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Transcriptional reprogramming of genes related to ascorbate and glutathione biosynthesis, turnover and translocation in aphid-challenged maize seedlings

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ABSTRACT

Aphids are pierce-sucking insects that severely affect redox homeostasis in the host tissues. Reduced ascorbate (AsA) and glutathione (GSH) serve as multifunctional molecules involved in constituting sophisticated plant defense responses to aphids' herbivory. This survey aimed to decipher transcriptional responses of forty-one genes associated with AsA and GSH biosynthesis, turnover and translocation in maize (*Zea mays* L.) seedlings infested with two hemipterans' species: bird cherry-oat aphid (*Rhopalosiphum padi* L.) and grain aphid (*Sitobion avenae* F). Cereal aphids' attack substantially stimulated the expression of fifteen maize genes – encoding L-galactose dehydrogenase (GalDH), L-galactono-1,4-lactone dehydrogenase (GLDH), ascorbate oxidase (AO), glutamate–cysteine ligase (GCL), glutathione synthetase (GS), two isozymes of glutathione peroxidase (GPX1, GPX3), five isoforms of glutathione transferase (GST9, GST11, GST16, GST31, GST38) and three GSH transporters (ABCC2, ABCC6, GT1). Transcriptional alternations of these genes were dependent on maize genotype, insect species, duration of infestation and aphids' abundance. Specific patterns of genes' expression as well as total activity of the corresponding enzymes in insect-challenged maize seedlings (cvs Ambrozja and Tasty Sweet, which are relatively resistant and susceptible, respectively) highlighted the molecular background of crucial part of the complex antioxidative responses of maize model plant toward cereal aphids' colonization.

1. Introduction

L-Ascorbic acid (ascorbate; AsA) and reduced glutathione (GSH; γ-Glu-Cys-Gly) have been considered as the most crucial low molecular hydrophilic antioxidants in foliar tissues of various cereal species (Wang et al., 2015). Transcriptional regulation of the selected genes involved in AsA and GSH metabolism and translocation was predominantly investigated in plants exposed to abiotic stressing factors, but surprisingly, alternations in these transcripts' abundance in the host tissues under insect herbivory are largely unknown (Liu et al., 2015b; Sytykiewicz, 2016).

Abbreviations: ABCC, ATP-binding cassette transporter subfamily C; AO, ascorbate oxidase; APX, ascorbate peroxidase; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; GalDH, L-galactose dehydrogenase; GCL, glutamate–cysteine ligase; GLDH, L-galactono-1,4-lactone dehydrogenase; GPX, glutathione peroxidase; GR, glutathione reductase; GS, glutathione synthetase; GST, glutathione transferase; GT1, glutathione transporter 1; MDHA, monodehydroascorbate; MDHAR, monodehydroascorbate reductase.

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Ascorbate serves as an electron donor in redox reactions catalyzed by numerous antioxidative enzymes and exhibits direct scavenging properties against reactive oxygen species (ROS) generated in the plant cells (Zhang et al., 2015). D-Mannose/L-galactose pathway has been recognized as the major route involved in *de novo* biosynthesis of ascorbate in the cereal plants (Castro et al., 2015). This anabolic pathway comprises six key enzymes: GDP-D-mannose pyrophosphorylase (GMP; E.C. 2.7.7.22), GDP-D-mannose-3′,5′-epimerase (GME; E.C. 5.1.3.18), GDP-L-galactose phosphorylase (GGP; E.C. 2.7.7.69), L-galactose-1-phosphate phosphatase (GPP; E.C. 3.1.3.93), L-galactose dehydrogenase (GalDH; E.C. 1.1.1.316) and L-galactono-1,4-lactone dehydrogenase (GLDH; E.C. 1.3.2.3) (Castro et al., 2015). In addition, a few minor routes (*i.e.*, *myo*-inositol, D-galacturonate and GDP-L-gulose pathways) linked to AsA biosynthesis under physiological and certain stressful conditions have been identified (Sanahuja-Solsona, 2013). It was found that rice plants exposed to exogenous application of L-galactose and L-galactono-1,4-lactone possessed substantially higher contents of AsA, but these effects were not observed in seedlings treated with different precursors utilized in alternative routes of AsA biogenesis (Fukunaga et al., 2010). Copper-containing ascorbate oxidase (AO; E.C. 1.10.3.3) is responsible for oxidation of L-ascorbic acid to monodehydroascorbate (MDHA), with reduction of oxygen into water in the plant tissues (Łukawska-Kuźma et al., 2012). The biosynthesis of reduced glutathione involves two enzymes: glutamate–cysteine ligase (GCL; E.C. 6.3.2.2, formerly named γ-glutamyl-L-cysteine synthetase; γ-ECS) and glutathione synthetase (GS; E.C. 6.3.2.3), encoded by *GCL* and *GS* genes, accordingly (Liu et al., 2015b). Glutamate–cysteine ligase preferentially catalyzes ATP-dependent γ-glutamyl-L-cysteine (γ-GC) formation using L-glutamate and L-cysteine as the substrates (Racchi, 2013). During the second step, GS enzyme catalyzes ATP-dependent GSH formation from γ-GC and glycine (Singh et al., 2015). GCL occurs in plastids, whereas GS is mainly localized in the cytosol (Gómez et al., 2004). Reduced glutathione may directly scavenge diverse ROS forms, and additionally, it may be utilized as a substrate in a variety of reactions processed by glutathione peroxidase (GPX; E.C. 1.11.1.9) and glutathione transferase (GST; EC 2.5.1.18) (Ozyigit et al., 2016). GPX isozymes have been considered to be involved in decomposition of hydrogen peroxide and lipid peroxides, thus counteracting the scale of oxidative damages within the structure of macromolecules and cellular organelles (Zhai et al., 2013). Primary functions of a wide battery of GST isoforms are associated with detoxification of endo- and exogenous compounds, redox signaling and participation in complex regulatory network of oxidative stress responses (Dixon and Edwards, 2009).

The results of previous experiments (Sytykiewicz, 2016) revealed profound genotype-dependent differential transcriptional responses of several *APX*, *GR*, *DHAR* and *MDHAR* genes related to AsA-GSH cycle as well as alternations in the content of reduced and oxidized forms of ascorbate and glutathione and their redox ratios in maize seedlings exposed to cereal aphids' herbivory. In addition, oligophagous bird cherry-oat aphid (*Rhopalosiphum padi* L.) evoked more severe changes in the vast majority of the analysed transcripts and compounds in comparison with changes induced by monophagous grain aphid (*Sitobion avenae* F.). Recently, it has been reported increasing abundance of these two insect pests colonizing maize crops in Poland (Bereś, 2015; Ruszkowska and Strażyński, 2015). Based on these data, there was a necessity to conduct much more complex molecular survey in order to gain new insights into transcriptional reprogramming of other genes linked to metabolism and transport of AsA and GSH molecules in aphid-infested maize plants. The primary purpose of the current work was to compare expression patterns of forty-one genes involved in AsA and GSH biosynthesis, turnover and translocation in seedlings of Ambrozja (highly resistant) and Tasty Sweet (susceptible) maize cultivars infested with *R. padi* or *S. avenae* aphids. The panel of the investigated genes embraced: ascorbate oxidase (*AO*) gene, six genes involved in AsA biogenesis (*GGP*, *GME*, *GMP*, *GPP*, *GalDH* and *GLDH*), *LPE1* gene encoding nucleobase-ascorbate transporter LPE1, two genes linked to GSH biosynthetic pathway (*GCL*, *GS*), four glutathione peroxidase genes (*GPX1*, *GPX2*, *GPX3*, *GPX4*), twenty *GST* genes (*i.e.*, *GST2*, *GST7*, *GST9*, *GST10*, *GST11*, *GST12*, *GST13*, *GST14*, *GST15*, *GST16*, *GST17*, *GST19*, *GST26*, *GST27*, *GST31*, *GST34*, *GST35*, *GST37*, *GST38* and *GST41*) that protein products comprise respective glutathione transferase isoforms, and seven genes linked to GSH transport (*GT1*, *ABCC2*, *ABCC3*, *ABCC4*, *ABCC5*, *ABCC6*, *ABCC7* – encoding glutathione transporter 1 /GT1/, and ATP-binding cassette /ABC/ transporters 2–7, respectively). In parallel, it was assessed insect-induced modulations in total activity of the seven enzymes (*i.e.*, AO, GalDH, GCL, GLDH, GPX, GS and GST) encoded by the examined genes that expression was induced in foliar tissues of the maize plants. Until now, there are no available reports regarding aphid-stimulated transcriptional reconfigurations of the analysed genes in seedlings of maize varieties with divergent resistance to the examined insects.

2. Materials and methods

2.1. Plant material

Seeds of maize (*Zea mays* L.) were obtained from grain trading companies, PNOS S.A. (Ożarów Mazowiecki, Poland) and Reheza (Moszna, Poland). The experiments involved two maize genotypes exhibiting distinct resistance degrees to both tested cereal aphids: cvs Ambrozja and Tasty Sweet – highly resistant and susceptible to the examined hemipterans, respectively. The seeds were surface-sterilized following the procedure of Sytykiewicz et al. (2014). Maize seedlings were grown in the environment chamber under controlled conditions as previously described (Sytykiewicz, 2016).

2.2. Cereal aphids

Wingless adult females (apterae) of the bird cherry-oat aphid (*Rhopalosiphum padi* L.) and the grain aphid (*Sitobion avenae* F.) were included in the leaf infestation biotests. Aphids' individuals were collected from virus-free stocks that were reared at