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Article Addendum

Ustilago maydis induced accumulation of putrescine in maize leaves

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Polyamines are implicated in the regulation of many processes in the plant cell, including functioning of ion channels, DNA replication, gene transcription, mRNA translation, cell proliferation and programmed cell death. Plant polyamines occur either in free form, covalently bound to proteins, or conjugated to hydroxycinnamic acids forming phenol amides. *Ustilago maydis* is a dimorphic and biotrophic pathogenic fungus responsible for common smut or "huitlacoche" in maize; and it is considered an excellent model for the study of plant-pathogen interactions. Recently, we reported alterations in polyamine metabolism of maize tumors induced on leaf blades by *Ustilago maydis* infection. Our data revealed a striking increase in maize polyamine biosynthesis, mainly free and conjugated putrescine in the tumors and in the green plant tissue surrounding the tumor. In this addendum, we describe that changes in polyamine metabolism take place even in earlier stages of maize plant infection with *Ustilago maydis*.

The diamine putrescine (Put), whose synthesis from amino acids (ornithine and arginine) can be catalyzed by ornithine decarboxylase (ODC, EC 4.1.1.17) or arginine decarboxylase (ADC, EC 4.1.1.19), constitutes an essential precursor for the synthesis of spermidine (Spd) and spermine (Spm). Events occurring through the successive activities of spermidine synthase (SPDS, EC 2.5.1.16) and spermine synthase (SPMS, EC 2.5.1.22) by the addition of aminopropyl groups. The aminopropyl moiety is derived from methionine, which is first converted into S-adenosylmethionine (SAM) and then decarboxylated via S-adenosylmethionine decarboxylase (SAMDC, EC 4.1.1.50). Besides the important functions of polyamines (PAs) in plant growth and development, they are also implicated in biotic stress responses.[1-4,10]

Conjugated PAs have been associated to reproduction and floral induction processes in plants.[5,6] These conjugates participate in the regulation of PA concentration inside the cell,[7] and contribute to the formation of a phenolic barrier made by ester-ether linkages, making cell walls more resistant to enzymatic hydrolysis.[8] Also, due to their anti-fungal properties,[9] a role in the protection against pathogens has been suggested.[3,6]

Accumulation of Free and Conjugated Putrescine in *Ustilago maydis*-Induced Chlorotic Lesions

Besides the analysis in maize plant tumors,[10] in the present Addendum we describe alterations in PA metabolism at earlier stages of maize plant infection with *U. maydis*. Specifically, those occurring two days post-inoculation in maize leaves (dpi; 8 d-old seedlings) and during the induction of chlorotic lesions (5 dpi; 11 d-old). In agreement with our previous data,[10] the most significant alterations were observed in the levels of free and conjugated Put (Fig. 1). At 2 dpi, an increase (1.7-fold) in free Put content takes place in the infected tissues (I) in comparison to the control non-infected ones; notably the higher accumulation was observed in the wounded (W or mock)-plants (3-fold; Fig. 1A). This result agrees with the observation that a transient accumulation of Put occurs as a characteristic event in response to mechanical wounding in plants.[11] In the case of Spd and Spm, a slight reduction in both infected (I) and wounded plants (W) is shown (Fig. 1B and C; 2 dpi). Later on, the chlorotic lesions (Chl) showed a pronounced increase (5-fold) in free Put levels (Fig. 1A; 5 dpi). Chlorosis is one of the early and characteristic symptoms in maize plants infected with *U. maydis*.[12] In these lesions, free Spd was also increased (2-fold; Fig. 1B; 5 dpi). However, Spm levels did not show any significant change (Fig. 1C).

Notably, free PA levels were found to be higher in younger maize plants than in the older ones. This is observed comparing the controls of the different time points monitored (8 and 11) and 15 d-old maize seedlings (tumor stage),[10] suggesting that at early stages of plant growth a higher pool of PAs is required. Regarding soluble conjugated PAs (phenol amides formed by conjugation with hydroxycinnamic acids), a specific accumulation was found in the chlorotic lesions while at 2 dpi they were undetectable

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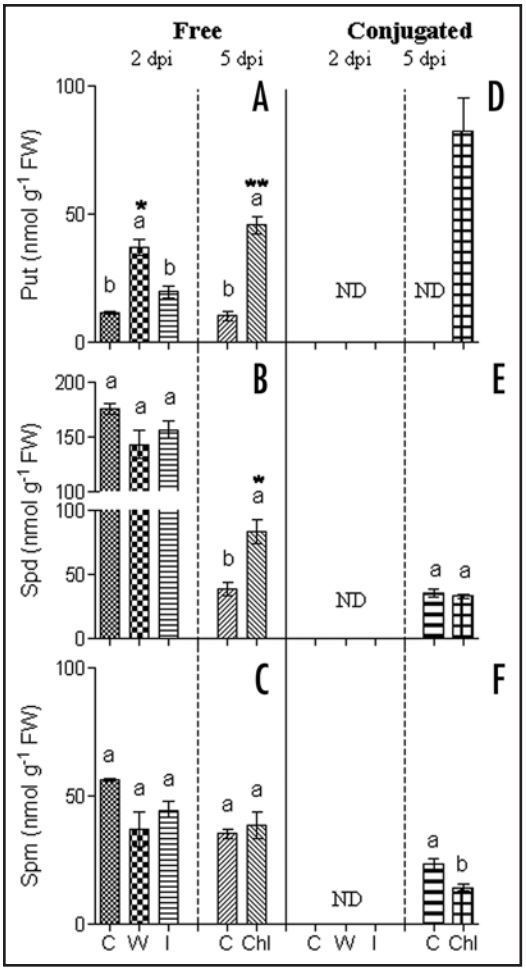

Figure 1. Determination of free and conjugated polyamine contents in 8 and 11 d-old maize plants infected (2 and 5 dpi) or not with *U. maydis*. (A and D) Put; (B and E) Spd; and (C and F) Spm. C, control plant; W, wounded plant; Chl, chlorosis; I, inoculated plant. Data are mean ± SE (n = 6) from two repeated experiments. ND, no detected. Different letters indicate significant differences between samples according to Tukey's multiple comparison tests at $p < 0.05$. Asterisks (* and **) indicate values that differ at $p < 0.01$ and $p < 0.001$ respecting control plants.

(Fig. 1D–F). Put conjugates were the most predominant ones, suggesting that it biosynthesis might be relevant in the chlorotic and tumor stage.[10] Recently, it was reported that the total content of hydroxycinnamic acids derivatives as well as the transcription and activity of the phenylalanine-ammonium lyase (PAL) are induced by *U. maydis* infection.[13] These changes are associated to increased cell wall synthesis and reinforcement during tumor development.[13]

The *Zmsamdc* Genes are Upregulated in the Chlorotic Lesions Induced by *Ustilago maydis*

Transcriptional profiling of *Adc*, *Samdc* (isoforms 1, 2, 3), *Zmspds1* and *Zmspms* genes of maize was performed by RT-PCR using specific oligonucleotides for each gene.[10,14] At 2 dpi, no alterations in the expression levels of these gene transcripts were recorded (data not shown). Conversely, in the chlorotic lesions

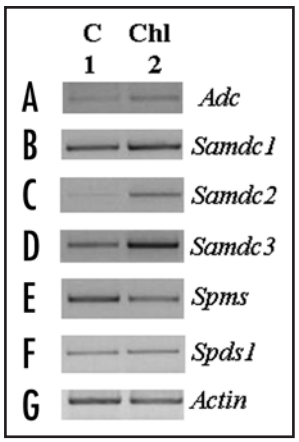

Figure 2. Expression of *Adc*, *Samdc* (isoforms 1–3), *Zmspds1* and *Zmspms* genes in leaf blades of 11 d-old maize plants infected (5 dpi) or not with *U. maydis*. Total RNA was isolated from leaf blades of C and Chl samples as in Figure 1.

the *Zmsamdc2* and *Zmsamdc3* transcripts were upregulated (Fig. 2C and D, lane 2). The *Adc* transcript was slightly affected (Fig. 2A, lane 2). In the case of the aminopropyltransferase genes, it was observed that *Zmspds1* expression suffered no alterations at this time point of the infection, whereas *Zmspms* transcript levels showed a reduction in the chlorotic areas (Fig. 2E and F, lane 2). In a similar fashion as in tumors,[10] an important regulation at the transcriptional level occurs at early stages of infection.

The overall changes in PA synthesis, might have several implications in the infection process. First, we suggest that soluble Put conjugates (hydroxycinnamic acids derivatives) might contribute to cell wall synthesis and reinforcement as a consequence of infection and tumor growth, as postulated by others.[13] On the other hand, we speculate that free Put might contribute to the enhanced cell division processes as well as pro-survival (anti-programmed cell death) role in tumor development.[15]

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