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FULL RESEARCH PAPER

Effect of leaf scald (*Xanthomonas albilineans*) on polyamine and phenolic acid metabolism of two sugarcane cultivars

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Abstract Polyamine and phenolic acid levels as well as activities of some enzymes of their biosynthetic metabolism were examined in two sugarcane (*Saccharum officinarum*) cultivars differing in susceptibility to leaf scald, a disease caused by the bacterium, *Xanthomonas albilineans.* Juice obtained from both infected cultivars showed significantly increased levels of free putrescine and ornithine decarboxylase activity. However, the pathogen induced different changes in the two cultivars in subsequent metabolic steps. Whereas acid insoluble conjugated spermidine completely disappeared from the highly susceptible cv. C 439-52, an increase in acid insoluble conjugated polyamines was observed in the moderately susceptible cv. L 55-5. Phenolic acid metabolism also differed in the two cultivars. Since total phenolic acid content and phenylalanine ammonium lyase activity was greater in both cultivars after infection, distribution of phenolic acids between free or conjugated forms diverted into different pathways. The level of susceptibility of the two cultivars is discussed in terms of changes in these compounds.

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Abbreviations

CAD	Cadaverine
HPLC	High performance liquid chromatography
LDC	Lysine decarboxylase
ODC	Ornithine decarboxylase
PACs	Phenolic acids
PAs	Polyamines
PAL	Phenylalanine-ammonium lyase
PCA	Perchloric acid
PH-PAs	Acid insoluble conjugated polyamines
PUT	Putrescine
S-PAs	Soluble polyamines
SH-PAs	Acid soluble conjugated polyamines
SPD	Spermidine
SPM	Spermine
TNBS	2,4,6-trinitrobenzene sulfonic acid
TNP	Trinitrophenyl

Introduction

Leaf scald is a vascular disease of sugarcane (*Saccharum officinarum*) caused by the bacterium *Xanthomonas albilineans*. Nowadays it is one of the most serious and devasting diseases affecting sugar cane throughout the world. The natural development of the disease comprises two consecutive phases: a

latent phase during which infected plants may not exhibit any symptoms, and an acute phase in which symptoms can vary from a white streak 'pencil line' 1–2 mm wide on the leaf to the death of the entire plant (Rott et al. 1994). Although planting healthy seed cane and using resistant cultivars were used as the most efficient means of control, assessment of cultivar reactions has not been easy as the development of symptoms can be influenced by the physiological stage of the plantation and environmental conditions (Rott et al. 1997). The definition of biochemical characteristics implicated in plant resistance needs to be updated.

Plants have a natural array of defence mechanisms to protect them from pathogenic organisms. Among chemical substances implicated in plant-pathogen interactions, polyamines and phenolic acids have been widely recognized. Polyamines are polycationic compounds that play important roles in a diverse developmental process (Slocum and Flores 1991). In cells, polyamines can exist as free molecules or be conjugated to negatively charged molecules such as DNA, RNA, phospholipids, sugars, and phenols. In higher plants, there are two main pathways by which polyamines are synthesized. The first pathway leads to the formation of putrescine (PUT), spermidine (SPD) and spermine (SPM). The other pathway leads to the formation of cadaverine (CAD). PUT can be directly synthesized from ornithine via ornithine decarboxilase (ODC) or formed through different intermediates derived from arginine decarboxylation. These pathways are related since the ODC substrate, ornithine, is produced by enzymatic hydrolysis of arginine via arginase. The polyamines SPD and SPM are formed by successive additions of an aminopropyl moiety to PUT and SPD, respectively. In higher plants, CAD seems to be mainly formed from lysine via lysine decarboxylase (LDC), although in recent work it was suggested a CAD formation pathway involving ODC could be involved (Lee and Cho 2001).

In many studies it has been shown that polyamine levels can vary greatly in infected plants (Walters 2003). These changes cannot only be accounted for by biosynthesis or catabolism but also by the hydrolysis or conjugation of free forms of polyamines (Coghlan and Walters 1990). Polyamines are commonly conjugated to cinnamic acids, preferentially to *p*-coumaric, ferulic and caffeic acids. The production of these compounds upon pathogenic plant infection have been associated with reduced fungal or viral dispersion, reduced germination of fungal spores, or reinforcement of plant cell walls making them more resistant to pathogenic hydrolytic enzymes (Ropenack et al. 1998).

Cinnamic acids are a widely distributed family of compounds and it is well known that they are implicated in plant-pathogen interactions. In fact, the level of the biosynthetic enzyme, phenylalanine ammonium lyase (PAL) is largely increased after infection with pathogens (Matern et al. 1995). Although most of the plant phenolic constituents occur constitutively, functioning as preformed inhibitors (phytoanticipins) associated with non-host resistance, others, such as hydroxyccinamic acid amides, function as phytoalexins being synthesized in response to pathogen attack.

Since PAs and phenolic acid are normally present in sugarcane juice and as their metabolism and interconversion between free and conjugated forms have been shown to be extensively altered by pathogens (Piñón et al. 1999; Fontaniella et al. 2003), the aim of the present study was to confirm possible differences in PAs and phenolic acids titres between healthy and *X. albilineans*-infected sugarcane juice obtained from two sugarcane cultivars with different susceptibilities to the disease. Subsequently, we determined to what extent these changes can be associated with susceptibility.

Materials and methods

Plant material

Plants of commercial *Saccharum officinarum* cvs L 55-5 and C439-52, field-grown in the Republic of Cuba, were used throughout this work. Cultivars were selected on the basis of their agronomic importance and susceptibility to leaf scald. Cultivar L 55-5 has been defined as moderately susceptible and cv. C 439-52 as highly susceptible to leaf scald (Dra. Piñón, INICA, Cuba, pers. comm.). Plants from both cultivars were obtained from healthy or scalded symptomatic plants.

Pathogen detection

An internode section from the lower part of the stalk from scalded plants was taken for pathogen detection.

The internode surface was blotted onto agar plates containing a selective medium described by Davis et al. (1994). Growth was recorded after 5 days at 28°C. To confirm the identity of *X. albineans* isolated colonies, a serological agglutination assay was performed according to Rott et al. (1994).

Extraction and preparation of raw sugarcane juice

Stalks from 12 month-old plants from the above mentioned sugarcane cultivars were cut and washed to remove dirt and foreign particles. Immediately after this, stalks were crushed and the extracted juice centrifuged at 5,000*g* for 15 min at 2°C. The supernatant, considered as the crude juice, was stored at 2°C until used. Each juice sample was obtained from the milling of four stalks from the same plant. All the analytical measurements were done in triplicate.

Protein measurement

Protein content of sugarcane juice was estimated according to Potty (1969) after precipitation of samples with 5% (w/v) trichloracetic acid (TCA) and using bovine seroalbumin as a standard.

Extraction and analysis of soluble and conjugated polyamines

Free and conjugated polyamines from sugarcane juice were analysed as dansyl-derivatives by high performance liquid chromatography (HPLC), according to the method described by Escribano and Legaz (1988) with some modifications. Juice samples of 2.5 ml were mixed with 6.0 ml of 5% (w/v) cold perchloric acid (PCA) and 150 µl of 5.0 mM *n*-butylamine (BUT) as an internal standard. The mixtures were stored overnight at 4°C in plastic tubes and then centrifuged at 17,000*g* for 20 min at 2°C. The supernatant containing both free (S-PAs) and acid soluble conjugated polyamines (SH-PAs), were stored at 4°C until used. The precipitate, containing conjugated acid insoluble polyamines (PH-PAs), was immediately resuspended in 6.0 ml of 1.0 M NaOH and 150 µl of 5.0 mM BUT as an internal standard and treated as previously described.

Acidic hydrolysis was carried out by mixing 2.0 ml of the supernatant (S + SH-PAs) and the precipitate (PH-PAs) with 2.0 ml of 4.0 N HCl. The mixtures were stored for 18 h at room temperature. After hydrolysis, polyamines were removed from various types of conjugates. According to the literature, polyamines removed from perchloric acid-soluble hydrolyzate (SH-PAs) are conjugated to small molecules (polyamine conjugates) such as saccharides or phenolic acids, whereas those liberated from the perchloric acid-insoluble hydrolyzate (PH-PAs) are conjugated to macromolecules (bound polyamines) such as DNA or proteins (Martín-Tanguy 1997).

After hydrolysis, samples were dansylated by adding 0.4 ml of saturated sodium carbonate and 0.4 ml of 55.6 mM dansyl-chloride (DNS-Chl) in acetone and incubated in the dark at 20°C for 18 h. Dansylated PAs were extracted twice from the mixtures with 3.0 ml of toluene (HPLC grade) and then cleaned according to Rodríguez et al. (2000).

PAs were eluted from a Kromasil C18 5 µm particle diam reverse phase column (250 mm × 4.6 mm ID) at 40°C, using an acetonitrile:water gradient. Detection was performed by fluorescence intensity measurements, at an excitation wavelength of 360 nm and an emission wavelength of 460 nm. PUT, CAD, SPD and SPM, from Sigma Chemical Co. (St. Louis, MO, USA) were used as standards.

Extraction and analysis of free and conjugated phenolic acids

Free phenolic acids and phenolic acid conjugates contained in the perchloric acid-soluble hydrolyzate (SH-PAs) and perchloric acid-insoluble hydrolyzate (PH-PAs) polyamine fractions were extracted and analysed by HPLC according to the method of de Armas et al. (1999) with some modifications.

Free phenolic acids (S-PAcs) contained in the (S + SH)-PAs fractions were extracted twice with 4.0 ml of diethyl ether:ethyl acetate (65:35, v/v). The aqueous phase, containing acid soluble conjugated phenolic acids (SH-PAcs) was stored until hydrolysis. Organic phases containing free phenolic acids (S-PAcs) were mixed and dried under an airflow. Residues were redissolved in 200 µl of acetonitrile and filtered through a 0.45 µm pore diam nylon filter before HPLC analysis.

Aliquots of 2.5 ml of (S + SH)-PAs and PH-PAs fractions were hydrolysed with 4.0 N HCl for 18 h at room temperature. After hydrolysis, phenolic acids were extracted twice with 4.0 ml of diethyl

ether:ethyl acetate (65:35, v/v). Organic phases were mixed, dried, and stored until HPLC analysis.

Phenolic acids were eluted from a Tracer-Excell ODS120-B column (240 mm × 4.6 mm) at 35°C using an acetonitrile (100%) acetic acid: milliQ-water (2:98, v/v) gradient. UV detection was performed at 270 nm. Protocatechuic, ferulic, *p*-coumaric, syringic, chlorogenic, caffeic and p-hydroxybenzoic acids, from Sigma Chemical Co. (St. Louis, MO, USA) were used as standards.

Assay of ornithine decarboxylase

ODC activity was assayed spectrophotometrically according to the method of Ngo et al. (1987) with the modifications described by Legaz et al. (2001). The method is based on the reaction of PUT, the product of ODC, with 2,4,6-trinitrobenzenesulfonic acid (TNBS) to give a coloured product, trinitrophenyl-putrescine (TNP-PUT) that was recovered in an organic, 1-pentanol phase. Absorbance was measured at 426 nm against a reagent blank. A standard curve was made with variable concentrations of PUT. A unit of specific activity was defined as 1.0 μg of PUT produced mg^{-1} protein min^{-1}.

Assay of lysine decarboxylase

LDC activity was assayed spectrophotometrically according to the method of Phan et al. (1982) with the modifications described by Legaz et al. (2001). The method is based upon the principle that CAD, a product of LDC activity, reacts with TNBS to give a product soluble in toluene. Absorbance was measured at 340 nm against a reagent blank. A standard curve was made with variable concentrations of CAD. A unit of specific activity was defined as 1.0 μg of CAD produced mg^{-1} protein min^{-1}.

Assay of phenylalanine ammonia lyase

PAL activity was assayed spectrophotometrically according to the method of Dickerson et al. (1984) with slight modifications. Substrate solution consisted of 1.0 mM EDTA, 1.0 mM DTT and 12.0 mM L-phenylalanine in 100 mM sodium borate buffer, pH. 8.8. Reaction was started by adding 400 μl of filtered sugarcane juice to 500 μl of substrate solution. Reaction mixture was then incubated at 30°C for 30 min and stopped by adding 1.0 ml of 4.0 N HCl. Controls were made from sugarcane juice previously inactivated with 4.0 N HCl. The amount of cinnamic acid produced by the enzyme action was measured spectrophotometrically by absorbance at 290 nm. A unit of specific activity was defined as 1.0 μg of cinnamic acid produced mg^{-1} protein min^{-1}.

Statistics

Statistical significance of the differences between means of the analysed parameters was evaluated by Student's *t*-test. The *F*-Test was used to test heterogeneity of variances. When needed, data were log transformed prior to analysis. Differences were considered significant when $P \geq 0.05$.

Results

Changes in the titre of polyamines under pathogenic infection

Polyamine analysis in healthy sugarcane juice revealed the presence of three polyamines: PUT, CAD and SPD (Figs. 1, 2). They were found either as free (S-PAs) (Figs. 1A, 2A) or conjugated to macromolecules (bound polyamines) in the acid-insoluble (PH-PAs) fraction (Figs. 1B, 2B). The tetramine SPM were not detected. No acid soluble conjugated polyamines (SH-PAs) were found in the two cultivars analysed. Comparison of these results to those obtained from other sugarcane juices revealed the low concentration and composition of this fraction (Fontaniella et al. 2003). In juice from healthy plants, the S-PAs fraction represented 76.8 and 58.2% of total polyamines in L 55-5 and C 439-52 cultivars, respectively (Figs. 1A, 2A). Total polyamine concentration including soluble and bounded PAs in the cv. L 55-5 (2.12 μg ml^{-1}) (Fig. 1A, B) was higher than that found for the cv. C 439-52 (1.53 μg ml^{-1}) (Fig. 2A, B) (Fig. 3).

The major change in juice from *X. albilineans*-infected plants with respect to healthy ones was related to S-PUT concentration. The amount of this polyamine was significantly greater in both cultivars when infected with *X. albilineans* (2.83 and 5.22 time-folds in L 55-5 and C 439-52, respectively) in comparison to that observed in healthy plants

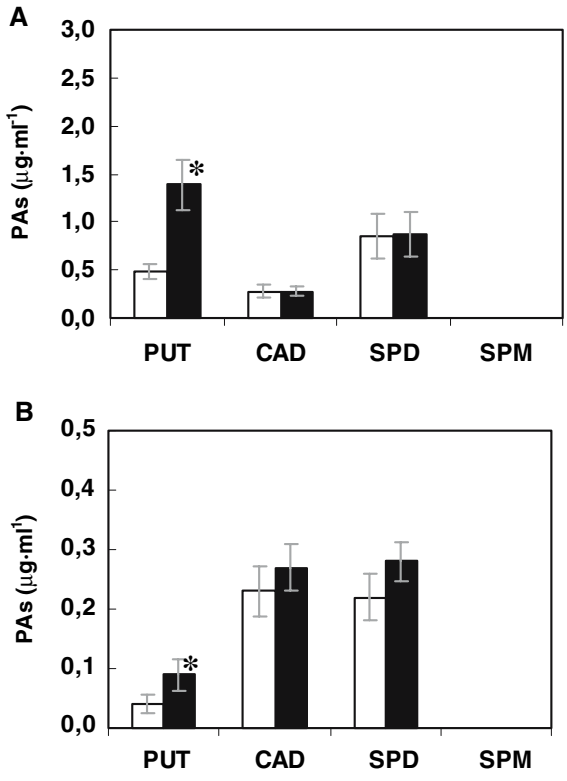

Fig. 1 Quantitation of free (S-PAs) (**A**) and acid insoluble conjugated (PH-PAs) polyamines (**B**) occurring in juice of cv. L 55-5 plants. Juice was obtained from healthy (white column) or *X. albilineans*-infected (black column) plants. Values shown are the means of three replicates ± S.E. * indicates values for diseased plants that differ at $P \leq 0.05$ from healthy plants

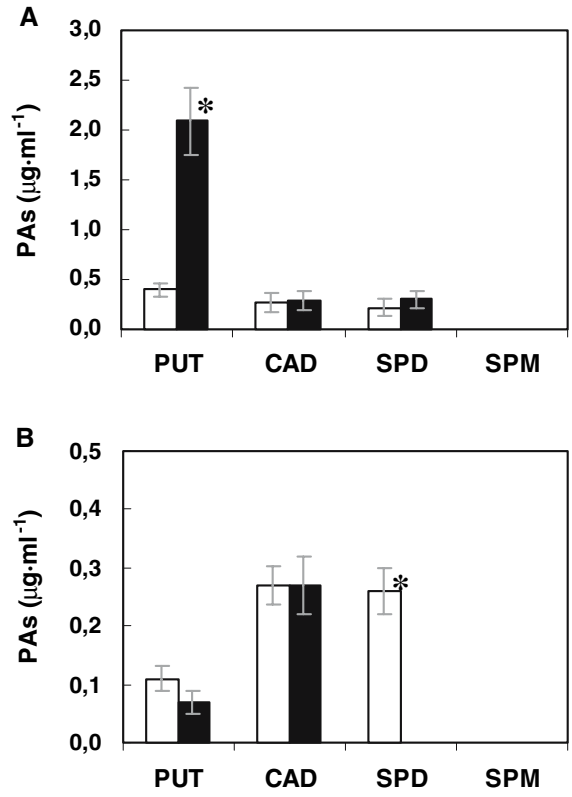

Fig. 2 Quantitation of free (S-PAs) (**A**) and acid insoluble conjugated (PH-PAs) polyamines (**B**) occurring in juice of cv. C 439-52 plants. Juice was obtained from healthy (white column) or *X. albilineans*-infected (black column) plants. Values are the mean of three replicates. Values shown are the means of three replicates ± S.E. * indicates values for diseased plants that differ at $P \leq 0.05$ from healthy plants

(Figs. 1A, 2A). The second significant variation attributed to bacterial infection is the content of bound polyamines. Although the concentration of the three bound PAs, PH-PUT, PH-CAD and PH-SPD were greater in juice of infected cv. L 55-5 plants, only PH-PUT was significantly different in healthy plants (Fig. 1B).

Juice from cv. C 439-52 contained the same three polyamine conjugates as found in L 55-5; however, variation of these conjugates in juice from infected plants was quite different. In these cultivars, PH-SPD completely disappeared whereas PH-PUT and PH-CAD were slightly lower (Fig. 2B).

Changes in the activity of arginase, ODC and LDC, enzymes of polyamine biosynthesis

In previous studies, it has been found that several enzymes related to polyamine biosynthesis occurred in sugarcane juice (Legaz et al. 2001). Arginase was shown to be a glycoprotein synthesized by sugarcane plants in response to *U. scitaminea* infection (Millanes et al. 2005). ODC and LDC have been shown to be highly active enzymes in sugarcane juice. Juice from healthy sugarcanes had moderate arginase activity, about 0.017 units. Arginase activity was lower in juice from infected plants of cv. L 55-5. In contrast, arginase activity in juice from infected cv. C 439-52 plants did not change, indicating the absence of any alteration when infected (Table 1). Juice from healthy plants of both cultivars showed similar values of ODC activity. Moreover, enzymatic activity of scalded plants of both cultivars was significantly higher; to a major extent for the cv. C 439-52 (8.10 times fold) and to a minor level for cv. L 55-5 (4.9 times fold) (Table 1).

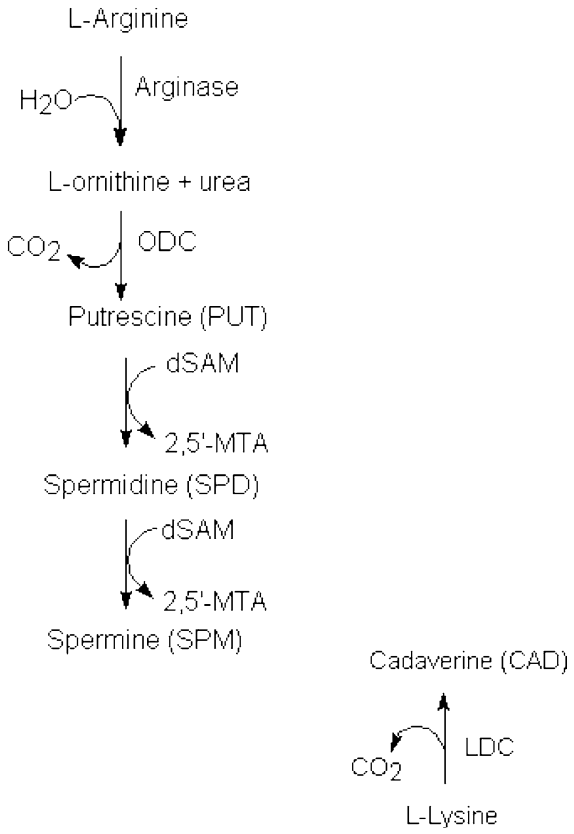

Fig. 3 Main pathway of polyamine biosynthesis, including CAD. *dSAM*: decarboxylated S-adenosyl methionine; *LDC*: lysine decarboxylase; *2,5′-MTA*: 2,5′-ethylthioadenosine; *ODC:* ornithine decarboxylase

In plants, CAD is mainly synthesized by the action of LDC which decarboxylates lysine to convert it into CAD. LDC activity differed between the two cultivars, with values 2.6 times higher in C 439-52 than in L 55-5. Enzymatic activity of juice from infected plants of both cultivars did not differ significantly from that obtained from healthy plants (Table 1).

Phenolic acids composition of healthy and infected juices

HPLC analysis of phenolic acid content of sugarcane juice revealed the presence of five phenolic acids: *p*-hydroxybenzoic, chlorogenic, caffeic, *p*-coumaric and ferulic acids. In both cultivars, phenolic acids were found both as free (S-PAcs) or conjugated (SH-PAcs) and bound (PH-PAcs) compounds, co-eluting with the corresponding polyamine fraction (Table 2). Syringic acid, one of the main phenolic acids that accumulates in sugarcane juice from other cultivars (de Armas et al. 1999), was not detected in the two samples analyzed in this work.

Only three phenolics, *p*-hydroxybenzoic, caffeic and *p*-coumaric acids, were detected as free form (S-PAcs). In both cultivars, caffeic acid occurred at the highest concentration. The major portion of the phenolic acids removed after hydrolysis was detected in the acid-soluble polyamine fraction (SH-PAs), while only a small part was detected in the acid-insoluble polyamine fraction (PH-PAs). Phenolic acid content of SH-fraction was about 79.3% and 54.4% of the total content in L 55-5 and C 439-52, respectively. Chlorogenic acid, a caffeic acid ester, occurred only in a conjugated form, being mostly accumulated in the SH fraction (Table 2). Although conjugates of polyamines and phenolic acids have been found to be common constituents of this fraction (Martin-Tanguy 1985), the absence of polyamines and the high amount of phenolic acids released after hydrolysis could indicate the existence of other types of conjugates. PH-fraction was revealed as the minor

Table 1 Specific activities of enzymes implicated in polyamine biosynthesis, arginase, ODC and LDC and in phenolic acids synthesis, PAL from juice of both healthy and *X. albilineans*-infected sugarcane plants of cvs L 55-5 and C 439-52

Cultiar		Specific enzyme activity (units)			
Arginase	ODC	LDC	PAL		
L 55-5	Healthy	0.016 ± 0.007	1.35 ± 0.24	0.092 ± 0.012	0.013 ± 0.04
	Infected	0.008 ± 0.004	6.61 ± 0.41[a]	0.130 ± 0.091	0.021 ± 0.009
C 439-52	Healthy	0.018 ± 0.010	1.45 ± 0.33	0.238 ± 0.048	0.008 ± 0.002
	Infected	0.016 ± 0.009	11.74 ± 0.47[a]	0.302 ± 0.061	0.014 ± 0.005

Values shown are the mean of three replicates ± SE

[a] indicate values for inoculated plants that differ at $P \leq 0.05$ from healthy plants

Table 2 Quantitation of free (S-PAcs), acid soluble (SH-PAcs) and acid insoluble (PH-PAcs) conjugated phenolic acids occurring in polyamine fractions obtained from both healthy and *X. albilineans*-infected plants of cvs L 55-5 and C 439-52

Phenolic acid μg ml^{-1}		Cultivar			
		L 55-5		C 439-52	
		Healthy	Infected	Healthy	Infected
S-PAcs	*p*-hydroxybenzoic	0.58 ± 0.09	1.71 ± 0.18*	1.37 ± 0.19	0.26 ± 0.04*
	Chlorogenic	n.d.	n.d.	n.d.	n.d.
	Caffeic	4.05 ± 0.59	5.25 ± 0.82	7.35 ± 1.22	2.58 ± 0.37*
	Syringic	n.d.	n.d.	n.d.	n.d.
	p-coumaric	n.d.	n.d.	0,80 ± 0,09	n.d.*
	Ferulic	n.d.	n.d.	n.d.	n.d.
	Total	4.63 ± 0.61	7.23 ± 0.91*	9.52 ± 1.47	2.84 ± 0.35*
SH-PAcs	*p*-hydroxybenzoic	5.25 ± 0.64	1.85 ± 0.20*	4.32 ± 1.73	n.d.*
	Chlorogenic	15.49 ± 2.06	23.68 ± 2.49*	8.95 ± 1.26	14.53 ± 2.04*
	Caffeic	n.d.	n.d.	n.d.	3.25 ± 0.40*
	Syringic	n.d.	n.d.	n.d.	n.d.
	p-coumaric	0.84 ± 0.09	0.77 ± 0.12*	2.37 ± 0.41	6.99 ± 1.07*
	Ferulic	1.38 ± 0.23	1.05 ± 0.14	0.06 ± 0.01	7.72 ± 1.12*
	Total	22.96 ± 3.04	27.35 ± 3.43	15.70 ± 2.88	32.49 ± 4.58*
PH-PAcs	*p*-hydroxybenzoic	0.09 ± 0.02	0.006 ± 0.002*	0.20 ± 0.03	n.d.*
	Chlorogenic	0.22 ± 0.03	0.18 ± 0.02*	n.d.	n.d.
	Caffeic	n.d.	n.d.	0,14 ± 0,01	n.d.*
	Syringic	n.d.	n.d.	n.d.	n.d.
	p-coumaric	0.84 ± 0.11	0.16 ± 0.03*	n.d.	n.d.
	Ferulic	0.34 ± 0.05	0.77 ± 0.10*	n.d.	n.d.
	Total	1.36 ± 0.18	1.22 ± 0.16	0.34 ± 0.06	–*
Σ(S + SH + PH)		28.95 ± 3.92	35.79 ± 4.88	25.56 ± 3.27	37.74 ± 5.02

Values shown are the mean of three replicates ± SE

* indicates values for inoculated plants that differ at $P \leq 0.05$ from healthy plants

fraction in both cultivars. Four phenolic acids were removed from the juice of L 55-5 plants after acidic hydrolysis, (*p*-hydroxybenzoic, chlorogenic, *p*-coumaric and ferulic acid), while, only *p*-hydroxybenzoic and caffeic acids were detected in C 439-52 plants (Table 2). Total phenolic acid content of this fraction also differed for both cultivars, showing higher values for L 55-5 than those found for C 439-52.

Juice from *X. albilineans*-infected plants from the two cultivars analysed showed higher content of total phenolic acids (S + SH + PH) than juice from healthy plants. However, changes in the three analyzed fractions differed between the two cultivars. Juices from infected plants of the most susceptible cv. C 439-52, showed a significant lower content of free phenolic acids and a significantly higher content of the phenolic acids associated to the SH polyamine fraction. Furthermore, phenolic acids associated with the PH polyamine fraction completely disappeared from infected plants. In juice obtained from infected plants of the moderately susceptible cv. L 55-5, free phenolic acids (S-PAcs) was the only phenolic fraction that showed significant differences in the total content of phenolic acids. Although no significant differences were found in the total content of phenolic acids liberated after hydrolysis from SH and PH polyamine fractions, HPLC analysis of each phenolic acid showed significant differences. It was concluded that both cultivars were able to synthesize a number of different phenols except syringic acid. The capability of conjugating chlorogenic acid to the SH fraction and ferulic acid to the PH fraction clearly

differentiates L 55-5 from C 439-52 cultivars, whereas the ability to increase the amount of free phenolics seems to be a characteristic of an enhanced level of resistance to disease in cv. L 55-5.

Changes in PAL activity after infection

PAL, a key enzyme for phenolic acids synthesis, catalysed the desamination of L-phenylalanine to produce cinnamic acid. Subsequent metabolic steps result in production of other phenolic acids. PAL activity in juice from healthy L 55-5 plants was higher than in juice from C 439-52 plants. Juice from scalded plants of both cultivars had higher levels of PAL activity than juice obtained from healthy plants (Table 1).

Discussion

The present study demonstrated that the composition of polyamines and phenolic acids in sugarcane juice can be altered by *X. albilineans* infection. Furthermore, our results indicate the existence of different metabolic pathways for polyamine and phenolic acids in the two cultivars analysed.

Both cultivars had a similar response to bacterial infection as shown by a significant increase in the content of S-PUT and ODC activity (Figs. 1A, 2A and Table 1, respectively). Furthermore, the incremental rate of both parameters seems to be positively correlated with susceptibility, since the increment in the most susceptible cv. C 439-52 was approximately twice that in the moderately resistant cv. L 55-5 (Figs. 2A, 1A, respectively). In spite of these common responses, results from the analysis of polyamines showed marked differences in subsequent metabolic steps and conjugation patterns between the two cultivars analysed. In the cv. L 55-5, defined as moderately susceptible, most of the PUT synthesized via ODC after bacterial infection was accumulated in the free form whereas another part was conjugated to the acid insoluble fraction as PH-PUT and PH-SPD. The ability to conjugate SPD to PH fraction seems to be a characteristic of cv. L 55-5 (Figs. 1A, B). Results from the analysis of CAD and LDC also revealed a slight increase of enzyme activity (Table 1) and in the conjugated PH-CAD in juice from infected plants (Fig. 1B).

Although polyamine metabolism of cv. C 439-52 (defined as highly susceptible) is also affected by bacterial infection, it is characterized by a lower level of polyamine conjugation since PH-PUT decreased and PH-SPD completely disappeared (Figs. 2A, B) in infected plants. Disappearance of *p*-hydroxybenzoic and caffeic acids from this fraction (Table 2) can also be evidence of the absence of conjugates formed among these acids and PUT or SPD.

Polyamine metabolism may be involved in a mechanism conferring resistance to a broad range of plant pathogens (Walters 2003). In the present work, increased ODC levels and decreased polyamine conjugates, mainly PH-SPD, seems to be correlated with plant susceptibility to bacterial infection. In previous work, it has been demonstrated that sugarcane polyamine biosynthesis and polyamine conjugate levels are changed by different pathogens such as phytoplasma or fungi (Fontaniella et al. 2003; Legaz et al. 1998). The role of polyamine-phenolic acid conjugates in plant-pathogen interactions, although extensively studied, is still controversial. In several studies, conjugation between polyamines and phenolic acids has been described as a defence mechanism against infection of several higher plants by viruses and fungi (Louis and Negrel 1991). Piñón et al. (1999) proposed that the susceptibility of sugarcane to smut was a consequence of the increased levels of polyamine conjugates to phenolic acids whereas resistance was related to accumulation of free phenolic acids. Increased production of phenolic acids in different plant species has been related to resistance against bacterial infections (Shekhawat and Chakravatti 1979; Reddy et al. 1977).

In the present work, and according to the results obtained, we propose that the high susceptibility of the cv. C 439-52 can be explained by the decrease in levels of free phenolic acids and the massive accumulation of phenolics conjugated to the SH fraction (Table 2). Thus, cv. L 55-5 is moderately susceptible to *X. albilineans* because of its capacity to increase the level of free phenolic acids.

However, removal of high amounts of phenolic acids from the acid-soluble fraction after acidic hydrolysis (-SH) (Table 2) and the absence of polyamines indicate the absence of monosubstitued hydroxycinnamic acid amides (HCAAs) in these fractions. On the other hand, as polysaccharides sterified to phenolic acid are common components of

sugarcane juice (Godshall et al. 2002), it is possible that the type of hydroxycinnamic acid conjugates from the SH fraction could be those formed between saccharides and phenolic acids.

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