Short communication

Effect of Atrazine on Glutathione Levels, Glutathione S-Transferase and Glutathione Reductase Activities in Pea and Wheat Plants

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Abstract

Miteva L.P-E., Ivanov S.V., Alexieva V.S., Karanov E.N. (2004): **Effect of atrazine on glutathione levels, glutathione S-transferase and glutathione reductase activities in pea and wheat plants**. Plant Protect. Sci., **40**: 16–20.

Changes were studied in the endogenous level of glutathione (total and oxidised), and in the amount of free thiol groups as caused by the herbicide atrazine on two species of plants with different sensitivity to it.The activities of two enzymes related to glutathione metabolism (glutathione reductase and glutathione S-transferase) were also determined. The application of the herbicide on leaf increased the levels of total and oxidised glutathione in pea and wheat plants. Increased activity glutathione S-transferase in wheat plants was found.

Keywords: atrazine; pea; wheat; glutathione; glutathione S-transferase; glutathione reductase

For more than 40 years, triazines have been among the most successful selective herbicides used in agriculture; their representative most widely used is atrazine: 2-(ethylamino)-4-chloro-6-isopropylamino-1,3,5-triazine. Like all *s*-triazines, atrazine is an inhibitor of photosystem 2 (PS2). As a result of this inhibition, the excitation energy generated by PS2 cannot be dissipated through the normal electron flow beyond Q_A, and singlet oxygen is generated (Cobb 1992). The application of herbicide concentrations of triazines enhanced the amount of hydrogen peroxide and also increased the amounts of oxidative lipid peroxidation products and ion fluxes in pea plants (Pallett & Dodge 1980; Ivanov *et al*. 2003).

Glutathione (GSH) plays important and well-defined roles in the metabolism and detoxification of numerous pesticides including herbicides that can initiate oxidative stress in plants (Kömives & Gullner 2000). GSH is a component of the glutathione-ascorbate shuttle which is part of the antioxidant system in plants (Foyer *et al*. 2001). Glutathione reductase (GR, EC 1.6.4.2) plays an important role in restoring reduced form of glutathione (Foyer & Halliwell 1976; Lascano *et al*. 2001). Detoxification of different pollutants, including atrazine, by conjugation is another of the glutathione functions (Shimabukuro *et al*. 1970). The process can take place spontaneously or as a result of the activity of the enzyme glutathione S-transferase (GST, EC. 2.5.1.18) (Kömives & Gullner 2000). Beside its function in detoxification, glutathione has an additional protective role in the reduction of cytotoxic hydroperoxides (which arise as a result of oxidative stress) to the respective alcohols (Dixon

et al. 1998). This reduction is catalysed by glutathione peroxidases and GST.

Recently, we reported on the effect of two herbicides – glyphosate and 2,4-dichlorophenoxyacetic acid – with a different mode of action on the endogenous glutathione concentration in pea and wheat plants (Miteva *et al*. 2003a, b). This paper also deals with the changes in the levels of glutathione and free thiol groups, and with the activities of GST and GR after atrazine treatment.

MATERIALS AND METHODS

The experiments were carried out with pea (*Pisum sativum* L., cv. Manuela) and wheat (*Triticum aestivum* L., cv. Sadovo-1) plants, grown as water cultures (Hoagland & Arnon nutrition medium) in growth chambers (photoperiod 12/12 h for pea, and 16/8 h for wheat, photon flux density 70 μmol/m^2/s, and temperature 25 ± 1°C). Plants were treated at 1st (wheat) or 3rd (pea) leaf stage by

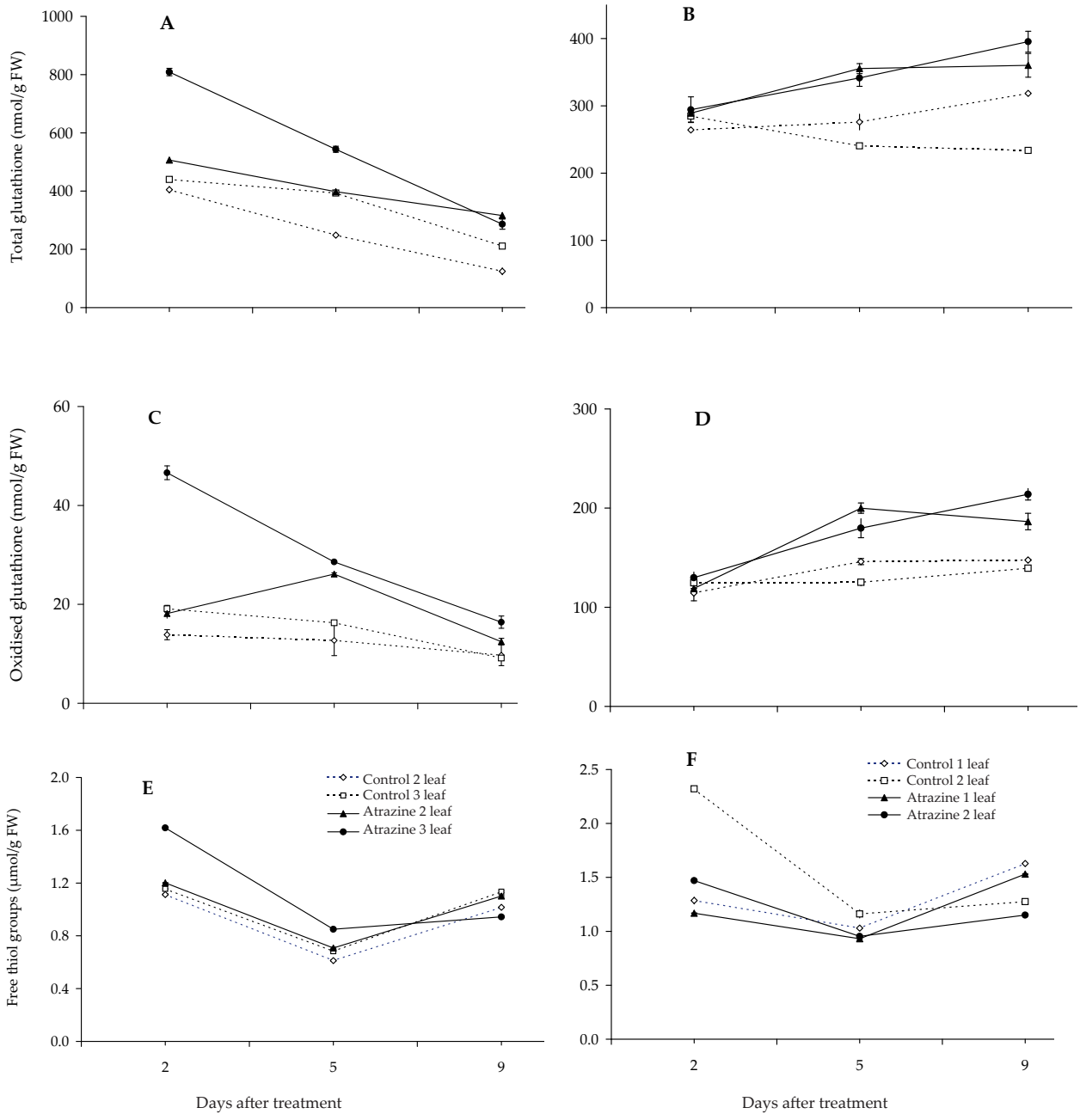

Figure 1. Effect of atrazine on total glutathione content in pea (A) and wheat (B) plants, oxidised glutathione content in pea (C) and wheat (D) plants, and on free thiol groups content in pea (E) and wheat (F)

spraying the leaves with 15mM atrazine. Analyses were carried out on the 2, 5, and 9 days after the treatment, and 1 and 2 leaves of wheat and from 2nd and 3rd leaves of pea.

Endogenous glutathione levels (as total and oxidised form), and the activity of GST were measured spectrophotometrically (Shimazu UV-VIS) detecting conjugation of CDNB (1-chloro-2,4-dinitrobenzene) with glutathione according to Gronwald *et al*. (1987). GR activity was determined by tracking down the reduction of DTNB (5,5′-dithio-bis(2-nitrobenzoic acid)) by the method described by Smith *et al*. (1988). The content of endogenous free SH-groups was evaluated using Elman's reactive according to the modified method of Edreva and Hadjiiska (1984). The results are the means from at least two independent experiments, each of them carried out in three replicates ± S.E.

RESULTS AND DISCUSSION

It was found that compared to the control, atrazine caused a rise of total glutathione levels in pea leaves during the whole experimental period (Figure 1A). Similarly, atrazine increased the content of oxidised glutathione (Figure 1C). On the last day of the stress programme, the GSSG/TG ratio in younger leaves of treated plants was lower than that in the control plants (data not shown). The opposite tendency was found with older leaves, where the GSSG/TG ratio was higher in the control plants. The herbicide treatment did not provoke significant changes in the activity of GST in pea plants (Figure 2A). GR activity (Figure 2C) gradually increased in the treated plants, and the effect was more pronounced in the upper leaf nodes. The absolute values of glutathione levels and enzyme activities during the period studied decreased in both experimental groups, which were better expressed by older leaves (2nd).

In wheat plants, total as well as oxidised glutathione contents in treated plants were higher than in the respective controls during the entire stress programme (Figure 1B). However, in contrast to pea, in wheat plants atrazine caused a rise in the GST activity in 2nd leaf node developed after treatment (Figure 2B). No significant differences in GR activity were observed (Figure 2D).

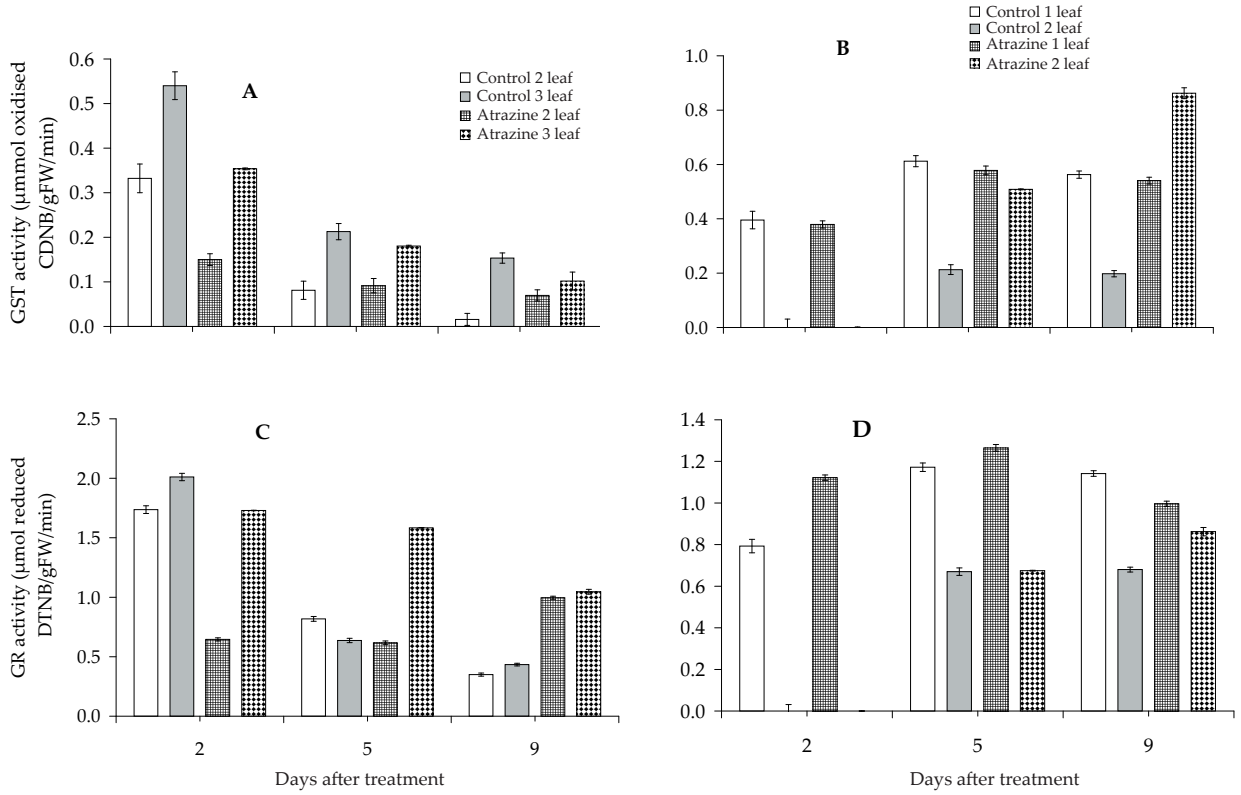

Figure 2. Effect of atrazine on glutathione-S transferase activity in pea (A) and wheat (B) plants and on glutathione reductase activity in pea (C) and wheat (D) plants

Free thiol group contents in treated pea plants were higher than in the control plants, following a tendency similar to that of the total glutathione (Figure 1A and 1E). In contrast, free thiol group contents in treated wheat plants were lower than in the control, which points to a different course of changes compared to total glutathione whose levels in sprayed plants were higher than in the control plants (Figures 1B and 1F). These data are in agreement with the suggestion that in different plant species the pool of total free thiols contains different low-molecular-weight thiols (homoglutathione, cystein, etc.) (Kranner & Grill 1993), which gives us aspects to investigate in future.

It is known that in both pea and wheat the main way for the detoxification of *s*-triazine herbicides is N-dealkylation (Shimabukuro *et al*. 1970). In pea plants, isoenzyme forms of GST detoxifying triazines were not detected (Marrs 1996). In wheat, the treatment with some xenobiotics (atrazine) led to an expression of GST 25 and GST 26, but no direct evidence exists for enzymic conjugation of atrazine and glutathione (Mauch & Dudler 1993).

On the other hand, an increase of the endogenous glutathione concentration and of the activity of enzymes involved in the glutathione-ascorbate cycle under stress conditions provoking the development of oxidative processes were reported by many authors (see the review by Foyer *et al*. 2001). Since the application of PS2 blockers (as triazines) leads to the singlet oxygen formation (Cobb 1992), we suppose that the enhanced glutathione levels in atrazine-treated plants are a consequence of oxidative stimulation of its biosynthesis. The increased GST activity observed in wheat plants was probably due to the transcription of an isoenzyme form catalysing the reduction of lipid peroxides.

Further investigations on the whole glutathione-ascorbate cycle will provide a better understanding of the role of these metabolites in the response of plants to the influence of herbicides with different modes of action.

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Souhrn

Miteva L.P-E., Ivanov S.V., Alexieva V.S., Karanov E.N. (2004): **Vliv atrazinu na hladinu glutathionu a na aktivitu glutathion S-transferasy a glutathion-reduktasy v rostlinách hrachu a pšenice**. Plant Protect. Sci., **40:** 16–20.

U dvou druhů rostlin, pšenice a hrachu, byly sledovány změny hladiny glutathionu (celkového a oxidovaného) a obsahu volných thiolových skupin, vyvolané použitím herbicidu atrazinu. Byly rovněž stanoveny aktivity některých enzymů účastných na metabolismu glutathionu (glutathion S-transferasy a glutathion-reduktasy). Aplikace herbicidu na list způsobila zvýšení hladiny celkového i oxidovaného glutathionu jak u pšenice, tak i u hrachu. V rostlinách pšenice byla současně zjištěna vyšší aktivita glutathion S-transferasy.

Klíčová slova: atrazin; hrách; pšenice; glutathion; glutathion S-transferasa; glutathion-reduktasa

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