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ARTICLE

Potencial role of salicylic acid in tolerance of maize to *Fusarium graminearum*

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ABSTRACT Six maize genotypes were examined to reveal correlations between stalk rot caused by *Fusarium graminearum* and the levels of cellulase activity and endogenous salicylic acid. Our results showed that in resistant genotypes the cellulase activities are lower than in sensitive and moderately resistant genotypes. This suggests that determining the cellulase enzyme activity in stalk tissue could provide a good indication of the stalk rot resistance of the genotypes. It was also demonstrated that Fusarium infection affected differently the salicylic acid content in the six genotypes.
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KEY WORDS

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Fusarium species are harmful fungi that cause vascular diseases of plants. These fungi do not only digest plant cell wall polymers by the synthesis of extra-cellular enzymes to obtain an important nutrient source but also degrade the cell wall enabling cell penetration and spread through plant tissue (Kikot et al. 2009). *Fusarium* fungi causing stalk rot are present worldwide where maize or cereals are cultivated. Infected plants fall down, this may cause hard yield losses. Data in the literature suggest that the yield losses due to *Fusarium* stalk rot may amount to 10–35% (Logrieco et al. 2002). Substantial differences in resistance to *Fusarium* stalk rot have been observed in various maize hybrids and inbred lines (Ledencan et al. 2003; Afolabi et al. 2008). Salicylic acid (SA) is an important signal involved in the activation of defence responses against abiotic and biotic stress (Catinot et al. 2008). Increases in endogenous SA level have been reported in pathogen challenged leaves of various plant species, furthermore exogenously applied SA can induce resistance against several biotic stresses (Chaturvedi and Shah 2007).

The aim of the present work to investigate correlation between levels of cellulase activity and endogenous SA content and the level of tolerance to *Fusarium* stalk rot.

Materials and methods

Six maize (*Zea mays* L.) genotypes were selected with well documented different level of tolerance to *Fusarium* stalk rots (2 sensitive: P05, P03; 2 moderately resistant: P01, P08 and 2 resistant: P07, P12). Plants were sown in plant pathology garden under the conditions described by Szőke et al. (2009). Maize plants were inoculated at adult stage with *Fusarium graminearum* isolate (FG36) according to Szőke et al. (2007). The extent to which the pith was rotted was determined using a computerised image analysis program. The stalk samples were photographed using a digital camera, and the size of the infected area was determined as a percentage of the whole area of the internode using the Colim 4.0 image analysis software. The principle behind the measurements is that healthy and diseased tissues form patches have a different intensity range. The stalks of control and infected maize plants were sampled for cellulase enzyme activity and HPLC analysis. The enzyme activity in the stalk tissue extracts was determined using the modified cup-plate method. The cellulase activity was determined from the diameter of the activity rings using the Colim 4.0 image analysing program followed by the calculation of the ring area in mm². Salicylic acid was measured according to Meuwly and Métraux (1993) and Pál et al. (2005). SA was quantified fluorimetrically (W474 scanning fluorescence detector, Waters, USA), with excitation at 305 nm and emission at 407 nm for SA.

Results

Significant differences were observed in the level of tolerance between the six genotypes, which were manifested in changes of cellulase enzyme activities (expressed as diameter of the activity rings) of the diluted tissue extracts. In control plants, the cellulase activities were 70.63, 18.47, 0.01, 0.01, 0.01, and 0.01 mm^2 in the case of P05, P03, P01, P08, P07 and P12, respectively. After infection the cellulase activities increased in all genotypes. The highest activity was observed in P05 and P03, in both case 137.7 mm^2. Activities were lower in the moderately resistant genotypes, 93.97 and 79.42 mm^2 in P01 and P08, respectively. In the two resistant genotypes (P07 and P12) the activities were 79.46 and 56.28 mm^2, respectively. Genotypes exhibiting less stalk rot damage in the field had lower levels of cellulase activity, while greater susceptibility

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to stalk rot was associated with higher cellulase activity. In the case of healthy plants, exhibiting no stalk rot damage, no cellulase enzyme activity could be detected. Our results showed that there is a close correlation between cellulase enzyme activity and the degree of tolerance against stalk rot.

In stalk tissue of the control plants the highest endogenous SA content was observed in the case of P05 genotype. *Fusarium* infection affected differently the SA content in the six genotypes. SA content decreased the in the case of the sensitive P05 genotype, did not change significantly in the sensitive P03 and P01 genotypes, and slightly decreased in the moderately resistant P08 genotype after infection. Significant increases were observed in the SA level of both resistant genotypes (P07 and P12) after infection, which were 371 and 49%, respectively.

Discussion

Our results showed that in resistant genotypes the cellulase activities are lower than in sensitive and moderately resistant genotypes. These results are in agreement with earlier data from the literature, which reported a positive correlation between the extent of stalk rot and cellulase enzyme activity (Ahmad et al. 2006; Szőke et al. 2009). Literary data reveal a positive correlation between the infectivity of *Fusarium* species and the quantity of enzyme they produce (Novo et al. 2006). The present data suggest that the enzyme activity values of genotypes can be used to characterise the susceptibility of the genotypes to stalk rot.

The present work also investigated changes in endogenous SA content in stalk tissue of maize genotypes with different tolerance to stalk rot caused by *Fusarium graminearum.* SA is synthesised by plants in response to challenge by various phytopathogens and is an essential signaling molecule involved in both local and systemic acquired resistance (Raskin 1992). In this paper we demonstrated that *Fusarium graminearum* infection decreased or did not changed SA content in sensitive and moderately resistant genotypes, while significantly increased in resistant ones. These results are in accordance with results of other authors, as in the above-ground parts of maize plants the content of free salicylic acid did not change, and the content of bound salicylic acid increased 1.7-fold in resistant, while the content of free salicylic acid decreased 4.8-fold, and content of bound salicylic acid decreased 2.14-fold in susceptible genotypes after infection with *F. verticillioides* (syn.: F. moniliforme) compared with control (Molodchenkova et al. 2002). But, not only infection able to induce SA-dependent stress responses. Infiltration of cellulase from *Trichoderma longibrachiatum* into melon cotyledons induced an oxidative burst which was followed by activation of ethylene and SA signaling pathways leading to the induction of peroxidase and chitinase activities (Martinez et al. 2001). SA-treated asparagus plants exhibited enhanced systemic resistance after inoculation with *Fusarium oxysporum* f.sp. asparagi, compared with untreated plants. SA activated peroxidase and phenylalanine ammonia-lyase, as well as lignification upon *Fusarium* attack (He and Wolyn 2005).

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