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GÜLSEREN COŞKUN

FİGEN ZİHNİOĞLU

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Effect of Some Biocides on Glutathione-s-transferase in Barley, Wheat, Lentil and Chickpea Plants

Gülseren COŞKUN, Figen ZİHNİOĞLU
Ege University, Faculty of Science, Department of Biochemistry, 35100 Bornova-İzmir - TURKEY

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Abstract: The conjugation of glutathione with herbicides and insecticides is considered to be one of the major detoxification mechanisms of plants. This conjugation is catalysed by a family of multifunctional proteins known as glutathione-s-transferases. Treatment of wheat (*Triticum aestivum* L. cv. Cumhuriyet-75), barley (*Hordeum vulgare* L. cv. Kaya), chickpea (*Cicer arietinum* L. cv. Ispanyol), and lentil (*Lens culinaris* Medik. cv. Kışlık-kırmızı-51) with the selective herbicides 2,4-D (2,4-Dichloro phenoxyacetic acid) and linuron (N-(3,4-dichlorophenyl)-N´-methoxy-N´-methyl urea), and insecticides parathion methyl (O,O-dimethyl O-(4-nitrophenyl) phosphorothioate) and malathion (S-1,2-bis(ethoxycarbonyl)ethyl O,O-dimethyl phosphorodithioate) caused a progressive increase in glutathione-s-transferase activity in roots and shoots.

The maximum increase in enzyme activity was observed in parathion methyl treated wheat roots: 485% of control with a parathion methyl concentration of 1.37 mM. A similar but lower increase in glutathione (GSH) levels was observed for roots and shoots, differing according to the origin and the type of pesticide.

Key Words: Glutathione-s-transferase, detoxification, pesticide

Arpa, Buğday, Mercimek ve Nohut Bitkilerindeki Glutation-s-transferaz Üzerine Bazı Biosidlerin Etkisi

Özet: Glutationun herbisitler ve insektisitlerle konjugasyonu, bitkilerin detoksifikasyon mekanizmalarının en önemlilerinden biridir. Bu konjugasyon, glutation-s-transferazlar olarak bilinen multifonksiyonel bir enzim ailesi tarafından katalizlenir. Buğday (*Triticum aestivum* L.cv.Cumhuriyet-75), arpa (*Hordeum vulgare* L.cv.Kaya), nohut (*Cicer arietinum* L.cv.İspanyol) ve mercimeğin (*Lens culinaris* Medik.cv.Kışlık-kırmızı-51) seçilen herbisitler; 2,4-D (2,4-dikloro fenoksiasetik asit), linuron (N-(3,4-diklorofenil)-N´-metoksi-N´-metil üre) ve insektisitler; paratiyon metil (O,O-dimetil O-(4-nitrofenil)fosforotiyoat), malatiyon (S-1,2-bis(etoksibikarbonil)etil O,O-dimetil fosforoditiyoat) ile muamelesi, köklerde ve filizlerdeki glutation-s-transferaz aktivitesinde yükselen bir artışa sebep olduğu görülmüştür.

Enzim aktivitesinde maksimum artış, 1,37 mM paratiyon metille muamele edilmiş buğday köklerinde kontrolün %485'i olarak gözlenmiştir. Benzer, fakat daha düşük bir artış, bitkilerin kök ve filizleri için, orijine ve pestisit türüne göre farklanarak glutation (GSH) düzeylerinde gözlenmiştir.

Anahtar Sözcükler: Glutation-s-transferaz, detoksifikasyon, pestisit

Introduction

The use of biocides is a well established necessity of modern agriculture. Extensive biochemical research on the development of plant resistance to several pesticides has ascertained the involvement of some major mechanisms [1,2]. The most general is probably metabolic detoxification, including the formation of conjugates with glutathione (γ-glutamyl-cysteinyl-glycine). The glutathione-glutathione-s-transferase (GSH-GST) system is widespread in nature. Glutathione-s-transferases (GSTs, EC 2.5.1.18) are enzymes that detoxify endobiotic and xenobiotic compounds by covalently linking glutathione to a hydrophobic substrate, forming a less reactive and more polar glutathione-s-conjugate [3,4]. In contrast to animals, where the conjugates are catabolized and excreted, plants have to store the soluble glutathione-s-conjugates in the vacuole, because of the lack of excretion pathways.

GSTs have been extensively studied in mammalian tissues [5-7]. As GSTs play a role in the cellular detoxification metabolism and the development of resistance towards carcinogens, drugs and pesticides in

different organisms, the isozyme multiplicity and physiological role of different GSTs have been studied in great detail. In plants, GSTs are well established as one of the major detoxification, selectivity factors in plants and environmental safety. These enzymes have been characterized from a number of plants [8,9] and their role in the protection against pesticides has been studied. However, the structural and functional relationship among these plant GSTs is still not as still well known as that among GSTs from higher organisms. Plant GSTs not only inactivate toxic compounds through conjugation, but also exhibit a number of additional functions, playing an important role in the plant's defence system. GSTs are involved in the inactivation of cytotoxic plant metabolites and in stress responses induced by pathogenic attack, oxidative stress and phytohormone treatment. In addition to their enzymatic function, some GSTs can also serve as ligand-binding proteins (Ligandin function), thus facilitating the intracellular transport and storage of hydrophobic non-substrate compounds, such as metabolites, drugs and hormones.

The activity of glutathione-s-transferases has been shown to increase in some plants in response to certain herbicides and herbicide antidote treatments [10].

2,4-D (2,4-dichloro phenoxyacetic acid) and linuron (N- (3,4-dichlorophenyl)-N'-methoxy-N'-methyl urea) are selective herbicides for controlling broad leaf weeds. On the other hand, parathion methyl (O, O-dimethyl O- (4-nitrophenyl) phosphorothioate) and malathion (S-l,2-bis(ethoxycarbonyl)ethyl O,O-dimethyl phosphoro-dithioate) are organophosphate insecticides which are also known as cholinesterase inhibitors [11]. Studies on the enzymatic detoxification of these four pesticides are limited.

The objective of this study was to determine the effect of various pesticide treatments (2,4-D, linuron, malathion and parathion methyl) on glutathione-s-transferase activity in wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), chickpea (*Cicer arietinum* L.), and lentil (*Lens culinaris* Medik.).

Materials and Methods

Chemicals

2,4-D (2,4-dichloro phenoxyacetic acid) and malathion (S-l,2-bis (ethoxycarbonyl) ethyl O,O-dimethyl phosphorodithioate) were provided by Hektas (Kocaeli, Turkey). Parathion methyl (O, O-dimethyl O- (4-nitrophenyl) phosphorothioate) was from Bayer (Leverkusen, Germany). Linuron (N- (3,4-dichlorophenyl)-N'-methoxy-N'-methyl urea) was obtained from AgrEvo (Berlin, Germany) (Figure 1). GSH, l-chloro-2,4-dinitrobenzene (CDNB), Bovine Serum Albumin and Ellmann's reagent (5,5'-dithiobis (2-nitrobenzoic acid)) were purchased from Sigma Chem. Co. (St Louis, MO). All other chemicals and reagents used were obtained from various commercial sources.

Plant Material

Plant seeds were washed and immersed in tap water for two hours, and then planted 1.5 cm deep in plastic trays containing moist cotton cloth and watered with distilled water in control treatments and with pesticides in other treatments. The pesticide treatment was applied to seeds: 2,4-D, 2 mM; linuron, 2 mM; malathion 0.73 mM; parathion methyl 1.37 mM. All the trays were covered with cellophane bags so as to create a closed system and the seeds were grown in growth chambers at 25°C. The

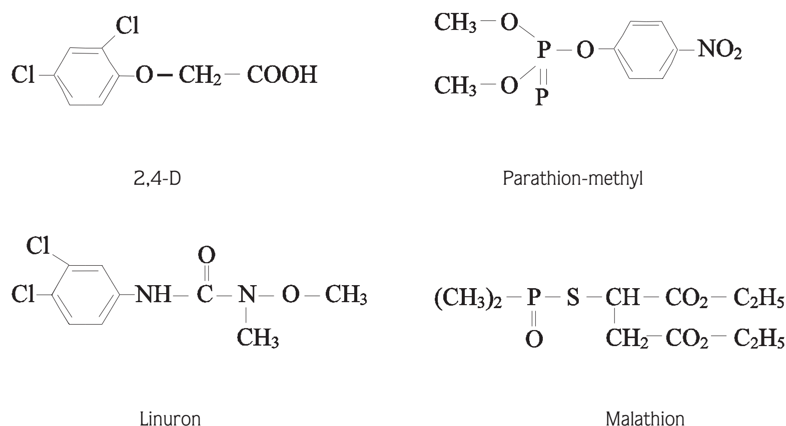

Figure 1. Chemical structures of pesticides used in this study

species studied were wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), chickpea (*Cicer arietinum* L.), and lentil (*Lens culinaris* Medik.). The plants were harvested 12 days after planting. Their shoots and roots were separated from the seed tissue and used for the determination of the glutathione-s-transferase activity, glutathione and protein contents.

Glutathione Assay

Fresh roots and shoots of the 12-day-old seedlings were washed and homogenized with 5% TCA (20% w/v). The homogenate was centrifuged at 10000 g for 15 minutes and the pH of the supernatant was adjusted to 4.0-5.0 with 1 M NaOH. The content of glutathione (GSH) in crude extract was determined using the Ellmann (DTNB: 5,5'-dithiobis (2-nitrobenzoic acid)) procedure [8], in which the reaction mixture comprised 0.1 ml of the sample, 2 ml of 100 mM pH 8.4 Tris HCl buffer and 0.1 ml Ellmann reagent (60 mg/100 ml Tris-HCl buffer 0.1 M, pH 7.0). The absorbance of the reaction mixture was read at 412 nm. The glutathione concentration in the samples was calculated from the standard curve using 2-20 µg/ml of GSH. Data are expressed as µg/g fresh weight of tissues.

GST Extraction and Assay

Treated and control plant tissues (shoots and roots) were rinsed with H_2O. All further steps were carried out at 4°C. Tissues were homogenized in 100 mM pH 7.0 phosphate buffer containing 0.05 mM DTE, 1 mM EDTA and 3.5% (w/v) PVPP. Slurry was filtered through cheese-cloth and centrifuged at 10000 g for 30 minutes. The pellet was discarded and the supernatant was used as the crude enzyme extract for the glutathione-s-transferase assay.

Glutathione-s-transferase activity was assayed spectrophotometrically at 340 nm by measuring the rate of l-chloro-2,4-dinitrobenzene conjugation with reduced glutathione as a function of time according to the established method of Habig [12]. The assay mixture contained 0.1 ml 30 mM GSH, 0.1 ml (approximately 3-4 mg protein.ml^{-1}) plant extract, 0.1 ml 30 mM CDNB and 2.7 ml 100 mM pH 6.5 phosphate buffer. The enzyme activity was expressed as nmolmin^{-1} and the specific activity as µmolmin^{-1} mg^{-1} protein.

All results were of at least three replicates. Protein contents were determined by the method of Lowry *et al.* [13] using bovine serum albumin as a protein standard.

Results and Discussion

The results are summarized in Figures 2-4.

The data in Fig. 2 show that pesticide treatment influenced the glutathione-s-transferase activities in all plants. The shoots and roots of the four plants exhibited

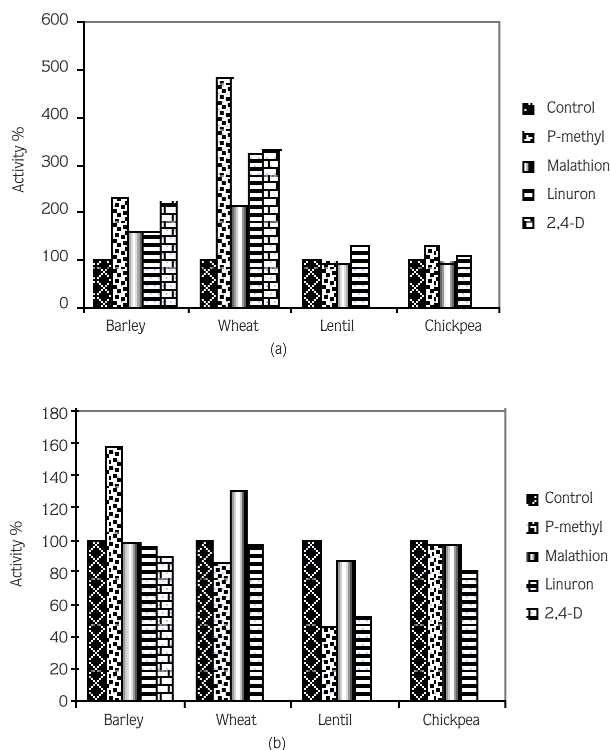

Figure 2. GST activity in roots (a), shoots (b) of different plants in control conditions and after treatment with herbicides and insectisides.
*Activity per gram tissue of GST of the herbicide treated/Activity per gram tissue of control x 100%
1 Unit GST activity: µmol/min/mg protein

different levels of enzyme activity upon exposure to various pesticide inductions. However, the highest GST activity was observed in the roots, especially for the parathion methyl treated wheat roots (484% of control). No significant increase in the enzyme activity was observed in lentil and chickpea. In contrast to the roots, the barley shoots treated with parathion methyl and the malathion treated wheat plant shoots had higher GST activity (150% and 130% of control, respectively). In Figure 3, the results of individual treatments of the four pesticides on the GSH content of wheat, barley, lentil and chickpea roots and shoots are shown. Results similar to those for GST activities were observed. In general, as a

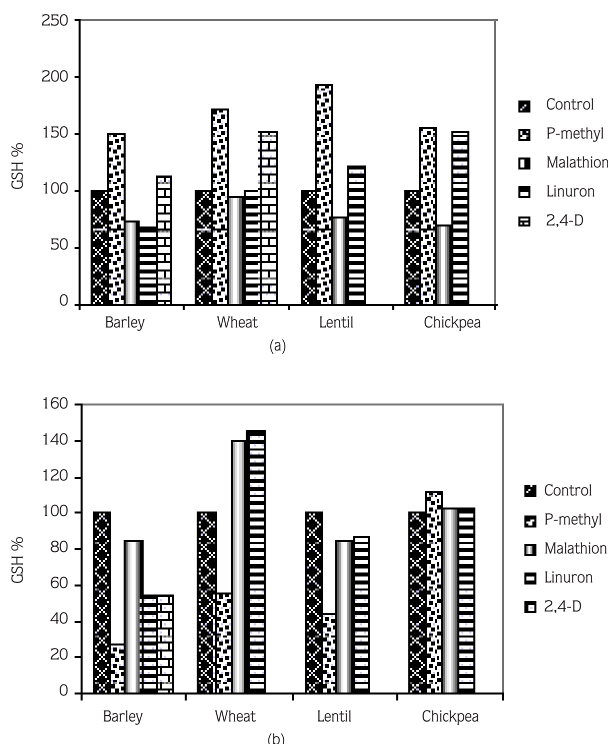

Figure 3. GSH content in roots (a), shoots (b) of different plants in control conditions and after treatment with herbicides and insecticides.
*μg g^{-1} tissue treated/μg g^{-1} control x 100%

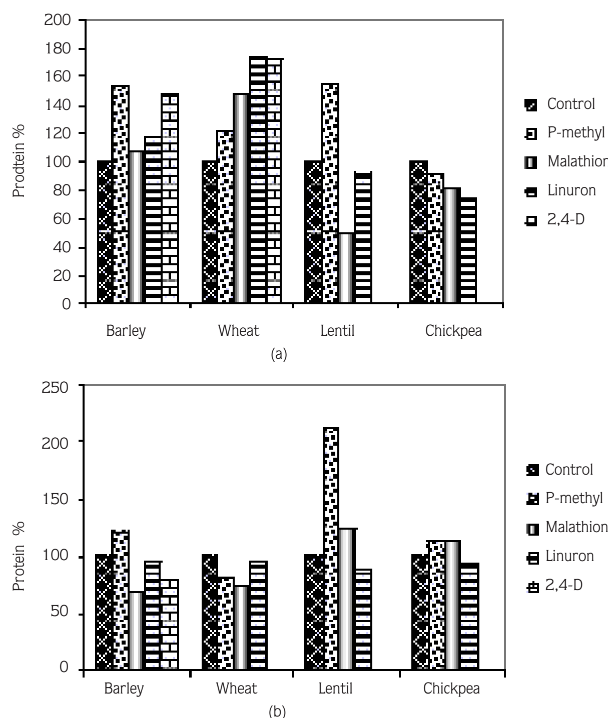

Figure 4. Protein content in roots (a), shoots(b) of different plants in control conditions and after treatment with herbicides and insecticides.

fold increase, the roots of the plants have better GSH levels, differing in range according to the plant source and the pesticide treatment. However, the levels of GSH in all plant roots were significantly increased by parathion methyl treatment (barley, 150%; wheat, 171%; lentil, 193%; and chickpea, 155% of control). In the case of plant shoots, a drastic decrease in wheat shoots was observed for parathion methyl, whereas linuron and malathion caused high GSH levels (146% and 140% of control respectively). When the data is expressed on the basis of μg GSH g^{-1} fresh weight, the GSH levels were highest in the shoots and lowest in the roots. An increase in activity was also reported in various plants by pesticide induction in previous works [14,15]. However, the cause of this fact is still not well understood, whether it could have resulted from either direct activation of a constitutively present glutathione-s-transferase isozyme(s) or an induction of new isozyme(s) at protein level.

Figure 4 shows the protein contents of all plants induced by pesticides via control. The protein contents of the plants were also found to differ with pesticide treatments except chickpea roots for all pesticides and, interestingly, lentil roots treated with malathion showed a marked decrease in protein concentration (50% of control). In contrast, lentil shoots induced with parathion methyl had the highest protein content (213% of control). These findings are not direct evidence for GST activity level control. The induction of plants by several pesticides can cause differentiation in the other enzyme levels, which are effective in the protective and tolerant system such as the other glutathione related enzymes, and mixed-function oxidases. The conjugation of glutathione with 1-chloro-2,4-dinitrobenzene is widely used for detecting glutathione-s-transferase activity but it may not detect all GST isozymes. The forms of GST specifically involved in the mechanism of tolerance to each molecule cannot be identified by the CDNB reaction; thus, it is possible that they differ or that they exhibit cross-reactivity with pesticide substrate [16]. Evidence supporting this hypothesis comes from the composite data of two corn GSTs active against CDNB [17,18]. The ability of pesticides to induce GST isozymes in corn [19] may be analogous to the effect of various xenobiotics on GST isozymes in mammalian tissues. Apparently this differential induction may be due to the ability of different xenobiotics to selectively activate transcription

of GST genes coding per enzyme subunits which exhibit particular substrate specificities [20]. Further studies are needed to determine the concentration dependence of the induction response. The results of this study and earlier reports [15,21] are consistent with the hypothesis that pesticides induce the GST activities in various ranges depending on the origin of the plant, the pesticide itself and treatment conditions.

In all the plants analysed, the GSH content was rather high and seemed to be induced by pesticide application in its cellular levels. The possible biological role of GSH relates to different aspects of the plant metabolism, in addition to its role in pesticide detoxification [22,23]. The multiplicity of functions that GSH can perform in cellular metabolisms suggests that GSH availability confers a general adaptability to stress conditions. These findings suggest a general role of the GSH-GST system in pesticide detoxification and in the plant's defence against toxic chemicals. On the other hand, the GST activity increase in monocotyledonous species (barley and wheat) was found to be higher than that of the dicotyledonous species (lentil and chickpea). This can be explained by the fact that the dicotyledonous species absorbed more pesticide than did the monocotyledonous species, but translocation was faster [24]. However, the biochemical mechanism is not yet known.

Overall, the data in this study show that the induction of plants with pesticides (2,4-D; parathion methyl; malathion; and linuron) may enhance the metabolic detoxification of these pesticides by stimulating the direct synthesis of glutathione-s-transferase and glutathione. Wheat (*Triticum aestivum* L.) treated with an organophosphorus insecticide, parathion methyl, was found to be the most induced. However, further studies are needed to elucidate the presence of specific parathion methyl GST isozymes in wheat and the ability of this pesticide to enhance selectively the activity of such GST isozymes.

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