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Transcriptional responses of catalase genes in maize seedlings exposed to cereal aphids' herbivory

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ABSTRACT

The performed study was aimed at assessing the influence of two species of cereal aphids (bird cherry-oat aphid, *Rhopalosiphum padi* L. and grain aphid, *Sitobion avenae* F.) on the transcriptional responses of all three catalase (*cat1*, *cat2*, *cat3*) genes in seedling leaves of maize (*Zea mays* L.) genotypes varying in resistance levels to the insects' infestation. Furthermore, time-course generation of hydrogen peroxide (H_2O_2) in the aphid-attacked plants was estimated. Colonization experiments were conducted on 14 day-old seedlings of six selected *Z. mays* genotypes (Tasty Sweet and Złota Karłowa – susceptible; Nana and Touran - moderately resistant; Ambrozja and Waza - highly resistant) that were artificially infested with adult apteral females of the tested hemipterans. Relative expression of target *cat* genes was monitored using real-time qRT-PCR technique, whereas hydrogen peroxide content was screened using a spectrophotometric microplate method. The obtained data indicated a crucial role of *cat1* and *cat2* genes in overcoming aphid-triggered disturbances in the redox homeostasis in the infested maize seedlings. It has been revealed dissimilar patterns of transcriptional reprogramming of the analysed *cat* genes in seedlings of resistant and sensitive maize cultivars. However, there were no significant changes in *cat3* gene expression in maize plants in response to cereal aphids' attack. It was also found that all tested *Z. mays* genotypes responded an elevation in H_2O_2 content compared to the uninfested control, and this accumulation was linked with resistance degrees to the aphids' colonization.

1. Introduction

Numerous studies evidenced that plant defence reactions towards biotic and abiotic stressing factors include a prompt and excessive generation of diverse reactive oxygen species (ROS), such as hydroxyl and superoxide anion radicals, and hydrogen peroxide (H_2O_2) (Gill and Tuteja, 2010; Li et al., 2014). Short-term ROS overproduction by oxidant-producing enzymes may result in activation of the signalling networks and triggering a sequence of local and/or systemic reactions (Foyer and Noctor, 2009). Nevertheless, the prolonged and exaggerated free-radicals' formation is associated with a depletion in the pool of intracellular antioxidants, and might be linked with severe oxidative damages of crucial biomolecules (e.g. proteins, lipids, nucleic acids, pigments and sugars) (Wang et al., 2014). Secondarily, the oxidative burst within the stressed plants may cause

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detrimental cytotoxic effects embracing plasma membrane injuries, cell cycle inhibition and programmed cell death (Vaculíková et al., 2014). In order to provide a tight regulation of ROS levels in living cells, plants have evolved a wide battery of enzymatic and non-enzymatic antioxidants responsible for maintaining the redox balance (Huang and Song, 2013).

Plant catalases (CATs; EC 1.11.1.6) have been considered as the major antioxidative enzymes involved in decomposition of hydrogen peroxide to water and molecular oxygen (O_2) (Afiyanti and Chen, 2014; Wang et al., 2014). CAT isozymes have been isolated from tissues of many plant species, such as barley (*Hordeum vulgare* L.), sunflower (*Helianthus annuus* L.), thale cress (*Arabidopsis thaliana* L.), maize (*Zea mays* L.), and tobacco (*Nicotiana tabacum* L.) (Afiyanti and Chen, 2014). For example, barley and potato plants possess two CAT isozymes, tobacco synthesises three isoforms, whereas *A. thaliana* at least six different types of the isozymes. In maize genome, it has been identified three catalase (*cat1*, *cat2* and *cat3*) genes, encoding the corresponding isozymes (CAT-1, CAT-2 and CAT-3) (Polidoros and Scandalios, 1997; Hu et al., 2010). The presence of multiple organ- or tissue-specific catalase isoforms indicates their distinct biological functions during plant growth and development, as well as adaptation to adverse exogenous stimuli (Wang et al., 2014). CAT activity in *Z. mays* plants has been substantially altered by numerous unfavourable environmental conditions, such as salinity (Kellos et al., 2008), drought (Chugh et al., 2011), chilling or high temperature (Erdal, 2012), UV irradiation (Wang et al., 2010), elevated magnetic field (Anand et al., 2012), heavy metals' exposure (Kumar et al., 2008; Alonso-Blázquez et al., 2015), growth in darkness, exogenous application of hydrogen peroxide, polyethylene glycol, abscisic (ABA) or salicylic (SA) acids (Kellos et al., 2008), and pathogen or insect attack (Ketabchi and Maryam, 2011; Świątek et al., 2014). Despite several reports evidenced modulations in activity of various CAT isoforms in many plant species subjected to stressing factors, there is a paucity of knowledge on regulation of the catalase genes, especially in the context of plant–insect interactions (Zhu-Salzman et al., 2004; Divol et al., 2005; Park et al., 2006). To date, there are no available reports comparing the intervarietal responses in abundance of various catalase transcripts in tissues of monocotyledonous plant species infested with aphids. Furthermore, there is very limited and contradictory information regarding the aphid-stimulated oxidative stress in the attacked host plants (Moloi and van der Westhuizen, 2006; Kuśnierczyk et al., 2008; Kerchev et al., 2012; Mai et al., 2013; Sytykiewicz, 2014). Moreover, the physiological consequences of insect-evoked H_2O_2 overproduction in the hosts and the aphid digestive tract, still remain largely unknown.

It has been hypothesized that cereal aphids' herbivory may be linked with considerable alternations in expression of *cat* genes as well as modulations in hydrogen peroxide content in seedlings of diverse *Z. mays* varieties. The primary objective of the study was to evaluate effects of the investigated cereal aphids (*Rhopalosiphum padi* L. or *Sitobion avenae* F.) herbivory on the transcriptional reprogramming of all three catalase (*cat1*, *cat2* and *cat3*) genes in the seedling leaves of six selected maize genotypes, exhibiting distinct resistance levels towards the tested hemipterans. In addition, the performed survey was aimed at establishing the influence of the cereal aphids' infestation on the amount of hydrogen peroxide in foliar tissues of the infested *Z. mays* varieties.

2. Materials and methods

2.1. Chemical reagents

All chemicals used in measurement of hydrogen peroxide content were purchased from Sigma–Aldrich (Poland) and were of high purity analytic grade.

2.2. Plant material

Seeds of the tested maize varieties: Ambrozja, Tasty Sweet and Touran were provided by commercial grain companies in Poland: Reheza (Moszna), PNOS S.A. (Ożarów Mazowiecki), and KWS Polska (Poznań). It has been previously reported that Tasty Sweet cultivar is susceptible for the cereal aphids' colonization, while Touran and Ambrozja genotypes represent moderate and high resistance levels, respectively (Sytykiewicz, 2014). Maize seeds were subjected to the following procedure of surface sterilization: i) stirring with 70% solution of ethanol (2 min), ii) treatment with 0.1% $HgCl_2$ (3 min), iii) the grains were washed with autoclaved deionized water at least five times (30 s each). Maize seedlings were kept in a climate chamber under the following environmental conditions: light intensity of 100 μM m^{-2} s^{-1}, long-day photoperiod (L:D, 16 h: 8 h), relative humidity of 65 ± 5%, and temperature of 22 ± 2 °C/16 ± 2 °C (day/night). Plants grown individually in plastic pots (10 × 9 cm), filled with the universal soil used in horticulture.

2.3. Insects

Wingless adult females of both investigated aphid species (*R. padi* and *S. avenae*) were sampled from cereal plants cultivated in the Siedlce district, Poland (52°09′54″N, 22°16′17″E). The relevant insect stocks were maintained for a year on *Triticum aestivum* L. *cv.* Tonacja in the laboratory of Department of Biochemistry and Molecular Biology (University of Natural Sciences and Humanities, Siedlce, Poland). Aphids' populations were reared on wheat plants under controlled conditions as specified in the passage 2.2.