Glutathione S-transferase activity in acetochlor, atrazine and oxyfluorfen metabolization in maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) and wheat (*Triticum aestivum* L.) (Poaceae)

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ABSTRACT. This experiment was conducted to evaluate the acetochlor, atrazine and oxyfluorfen herbicides plant selectivity, in relation to glutathione S-transferase activity (GST) in maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) and wheat (*Triticum aestivum* L) (Poaceae) plants. GST activity was detected 24, 48 and 72 hours after treatment applications. The experiment's treatments consisted of spraying plants with water (control), acetochlor (3 $L.ha^{-1}$)`, atrazine (4 $L.ha^{-1}$) and oxyfluorfen (1 $L.ha^{-1}$). The highest GST activities were observed in presence of acetochlor, mainly at 48 hours after treatment. These increments were 105, 148 and 118% when compared to maize, sorghum and wheat control groups, respectively. It is suggested that the GST may have a role in acetochlor degradation and it may be a reason for this herbicide's selectivity in these crops.

Key words: glutathione S-transferase, atrazine, acetochlor, oxyfluorfen, herbicides, selectivity.

RESUMO. Atividade de glutationa S-transferase na metabolização de acetochlor, atrazine e oxyfluorfen em milho (*Zea mays* L.), sorgo (*Sorghum bicolor* L.) e trigo (*Triticum aestivum* L.) (Poaceae). Este experimento foi conduzido para avaliar a seletividade em plantas dos herbicidas acetochlor, atrazine e oxyfluorfen em relação à atividade da glutationa S-transferase (GST) em plantas de milho (*Zea mays* L.), sorgo (*Sorghum bicolor* L.) e trigo (*Triticum aestivum* L.) (Poaceae). A atividade da GST foi detectada às 24, 48 e 72 horas após as aplicações dos tratamentos. Os tratamentos do experimento consistiram de aplicação com água (controle), acetochlor (3 $L.ha^{-1}$), atrazine (4 $L.ha^{-1}$) e oxyfluorfen (1 $L.ha^{-1}$). As maiores atividades de GST foram observadas na presença de acetochlor, principalmente às 48 horas após o tratamento. Esses aumentos foram 105, 148 e 118% em relação ao controle para milho, sorgo e trigo, respectivamente. É sugerido que a GST pode ter papel na degradação de acetochlor e pode ser uma das razões para a seletividade desse herbicida para essas culturas.

Palavras-chave: glutationa S-transferase, atrazine, acetochlor, oxyfluorfen, herbicidas, seletividade.

Introduction

Glutathione S-transferases (GSTs, EC 2.5.1.18) are very often thought as detoxification enzymes and, indeed, they were first discovered for their ability to metabolize a wide variety of toxic exogenous compounds (xenobiotics), via glutathione conjugation (Mannervik and Danielson, 1988). The glutathione level is known to correlate with plants tolerance of xenobiotics (May *et al*., 1998). This enzyme catalyzes the tripeptide glutathione (GSH, γ-glutamyl-cysteinyl glycine) conjugation to a variety of hydrophobic, electrophylic, and usually cytotoxic substrates (Daniel, 1993; Wilce and Parker, 1994), including herbicides and other xenobiotics (Timmerman, 1989; Fuerst *et al*., 1993; Irzyk and Fuerst, 1993; Jepson *et al.,* 1994). The addition of GSH, via cysteine thiol group, to an electrophylic site of a xenobiotic substrate, produces water-soluble conjugates with greatly reduce toxicity (Timmerman, 1989; Dean *et al.,* 1990; Fuerst *et al*., 1993; Irzyk and Fuerst, 1993; Jepson *et al*., 1994). This mechanism is often a key step in xenobiotics detoxification and elimination from the cytoplasm (Kreuz *et al*., 1996; Reinemer *et al*., 1996).

The first known function for plant glutathione S-transferases was in the herbicide metabolism to nontoxic forms (Frear and Swanson, 1970, Shimabukuro *et al*., 1970, 1971). There are many documented cases where the herbicide-GSH conjugate formation in the resistant (but not in the susceptible) species is responsible for herbicide selectivity (Lay and Casida, 1976; Komvies *et al*., 1985; Fuerst and Gronwald, 1986; O'Connell *et al*., 1988). It has been reported that chloroacetanilide herbicides are metabolized more readily in tolerant plants through the formation of predominantly water-soluble metabolites, which are mainly conjugates with glutathione or homoglutathione by glutathione S-transferases enzymes, or catabolites of such conjugates (Breaux, 1987).

The present study aims to evaluate the effect of the herbicides acetochlor, atrazine and oxyfluorfen on GST activity in maize, sorghum and wheat seedlings (Poaceae).

Material and methods

Plants and chemicals

Maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) and wheat (*Triticum aestivum* L.) were used in this work. All reagents used in this investigation were purchased from Sigma Chemical Co (USA).

Cultivation of plants and herbicide treatments

Fifteen seeds were planted 1.5cm deep in plastic trays containing 3 liters of soil. The trays were kept at 25^0C in a greenhouse, and watered when necessary. After fourteen days, oxyfluorfen (one liter per hectare), acetochlor (three liters per hectare) and atrazine (four liters per hectare) were sprayed on these crops with a backpack sprayer, with one dg 110.02 nozzle and 35 psi pressure, delivering 200 liters per hectare. The seedlings were harvested 24, 48 and 72 hours later.

GST extraction and assay

Samples of treated and control seedlings shoots tissues were rinsed with distilled water and dried by blotting with filter paper. The shoot segments (1 g fresh weight) were homogenized for enzyme extraction with a pestle in ice-cold mortar, with a small amount of quartz sand on ice, in 5ml of 50 mM Tris-HCl buffer, pH 7.0, containing 20% (v/v) glycerol, 1 mM ascorbic acid, 1 mM dithiothreitol, 1 mM EDTA, 1 mM reduced glutathione (GSH) and 5 mM $MgCl_2$, including 1% (w/v) polyvinylpyrrolidone (PVP). After two centrifugation steps (6 min. at 12,000 g and 16 min. at 26,000 g) at 4^0C, the supernatant was used as crude extract for GST activity determination (Knörzer *et al.*, 1996). GST activity was determined according to Wu *et al.* method (1996), using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The 3 mL reaction mixture contained 30 µL of the enzyme extract, 2 ml of 100 mM potassium phosphate buffer (pH 6.9), 0.9 ml of 3.3 mM GSH and 100 µL of 30 mM CDNB in 96% alcohol. The mixture was incubated at room temperature (25^0C) for 60 min. The reaction was started by the addition of CDNB and the change in absorbance, due to GSH-CDNB conjugate formation in time ratio, was spectrophotometrically measured at 340mm. A molar extinction coefficient of 9.6 mM cm^{-1} (Habig and Jakoby, 1981) was used to calculate enzyme activity, which was corrected for nonenzymatic conjugation. The rate of nonenzymatic conjugation was determined by using the same reaction mixture without the crude plant extract. The GST specific activity was expressed as nmol min^{-1} per mg protein. Protein concentrations in the enzymatic extracts were determined by Lowry *et al*. method (1951), using bovine serum albumin as standard.

Statistical analysis

The difference significance was tested through variance analysis and Student's t multiple test to compare each treatment group with the control groups and with the others ($p \leq 0.05$). Values are presented as ± SEM means.

Results and discussion

Table 1 describes the GST activity in maize seedlings submitted to a treatment with atrazine, acetochlor and oxyfluorfen. The GST activity was significantly higher in presence of acetochlor, obtaining a 105% increase 48 hours after the treatment application. Atrazine induced 31 and 38% GST activity elevation, 24 and 48 hours after herbicide application, respectively, while oxyfluorfen caused a 57% GST activity increment, but only at 48 hours. These results are consistent with Jablonkai and Hatzios (1991) findings, who evaluated the GST activity role in maize and wheat response to acetochlor, a chloroacetanilide herbicide. These authors demonstrated the important role of GST activity endogenous levels in chloroacetanilide herbicide detoxification and selectivity. The maize resistance to atrazine has been proved caused by the activity of a soluble GST, efficient at detoxifying S-triazines (Shimabukuro *et al*., 1970). Sommer and Böger (1999) identified several maize GSTs isoforms for their ability to conjugate herbicides with reduced glutathione, and some GST isoforms

may be involved in the defense response to oxidative stress in plants.

Table 1. Glutathione S-transferase activity (nmol min^{-1} per mg protein) in shoots of maize (*Zea mays* L.) seedlings harvested 24, 48 and 72 hours after treatment with the herbicides atrazine, acetochlor and oxyfluorfen (3, 1 and 4 liters/hectare, respectively)

Treatment (hours)	Herbicides			
	Control	Atrazine	Acetochlor	Oxyfluorfen
24	13.43±1.45 ^{B}	17.66±1.74 ^{A} ⋆(131%)	18.45±1.58 ^{A} ⋆(137%)	15.02±1.68 ^{B} ⋆(112%)
48	14.81±1.94 ^{C}	20.41±2.01 ^{B} ⋆(138%)	30.37±1.78 ^{A} ⋆(205%)	23.33±1.87 ^{B} ⋆(157%)
72	13.31±1.47 ^{B}	15.96±1.75 ^{B} ⋆(120%)	20.03±1.95 ^{A} ⋆(150%)	13.09±1.65 ^{B} ⋆(98%)

Values correspond to the average of five separate determinations±standard deviation. Means followed by the same letter (horizontal) are not significantly at the 0.05 level according to the ANOVA-test; ⋆(percentage regarding to control)

In comparison with the control plants, a GST activity 148% increment was observed in sorghum seedlings (Table 2), 48 hours after acetochlor treatment. GST activity was increased in wheat (Table 3) with acetochlor (increment of 118%) and atrazine (increment of 59%), 48 hours after treatment. An increase of enzyme activity was observed 72 hours after the application of the three herbicides: 95%, 78% and 42% increment for atrazine, acetochlor and oxyfluorfen, respectively. It has been reported that closely-related *p*-nitrodiphenyl ether herbicide acifluorfen, as well as other diphenyl ethers, is decomposed in higher plants via conjugation with glutathione by GST (Frear *et al*., 1983; Lamoureux *et al*., 1991).

Table 2. Glutathione S-transferase activity (nmol min^{-1} per mg protein) in shoots of sorghum (*Sorghum bicolor* L.) seedlings harvested 24, 48 and 72 hours after treatment with the herbicides atrazine, acetochlor and oxyfluorfen (3, 1 and 4 liters/hectare, respectively).

Treatment (hours)	Herbicides			
	Control	Atrazine	Acetochlor	Oxyfluorfen
24	9.62±1.75 ^{AB}	7.41±1,68 ^{B} ⋆(77%)	11.14±1.59 ^{AB} ⋆(116%)	13.07±1.74 ^{A}⋆(136%)
48	10.01±1.91 ^{B}	12.92±1.44 ^{B} ⋆(129%)	24.78±2.13 ^{A} ⋆(248%)	12.32±1.89 ^{B}⋆(123%)
72	9.15±1.65 ^{A}	11.84±1.72 ^{A} ⋆(129%)	9.68±1.58 ^{A} ⋆(106%)	9.87±1.62 ^{A}⋆(108%)

Values correspond to the average of five separate determinations±standard deviation. Means followed by the same letter (horizontal) are not significantly at the 0.05 level according to the ANOVA-test; ⋆(percentage regarding to control)

The principal basis of the herbicides practical application is their plant selectivity. A given plant's inherent ability to detoxify a given herbicide has long been established as a major factor determining the selective action of most herbicides. Glutathione S-transferase are key metabolic enzymes involved in the detoxification of several herbicides in plants (Timmerman, 1989; Fuerst *et al*., 1993; Irzyk and Fuerst, 1993; Jepson *et al*., 1994).

Table 3. Glutathione S-transferase activity (nmol min^{-1} per mg protein)in shoots of wheat (*Triticum aestivum* L.) seedlings harvested 24, 48 and 72 hours after treatment with the herbicides atrazine, acetochlor and oxyfluorfen (3, 1 and 4 liters/hectare, respectively).

Treatment (hours)	Herbicides			
	Control	Atrazine	Acetochlor	Oxyfluorfen
24	10.93±1.69 ^{A}	10.67±1.84 ^{A} ⋆(98%)	11.21±1.74 ^{A} ⋆(103%)	9.92±1.91 ^{A}⋆(91%)
48	8.42±1.79 ^{C}	13.42±1.65 ^{B} ⋆(159%)	18.36±1.91 ^{A} ⋆(218%)	9.35±1.74 ^{C}⋆(111%)
72	8.62±1.61 ^{C}	16.85±2.01 ^{A} ⋆(195%)	15.34±1.75 ^{A} ⋆(178%)	12.21±1.89 ^{B}⋆(142%)

Values correspond to the average of five separate determinations±standard deviation. Means followed by the same letter (horizontal) are not significantly at the 0.05 level according to the ANOVA-test; ⋆(percentage regarding to control)

It has been shown that the maize tolerance to atrazine is due to the activity of a soluble GST, efficient at detoxifying S-triazines (Shimabukuro *et al*., 1970; 1971). Gronwald and Plaisance (1998) purified the glutathione S-transferase from sorghum shoots, which exhibited GST activity with 1-chloro-2,4-dinitrobenzene but little or no activity with metolachlor, a chloroacetanilide herbicide. It is well established that maize and sorghum tolerance to atrazine is due to the GSH and GST (atrazine) high levels in these species, which facilitates the herbicide detoxification via GSH conjugation. In maize, GST (atrazine) activity is constitutively expressed in leaf and stem tissues (Ezra and Stephenson, 1985; Timmerman, 1989).

In higher plants, GSTs have been studied from the resistance to herbicides viewpoint, and GSTs that metabolize various herbicides have been identified (Dean *et al.,* 1990; 1991). In fact, increased glutathione (GSH) levels and GST activities were found in different plants exposed to a wide range of stress effects, including exposure to herbicides. GSH and GST have well-defined roles in the metabolism of several herbicides. Following conjugation with GSH, herbicides are exported into the vacuole by means of an ATP-dependent pump in barley (Martinoia *et al*., 1993). Crude extracts of pea seedling showed high activity toward a range of GST substrates including 1-chloro-2,4-dinitrobenzene, but low activities toward herbicides chloroacetanilides and atrazine (Edwards, 1996). The conjugation of glutathione with 1-chloro-2,4-dinitrobenzene is widely used for detecting GST activity, but it may not detect all GST isozymes.

The data presented in this work describe the 1-chloro-2,4-dinitrobenzene conjugation activity elevation by acetochlor, atrazine and oxyfluorfen.

These data were more evident in all seedlings submitted to a treatment with acetochlor, indicating higher plant protection against acetochlor than atrazine and oxyfluorfen. In conclusion, the data presented here suggest that the enhanced GST activity may have a role in acetochlor degradation in maize, sorghum and wheat.

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