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Aphid-stimulated transcriptional reconfigurations of chlorophyllase-2 gene in maize (*Zea mays* L.) seedlings

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ABSTRACT

The purpose of the survey was to evaluate impact of bird cherry-oat aphid (*Rhopalosiphum padi* L.) herbivory on transcriptional responses of chlorophyllase-2 (*CLH2*) gene, total activity of chlorophyllase (CLH) enzyme and content of chlorophyll *a* in maize (*Zea mays* L.) leaves. Insect infestation assays were performed on 14-day-old seedlings of six maize varieties (*i.e.*, Ambrozja and Waza – highly resistant; Eleganza and Touran – intermediately resistant; Tasty Sweet and Złota Karłowa – sensitive towards the examined aphids). Maize plants were artificially infested with mature wingless females of *R. padi*. Abundance of *CLH2* transcript was quantified with the use of real-time qRT-PCR technique, whereas chlorophyllase activity and amount of the tested pigments were estimated using microplate spectrophotometric methods. Aphids' feeding markedly upregulated relative expression of *CLH2* gene and enhanced total activity of CLH in maize seedlings. Conversely, hemipterans' attack caused significant depletion in content of chlorophyll *a* in tested plants. Considerably stronger effect of *R. padi* colonization on level of all tested parameters was found in seedling leaves of susceptible maize cultivars in comparison with more resistant ones. This is the first survey unravelling insect-stimulated modulations in both expression of *CLH2* gene and chlorophyllase activity in tissues of maize genotypes displaying divergent resistance to aphids' infestation.

1. Introduction

Plant chlorophyllases (CLHs) comprise a group of chlorophyllido-hydrolases (EC 3.1.1.14), responsible for cleavage of chlorophylls into a variety of molecular forms of chlorophyllide (Hu et al., 2016; Huang et al., 2016). It has been documented that alternations in expression of chlorophyllase genes and/or CLH activity may vary significantly in plants exposed to

Abbreviations: Chl *a*, chlorophyll *a*; *CLH2*, chlorophyllase-2 gene; CLH, chlorophyllase enzyme; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SA, salicylic acid; SPD, spermidine.

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multifarious exogenous stimuli (e.g., heavy metals, chilling, high temperature, salinity, drought, wounding, attack of pathogens, parasites or insects, application of salicylic acid/SA/or spermidine/SPD/) (Saidi et al., 2012; Hu et al., 2015; Roychoudhury et al., 2016). It was reported the crucial role of ethylene in acceleration of the chlorophyllase activity in plants grown under unfavourable environmental conditions (Azoulay-Shemer et al., 2011). Additionally, Banaś et al. (2011) revealed that abundance of two chlorophyllase transcripts (*i.e.*, *AtCLH1* and *AtCLH2*) in *Arabidopsis thaliana* L. plants strongly depended on light intensity. Schenk et al. (2007) evidenced the occurrence of chlorophyll depletion in *A. thaliana* mutants with knockouts of the two *CLH* genes. In this context, it should be underlined that catabolic pathways of chlorophylls' degradation in plant tissues are not completely recognized.

Aphids are pierce-sucking phloem feeders causing a number of injuries in infested cereal hosts. It may include ultrastructural damages of cells, disturbances in the course of many biochemical processes and cycles, decline in content of photoassimilates, induction of chlorosis, necrosis, morphological deformations, transmission of plant viruses and severe yield loss (Yu et al., 2016; Zeb et al., 2016). Few studies indicated that aphids' infestation man result in profound diminution in amount of the green pigments in the hosts (Goławska et al., 2010; Sytykiewicz et al., 2013a). Chlorophylls are the most abundant pigments in photosynthetic apparatus, therefore, insect-evoked decrease in content of these compounds may lead to a considerable decrement in efficiency of photosynthesis process, that secondarily suppresses plant growth and development (Lin et al., 2014).

Based on previous experiments, it has been assumed that *Rhopalosiphum padi* L. aphids' feeding may influence relative expression of chlorophyllase-2 (*CLH2*) gene and/or total activity of CLH enzyme in maize plants exhibiting diverse sensitivity to the tested hemipterans. Until now, there are no available reports evidencing the impact of aphids' attack on levels of tested parameters in different maize varieties. Hence, the major purpose of the study was to evaluate the effect of bird cherry-oat aphid females' herbivory on transcriptional reprogramming of *CLH2* gene and level of CLH activity in seedlings of aphid-resistant (Ambrozja, Eleganza, Touran and Waza) and aphid-susceptible (Tasty Sweet and Złota Karłowa) *Zea mays* L. genotypes. In parallel, at the metabolite level, insect-evoked changes in Chl *a* content in tested host plants were estimated.

2. Materials and methods

2.1. Plant material

Seeds of six examined *Z. mays* cultivars (*i.e.*, Ambrozja, Eleganza, Tasty Sweet, Touran, Waza and Złota Karłowa) were purchased from local grain companies, such as KWS (Poznań, Poland), PNOS S.A. (Ożarów Mazowiecki, Poland), Reheza (Moszna, Poland) and W. Legutko (Jutrosin, Poland). Ambrozja and Waza maize genotypes have been classified as highly resistant to *R. padi* apterae infestation, Eleganza and Touran varieties were moderately resistant, whereas Tasty Sweet and Złota Karłowa cultivars were found to be susceptible (20). The seeds were sterilized in 70% ethanol (2 min), soaked in 0.1% $HgCl_2$ (3 min), and then, they were rinsed with deionized water. Seeds were placed separately in plastic containers (10 × 9 cm; diameter × height), filled with universal garden soil (Kronen, Agro Market, Poland). The seedlings were grown in a climate chamber under controlled conditions: photoperiod (L:D, 16 h:8 h), light intensity of 100 μM m^{-2} s^{-1}, 65 ± 5% humidity, temperature of 22 ± 2 °C (day) and 16 ± 2 °C (night).

2.2. Aphids

Adult parthenogenetic females (apterae) of *R. padi* were gathered from cereal crops grown within the Siedlce district, Poland (52°09′54″N, 22°16′17″E). Virus-free aphids' stocks were reared on wheat plants (cv. Tonacja) in laboratory of Department of Biochemistry and Molecular Biology, at the University of Natural Sciences and Humanities (Siedlce, Poland).

2.3. Infestation experiments

The insect infestation biotests were performed in triplicates, and each round of the experiments was conducted on leaves of 14-day-old maize seedlings. Each time, 20 plants of a given genotype were artificially infested with adult wingless females of *R. padi*, and the same number of plants were uninfested (control). All maize seedlings (insect-colonized and control ones) were individually isolated using transparent plastic cages (20 × 40 cm; diameter × height), and covered with nylon mesh. First phase of the study was performed on seedlings of Waza and Złota Karłowa cultivars (highly resistant and sensitive to *R. padi* aphids, respectively). Transcriptional reconfigurations of *CLH2* gene, total activity of chlorophyllase, and content of Chl *a* in aphid-infested and control maize plants were measured at 0, 1, 3, 6, 12, 24, 48, 72, 96, 120 and 144 h after insect infestation (hpi). The effect of two levels of insects' infestation was evaluated: 40 and 60 aphids per plant (8 and 12 apterae per leaf, accordingly). Second stage of the survey was carried out on seedlings of six maize genotypes (Ambrozja, Eleganza, Tasty Sweet, Touran, Waza, Złota Karłowa) characterized with diverse susceptibility levels to the examined hemipterans. During this phase of the experiments, host plants were colonized by higher number of insects (80 *R. padi* females per plant; 16 aphids per leaf), whereas control seedlings remained non-infested. All investigated parameters (*i.e.*, *CLH2* gene expression, CLH activity and Chl *a* accumulation) in aphid-stressed and uninfested maize seedlings were monitored at 0, 24, 72, 96 and 144 hpi.