

Selective Induction of Glutathione S-Transferase Subunits in Wheat Plants Exposed to the Herbicide Acifluorfen

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Exposure to the herbicide acifluorfen resulted in marked increase of glutathione S-transferase (GST) enzyme activity in wheat seedlings, primarily in shoot tissues. From the six major, constitutively expressed GST subunits found in untreated wheat shoots subunits 2 and 3 were selectively induced by acifluorfen. No new subunit could be detected. The induced subunits belong to those GST isoenzymes, which metabolize diphenyl ether herbicides.

Introduction

The glutathione S-transferase (GST, E. C. 2.5.1.18.) isoenzyme family has well-defined roles in detoxification reactions in plants (Marrs, 1996; Dixon *et al.*, 1998; Sommer and Böger, 1999). These enzymes are homo- or heterodimeric combinations of different subunits. GST isoenzymes catalyze the conjugation of glutathione (GSH) to various xenobiotics (including numerous herbicides) and their electrophilic metabolites to produce less toxic and more water-soluble thioether conjugates. They are also capable of catalyzing the breakdown of toxic lipid hydroperoxides and thereby contribute to the protection of plants against oxidative membrane damage (Marrs, 1996). The nitrodiphenyl ether herbicide acifluorfen (5-[2-chloro-4-(trifluoromethyl)-phenoxy]-2-nitrobenzoic acid) is known to induce the accumulation of protoporphyrin IX and other tetrapyrrole derivatives in plant leaves (Matringe and Scalla, 1988; Sandmann and Böger, 1988). In the presence of light and molecular oxygen, the accumulation of protoporphyrin IX results in the increased production of reactive oxygen species, photooxidative membrane damage and, ultimately, cell death. Acifluorfen and other nitrodiphenyl ether herbicides are metabolized *via* GSH-conjugation in plants, and their selective action is primarily due to differences in herbicide detoxification rates (Kömives and Gullner, 1994). Exposure to acifluorfen and to the structurally related herbicide ox-

yfluorfen led to considerable GST induction in dicotyledonous plants (Gullner *et al.*, 1991; Knörzer *et al.*, 1996), but no data have been reported about their effects on the monocotyledonous wheat. Recently the total subunit complement of wheat GSTs has been characterized (Pascal *et al.*, 1998), and the inducibility of GST subunits by a herbicide safener has been reported (Pascal and Scalla, 1999). In the present study we have investigated the induction of various GST subunits in wheat seedlings exposed to acifluorfen.

Materials and Methods

Seeds of wheat plants (cv. Courtot) were sown in quartz sand. Seedlings were cultivated in a growth chamber set at 25/20 °C (day/night) and 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. They were supplied with one-fifth strength Hoagland solution. Five-day-old seedlings were treated with 50 μM acifluorfen dissolved in the nutrient solution. At one-day intervals, tissues were frozen in liquid nitrogen and the soluble proteins extracted as previously described (Pascal and Scalla, 1999). GST activities were measured in 16500 \times g supernatants of shoot and root tissues extracts, by using 1-chloro-2,4-dinitrobenzene as substrate (Pascal *et al.*, 1998). The total GST complement was purified from shoots by affinity chromatography on GSH-agarose gel and the subunit composition was investigated by reversed-phase HPLC as described earlier (Pascal and Scalla, 1999). Protein contents of plant ex-



tracts were measured by the method of Bradford (1976) using bovine serum albumin (BSA) as a standard. The total amount of chlorophylls was measured in 80% acetone extracts following the equation used by Arnon (1949).

Results and Discussion

Exposure of wheat seedlings to 50 μM acifluorfen in the light resulted in an immediate inhibition of growth. Fresh weights of shoots increased by 126% in five days for control seedlings, and only by 43% for acifluorfen-treated seedlings. During the same period, root fresh weight increased by 45% for control plants, and only by 3% for acifluorfen-treated plants. After 2 days of treatment, the fresh weights of shoots and roots of the herbicide-treated plants amounted to $68.8 \pm 3.0\%$ and $85.6 \pm 11.8\%$ of the controls, respectively. Acifluorfen treatment also induced photobleaching in shoot tissues. Bleaching was first visible 2 days after treatment, and concerned especially the top half of the primary leaves at day 5. In control shoots, the chlorophyll content slightly increased from day 0 to day 5 (from 1.19 to 1.44 mg per g fresh weight). In acifluorfen-treated shoots, it decreased during that period from 1.19 to 0.807 mg per g fresh weight, and it amounted to 86.2% of the controls after 2 days of treatment.

At the same time as these phytotoxic effects, acifluorfen treatments led to the marked induction of GST activities, particularly in shoots. The GST activity in acifluorfen-treated shoots was 2.8-fold higher than in controls two days after the herbicide treatment (Fig. 1). This increase persisted at least until 5 days following exposure, since at that time the activity in treated shoots was 2.9 times higher than in controls. GST activity was induced also in the roots, but to a much lesser extent than in the shoots.

To characterize the effect of acifluorfen on wheat GST enzymes, the total GST content was extracted from shoot tissues, purified by affinity chromatography and the GST subunit composition was investigated by reversed-phase HPLC (Fig. 2). In accordance with earlier studies (Pascal and Scalla, 1999), at least six major GST subunits, numbered 2, 3, 4, 5, 8 and 9, were found in the extracts of untreated shoots. Their molecular masses ranged between 23 140 and 24 958 Da (Pas-

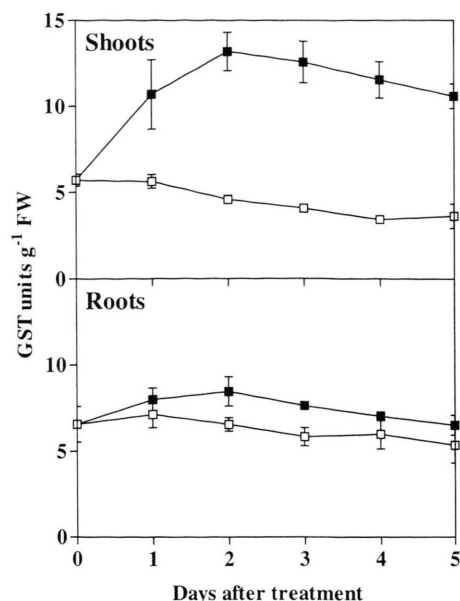


Fig. 1. Induction of glutathione S-transferase activity in the shoots and roots of wheat seedlings exposed to 50 μM acifluorfen. Enzymatic activities were estimated in 16500 \times g supernatants of tissue extracts. Data are mean values \pm SD of 4 replicates. □ : untreated seedlings; ■ : seedlings exposed to acifluorfen. FW, fresh weight.

cal and Scalla, 1999). Acifluorfen treatment (50 μM) did not result in the appearance of any new subunits. However, the levels of the major subunits 2 and 3 were selectively increased, and 2 days after treatment their peak areas were 3.2- and 3.9-fold higher than in untreated controls, respectively (Fig. 2). Several unidentified minor peaks eluting at 34–37 mins were also more abundant in acifluorfen-treated samples, but they have not been characterized. The levels of the other major subunits were unchanged or only slightly modified by acifluorfen.

Limited information is available about GST isoenzymes in wheat (Cummins *et al.*, 1997; Pascal and Scalla, 1999). Pathogen attack was shown to induce the transcription of a gene coding for a 29 kDa GST subunit. Two more GST subunits of apparent molecular masses of 25 and 26 kDa were also identified, which were inducible by heavy metals and herbicides (Mauch and Dudler, 1993). Earlier studies showed that subunits 2 and 3 observed in our experiments possess molecular masses of 24 924 and 24 958 Da, respectively (Pascal and Scalla, 1999). In addition, the N-terminal

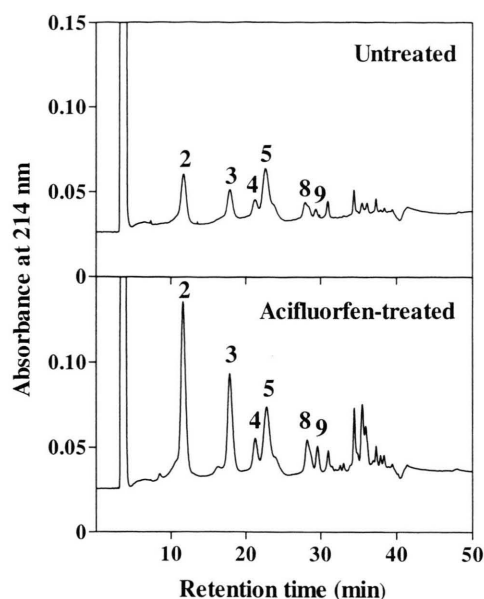


Fig. 2. Reversed-phase HPLC analysis of glutathione S-transferase preparations from wheat shoots after purification by affinity chromatography. Herbicide-treated seedlings were exposed to 50 μ M acifluorfen for 2 days. All samples were from 1 g fresh weight shoot tissues. The GST subunit peaks are labelled according to their elution order. Representative elutions chosen from several experiments are shown.

amino acid sequence of wheat subunit 2 was shown to be very close to that of maize GST 27, which is inducible by chemicals (Pascal *et al.*, 1998). A GST isoenzyme consisting of subunit 2 and 3 purified from wheat shoot was able to metabolize the diphenyl ether herbicide fluorodifen, but did not exhibit any glutathione peroxidase activity (Pascal and Scalla, 1999). These results suggest that the wheat GST subunits 2 and 3 induced by acifluorfen treatment could participate rather in the detoxification of acifluorfen than in the protection against the oxidative stress caused by the herbicide.

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- Arnon D. I. (1949), Copper enzymes in isolated chloroplasts. *Plant Physiol.* **24**, 1–15.
- Bradford M. M. (1976), A rapid and sensitive method for the quantitation of microgram quantities of protein using the principles of protein dye-binding. *Anal. Biochem.* **72**, 248–254.
- Cummins I., Cole D. J. and Edwards R. (1997), Purification of multiple glutathione transferases involved in herbicide detoxification from wheat (*Triticum aestivum* L.) treated with the safener fenchlorazole-ethyl. *Pestic. Biochem. Physiol.* **59**, 35–49.
- Dixon D. P., Cole D. J. and Edwards R. (1998), Purification, regulation and cloning of a glutathione transferase (GST) from maize resembling the auxin-inducible type-III GSTs. *Plant Mol. Biol.* **36**, 75–87.
- Gullner G., Kömives T. and Király L. (1991), Enhanced inducibility of antioxidant systems in a *Nicotiana tabacum* L. biotype results in acifluorfen resistance. *Z. Naturforsch.* **46c**, 875–881.
- Knörzer O. C., Durner J. and Böger P. (1996), Alterations in the antioxidative system of suspension-cultured soybean cells (*Glycine max*) induced by oxidative stress. *Physiol. Plant.* **97**, 388–396.
- Kömives T. and Gullner G. (1994), Mechanisms of plant tolerance to photodynamic herbicides. In: *Porphyrin Pesticides: Chemistry, Toxicology, and Pharmaceutical Applications* (Duke S. O. and Rebeiz C. A., eds.). ACS Symp. Ser. 559, Amer. Chem. Soc., Washington DC, pp. 177–190.
- Marrs K. A. (1996), The functions and regulation of glutathione S-transferases in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 127–158.
- Matringe M. and Scalla R. (1988), Studies on the mode of action of acifluorfen-methyl in nonchlorophyllous soybean cells. *Plant Physiol.* **86**, 619–622.
- Mauch F. and Dudler R. (1993), Differential induction of distinct glutathione-S-transferases of wheat by xenobiotics and by pathogen attack. *Plant Physiol.* **102**, 1193–1201.
- Pascal S., Debrauwer L., Ferte M.-P., Anglade P., Rouimi P. and Scalla R. (1998), Analysis and characterization of glutathione S-transferase subunits from wheat (*Triticum aestivum* L.). *Plant Sci.* **134**, 217–226.
- Pascal S. and Scalla R. (1999), Purification and characterization of a safener-induced glutathione S-transferase from wheat (*Triticum aestivum*). *Physiol. Plant.* **106**, 17–27.
- Sandmann G. and Böger P. (1988), Accumulation of protoporphyrin IX in the presence of peroxidizing herbicides. *Z. Naturforsch.* **43c**, 699–704.
- Sommer A. and Böger P. (1999), Characterization of recombinant corn glutathione S-transferase isoforms I, II, III, and IV. *Pestic. Biochem. Physiol.* **63**, 127–138.