Journal of Plant Physiology 221 (2018) 62–65

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Short communication

Possible involvement of glutathione S-transferases in imazamox detoxification in an imidazolinone-resistant sunflower hybrid

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ARTICLE INFO

Keywords:
Sunflower
Imazamox
Glutathione (GSH)
Glutathione S-transferases (GSTs)
Non-target resistance
Clearfield

ABSTRACT

The resistance of crops to herbicides can be due to target site based resistance or non-target site based resistance mechanisms or a combination of both. In non-target site resistance, the detoxification efficiency plays a major role by involvement of enzymes such as P450s, GTs, GSTs and ABC transporters. The resistance of the first commercial Clearfield sunflower hybrids (*Imisun* trait) to herbicides of imidazolinone group is based on a combination of both types of resistance. The target site resistance consists of a mutation in *Ahasl1* gene, encoding the synthesis of the AHAS enzyme. The non-target site resistance is supposed to be due to intensified herbicide disposal and is not fully understood. The objective of this study was to detect the fast response of the glutathione-mediated detoxification system in IMI-R and IMI-S sunflower hybrids to the herbicide imazamox and to study the possible participation of GSTs in the enhancement of the hybrids' tolerance. The obtained results allow to presume that GSTs are involved in imazamox detoxification in the sunflower Imisun trait and thus contributing to its non-target site resistance.

1. Introduction

Sunflower (*Helianthus annuus*) is an economically important oilseed crop. The Clearfield® technology in sunflower allows post-emergence control of a variety of broad leaf and parasitic weeds (Pfenning et al., 2008). This technology includes the use of an imidazolinone herbicide (imazamox) in combination with imidazolinone resistant (IMI-R) sunflower hybrids. Imazamox kills weeds by inhibiting acetolactate synthase (ALS), which is the first common enzyme in the synthesis of the branched-chain amino acids leucine, isoleucine and valine in plants (Duggleby and Pang, 2000). Conventional sunflower hybrids are sensitive to imazamox while Clearfield® sunflower hybrids resist application rates of these herbicides that are lethal for other plants. The resistance of Clearfield® hybrids to imidazolinone herbicides is based on a mutation in the *AHAS1* gene that spontaneously developed. It results in site of action resistance of the enzyme ALS. In addition to this gene-based resistance, non-target site mechanisms of resistance were described to be involved in the imidazolinine resistance of the *Imisun* Clearfield® sunflower hybrids (Sala et al., 2012).

The knowledge concerning the detoxification of imazamox in IMI-R plants is still limited and insufficient. Until now, participation in non-target herbicide resistance has only been recognised for P450s, GSTs, glycosyltransferases and ABC transporters (Yuan et al., 2006). Kaspar et al. (2011) have shown a significantly higher resistance of sunflower to imazamox by inhibiting the activity of P450 monooxygenases (P450). More recently Breccia et al. (2017) also suggested the involvement of different P450 isozymes in providing imazapyr resistance to *Imisun* cultivars. In resistant cultivars of beans and wheat, imazamox metabolism involves oxidative hydroxylation of the pyridine ring, and subsequent carbohydrate conjugation (Rojano-Delgado et al., 2014). However, the best-investigated group of plant enzymes implicated in herbicide detoxicification are undoubtedly the GSTs (Riechers et al., 2010). A study of transcript profiles of wild type imidazolinone sensitive (IMI-S) and IMI-R genotypes of *Arabidopsis thaliana* showed that in wild-type plants, the first genes responding to imazapyr application were related to detoxicification such as cytochrome P450s, glycosyl transferases, and ABC-transporters, but also GSTs (Manabe et al., 2007). García-Garijo et al. (2014) also reported enhanced activities of GSTs in imazamox-exposed IMI-R seedlings of *Phaseolus vulgaris* and *Vicia sativa*. In contrast, IMI-S cultivars metabolized the herbicide either slowly or even not at all (Rojano-Delgado et al., 2014). Nonetheless, up till now, it has not been unquestionably confirmed that GSTs are involved in detoxification of imidazolinone herbicides. We assume that glutathione could play a role in imazamox disposal in sunflower plants as an additional biochemical pathway resulting in more effective herbicide detoxification. To test this hypothesis, we examined the redox status of

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https://doi.org/10.1016/j.jplph.2017.12.008
Received 23 October 2017; Received in revised form 1 December 2017; Accepted 4 December 2017
Available online 09 December 2017
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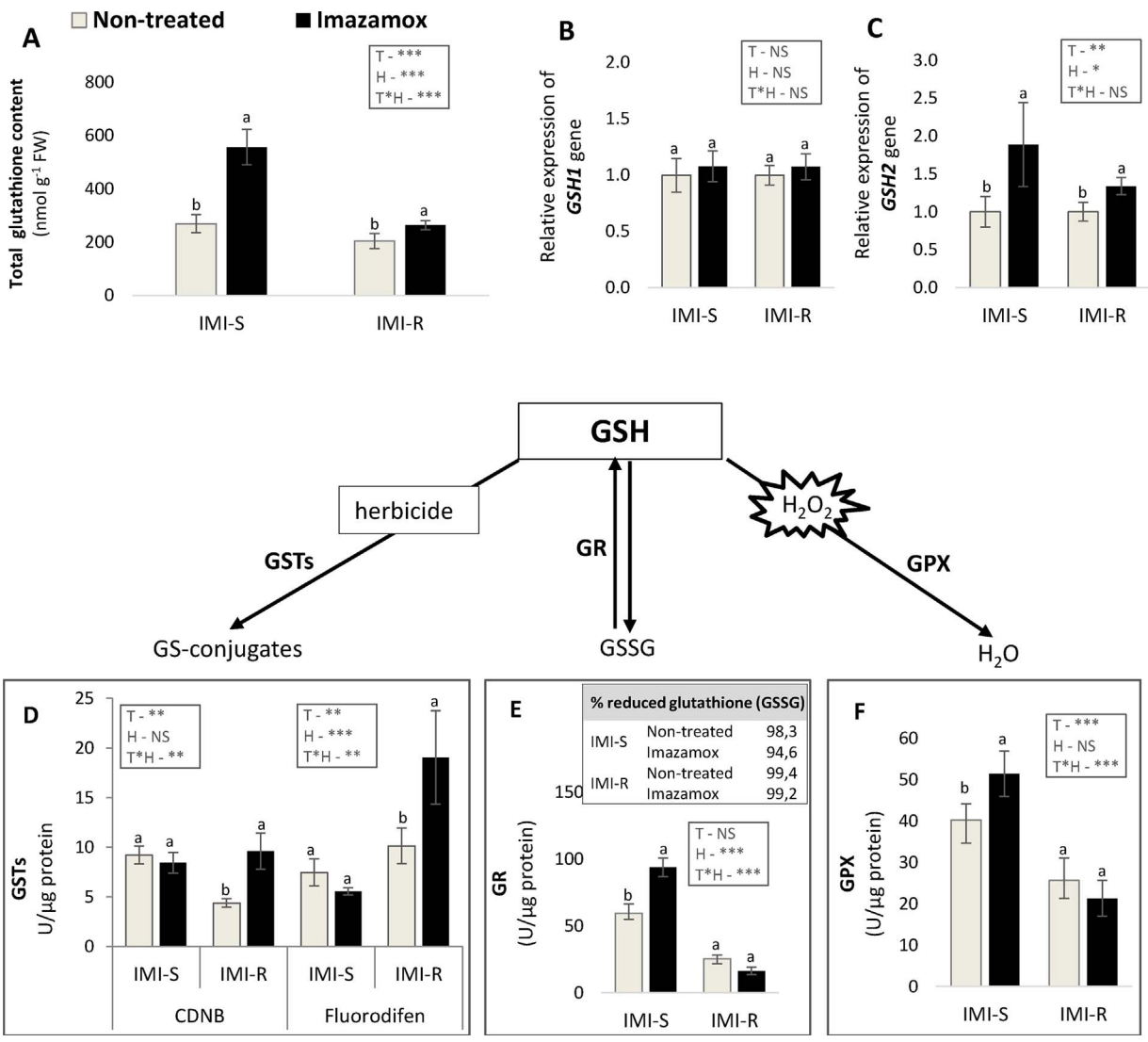

Fig. 1. Overview of the relative gene expressions, enzyme activities and metabolites contents that play a role in either the detoxification of herbicides or the antioxidant defence in leaves of IMI-S and IMI-R sunflower hybrids treated with the herbicide imazamox: **A** – total glutathione (GSH) content; **B** – relative gene expression of GSH1 gene, encoding the enzyme gamma-glutamylcysteine synthetase (EC 6.3.2.2.); **C** – relative gene expression of GSH2 gene, encoding the enzyme glutathione synthetase (EC 6.3.2.3.); **D** – glutathione-S-transferase (GSTs) activity (substrates chlorodinitrobenzene and fluorodifen); **E** – glutathione reductase (GR) activity and percentages of reduced glutathione; **F** – glutathione peroxidase (GPX) activity. Error bars indicates standard deviation (SD). The values represent the mean of three biological replicates. Different letters (a, b) express significant differences between treatments ($P < 0.05$). The small text boxes within each graph show the significance levels of 2-way analysis of variance (ANOVA): * $P = 0.05$, ** $P = 0.01$, *** $P = 0.001$, NS – not significance).

glutathione and the gene expression and activities of glutathione-dependent enzymes in IMI-S and IMI-R sunflower hybrids 24 h after application of imazamax. The aims of our study were (1) to detect the herbicide-induced fast responses in IMI-S and IMI-R sunflower plants as well as (2) to figure out whether the GSTs enzyme family is participating in imazamox detoxification in IMI-R sunflower plants.

2. Materials and methods

2.1. Plant material and treatment

Seeds of the sunflower Clearfield® hybrid Mildimi (IMI-R, *Imisun* trait) and the conventional IMI-sensitive hybrid Albena (IMI-S) were hydroponically grown (4 plants per pot; ½ modified Hoagland nutrient solution) in controlled conditions in a plant growth chamber (photoperiod 14/10 h (light/dark), 250 µmol m^{-2} s^{-1} photosynthetic active radiation (PAR) at leaf level, temperature 24/22 ± 1 °C day/night and 60% relative air humidity. The nutrient solution was continuously aerated. The imazamox was applied at the 2–3 leaf pair stage, by spraying it on the leaves, at a rate of 132 µg per plant, equivalent to 40 g active ingredient ha^{-1} (recommended rate 48 g a i ha^{-1}).

2.2. Determination of glutathione content

The method described by Quevel and Noctor (2007) was used to determine reduced (GSH) and oxidized (GSSG) glutathione. Estimations of GSH and GSSG are based on the glutathione reductase dependent reduction of 5,5-dithiobis(2-nitro-benzoic acid) (DTNB), monitored at 412 nm.

2.3. Determination of enzyme activities

- Glutathione peroxidase (GPX, EC 1.11.1.9) was determined spectrophotometrically according to Dixon et al. (1998). The decrease in absorbance at 340 nm, due to NADPH consumption, was determined ($\varepsilon = 6.22\ mM^{-1}\ cm^{-1}$).
- Glutathione reductase (GR, EC 1.8.1.7) was determined spectrophotometrically according to Zhang and Kirkham (1996), based on the reduction of GSSG, using NADPH. The oxidation of NADPH was measured at 340 nm ($\varepsilon = 6.22\ mM^{-1}\ cm^{-1}$).