the accumulation of glycosides of vanillic, caffeic and *p*-hydroxybenzoic acids (F_4) coincided with the decrease in their free forms (F_1). The gradual rise of glycoside-bound ferulic acid (F_4) might be associated with the decrease in the contents of its esters (F_2, F_3). Simultaneous increases in free and glycoside-bound forms of chlorogenic acid were found in tubers from both control and treated plants at the beginning of sprouting (Fig. 3).

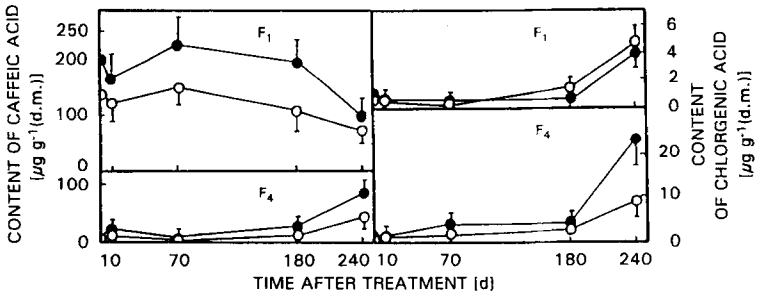

Fig. 3. Time courses of changes in the levels of caffeic (CaA) and chlorogenic (ChA) acids in the periderm of tubers from control (*open circles*) and CEPA-treated (*closed circles*) plants. F_1 - free phenolic acids, F_4 - glycoside-bound phenolic acids. Note the differences in the y-axis scales. Bars represent the standard errors of 4 - 5 replicates in two independent experiments.

Discussion

The effect of ethylene-releasing compounds on potato dormancy and sprouting depends on the concentration of the applied compound and the duration of treatment (Rylski *et al.* 1974). Treatment of vegetative parts of potato plants with 0.2 % CEPA in our experiments delayed the sprouting of tubers by about two months and increased the resistance of tubers to infections caused by *Phythophtora infestans*, *Erwinia carotovora* and *Fusarium* spp. during the storage period. Our investigations

showed that CEPA treatment of potato plants brought about a significant rise in the total content of phenolic acids. It has been shown that treatment of plant tissue with various ethylene-releasing compounds can induce defence genes involved in the phenylpropanoid and flavonoid biosynthetic pathways (Ecker and Davis 1987). There is also a possibility of causal relationship between the increased level of ethylene and metabolism of phenolics mediated through the induction of phenylalanine ammonia-lyase after CEPA treatment (Hyodo *et al.* 1978).

The enhancement of phenolics in the periderm of tubers from treated plants was caused primarily by the rise in the contents of free vanillic, caffeic and *p*-hydroxybenzoic acids and by the increase in the amounts of cell wall-bound ferulic, vanillic and *p*-coumaric acids. Increases in the level of esterically bound caffeic acid might be expected but we are not able to determine it with the method used in our experiment. The level of chlorogenic acid did not increase significantly before the period of sprouting.

The data on the antibacterial activity of different phenolic acids identified in potatoes revealed that caffeic, ferulic and vanillic acids significantly inhibited the growth of *Erwinia carotovora* at concentrations of 0.5 mg cm^{-3} (vanillic acid was the strongest inhibitor; Lyon and Mc Gill 1988) while there are conflicting accounts of the activity of chlorogenic acid against various microorganisms. Kuc *et al.* (1956) suggested it to be antifungal while Lyon and Mc Gill (1988) did not detect its antibacterial activity even at the concentration 1 mg cm^{-3} in liquid media. Clerivet and Macheix (1993) found that level of chlorogenic acid, prevalent in *Solanum gilo* leaves, was related to foliar resistance to *Stemphylium floridanum.* However, for sufficient level of resistance of *S. gilo* leaves the role of other identified phenolic compounds was also proposed. A direct relationship between the total phenolic content and resistance against *Erwinia carotovora* has been recently shown in tubers of cultivars with different resistance to this pathogen (Kumar *et al.* 1991).

Prevention of penetration of pathogen into the tuber tissue has been shown to be a highly effective resistance mechanism (Vaughn and Lulai 1991). We found in the periderm from tubers with CEPA-prolonged dormancy more than 100 % higher contents of cell wall-bound ferulic, *p*-coumaric and vanillic acids. Bound phenolic material containing oxidized chlorogenic acid, esterified *p*-coumaric and ferulic acids, lignin and suberin have been implicated in the non-race-specific resistance of potato tubers discs to *Phytophthora* and *Phoma spp.* (Ampomah and Friend 1988). The unchanged level of soluble esters in our experiment may be explained by one of their physiological functions in plants: they are considered to be direct precursors of lignin or cell wall-bound phenolics and they do not accumulate in lignifying tissue (Fuchs and De Vries 1969). Phenolic glycosides, whose content increased at the beginning of sprouting, may represent the "inactivated" forms of free acids. The increased accumulation of free chlorogenic acid and its glycoside might be associated with the "inactivation" of caffeic acid, which decreased significantly during this period. Despite its abundance, the biosynthesis of chlorogenic acid and its function in potato is not completely understood (Hahlbrock and Scheel 1989).

We have shown that the CEPA treatment of potato plants increased the resistance of tubers to infections caused by *Phytophthora infestans, Erwinia carotovora* and

Fusarium spp. We can conclude that the increased accumulation of free phenolic acids and cell wall ester-bound phenolics in the periderm of tubers from CEPA treated plants support the importance of a preinfection chemical barrier and that phenolic acids may play an important role in higher resistance to pathogens observed after CEPA treatment.

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