Pure Appl. Chem., Vol. 82, No. 1, pp. 289–297, 2010.
doi:10.1351/PAC-CON-09-01-17

Effects of tribenuron-methyl treatment on glutathione *S*-transferase (GST) activities in some wheat and barley varieties*

Elif Oztetik‡

Science Faculty, Department of Biology, Anadolu University, 26470, Eskisehir, Turkey

Abstract: For efficient and profitable crop production, appropriate weed management is essential. Today, herbicides are an integral part of modern farming practice globally, as they assure the convenient method of weed control chemically. Glutathione *S*-transferases (GSTs, EC.2.5.1.18) are a superfamily of multifunctional enzymes that detoxify endo- and xenobiotic compounds by conjugating glutathione (GSH) to a hydrophobic substrate. Plant GSTs have been a focus of attention because of their roles in herbicide detoxification and environmental safety. In this study, the application of herbicide called tribenuron-methyl to the cultivars of wheat (*Triticum aestivum* L. cv. Izgi-2001, *Triticum aestivum* L. cv. Alpu-2001) and barley plants (*Hordeum vulgare* L. cv. Bilgi-91, *Hordeum vulgare* L. cv. Kalayci-97) caused an increase in GST activities of both in roots and shoots. Total GSH and protein contents were also determined for all above-mentioned plants. As a conclusion, our results indicate that depending on the herbicide itself, treatment conditions and the origin of the plant, tribenuron-methyl had an effect on the parameters measured in this study, including the GST activities and synthesis of GSH. The maximum increase in enzyme activity was observed in herbicide-treated *Triticum aestivum* L. cv. Izgi-2001 roots: 192 % of control with a tribenuron-methyl concentration of 1.5 M. However, further investigations are needed to elucidate the presence of specific tribenuron-methyl GST isozymes in this plant.

Keywords: detoxification; glutathione-*S*-transferase; tribenuron-methyl; wheat and barley.

INTRODUCTION

Weeds are undesirable plants growing within a crop. Crop yields can be significantly reduced up to 40–100 % without weed control. To achieve chemical weed management, herbicides with diverse mode of actions are in use all over the world. Glutathione-*S*-transferases (GSTs, EC.2.5.1.18) are found in all aerobic organisms, where they catalyze the conjugation of the thiol group of the glutathione (GSH) to diverse electrophilic centers on lipophilic molecules, including herbicides [1]. The conjugation to GSH usually results in the alleviation of toxicity through formation of rather less active end-products. While the resulting glutathione *S*-conjugates catabolized and excreted in animals [2], they are tagged and transported from the cytosol into the vacuole via the ATP-binding cassette (ABC) transporters (glutathione pump) for storage excretion in plants [3,4].

*Paper based on a presentation at the 13th International Biotechnology Symposium (IBS 2008): "Biotechnology for the Sustainability of Human Society", 12–17 October 2008, Dalian, China. Other presentations are published in this issue, pp. 1–347.
‡Tel.: +90 222 335 05 80, ext: 4730; Fax: +90 222 320 49 10; E-mail: eoztetik@anadolu.edu.tr

Plant GSTs were discovered with regard to their role in herbicide detoxification and environmental safety. The identification of an atrazine–GSH conjugate was the first report of herbicide metabolism in plants by GSH conjugation [5]. Subsequent research in many plant species has shown the increase in GST activity and existence of GST isoenzymes in response to treatment of various herbicide classes [6–9]. Today it was shown that GSTs are able to catalyze the first step in the biodegradation of several major classes of herbicides, including sulfonylureas [10–12], triazines (atrazine), chloroacetanilides [13,14], thiocarbamate sulfoxides [15], diphenylethers [16], and many more. The plant GSTs, in addition to their enzymatic activities, have less well characterized roles in endogenous metabolism, including functioning as GSH-dependent peroxidases counteracting oxidative stress [17–19], or GSH-dependent isomerases [20,21], and noncatalytically acting as flavonoid-binding proteins [22], stress signalling proteins [23], and regulators of apoptosis [24].

In this study, a herbicide called tribenuron-methyl ({methyl 2-[4-methoxy-6-methyl-1,3,5-triazin-2-yl(methyl)carbamoylsulfamoyl]benzoate}, commercially available as Granstar) was used (Fig. 1). It belongs to triazinylsulfonylurea herbicides and a derivative of tribenuron. It has excellent crop selectivity and a broad spectrum for control of broadleaf weeds and clovers in wheat and barley. Table 1 lists the weeds that are controlled by tribenuron-methyl treatment in wheat and barley [25]. After application, tribenuron-methyl enters into the weeds through their green leaves and roots, blocks the cell division, and avoids the growth of weeds [25]. In the literature, there are few reports about the efficacy of tribenuron-methyl to control weeds in crops by comparing to other broadleaved herbicides [26–28]. However, the studies on the enzymatic detoxification of this herbicide are limited.

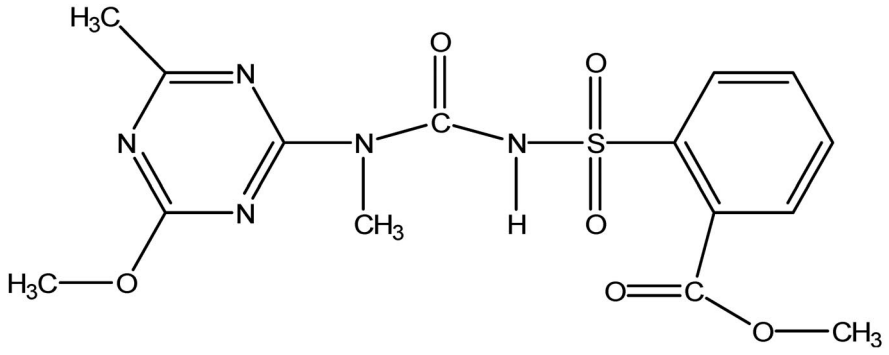

Fig. 1 Chemical structure of tribenuron-methyl ($C_{15}H_{17}N_5O_6S$).

Table 1 Weeds that are controlled by tribenuron-methyl treatment in wheat and barley [25].

Cereals	Name of weed
Wheat and barley	*Vaccaria pyramidata*
	Lamium spp.
	Ranunculus arvensis
	Papaver rhoeas
	Myagrum perfoliatum
	Bifora radians
	Boreava orientalis
	Cirsium arvense
	Buglossoides arvense
	Silene copoidea
	Geranium tuberosum
	Isatis tinctoria
	Vicia spp.
	Raphanus sp.
	Sinapis arvensis
	Galium tricorne
	Asperula arvensis
	Neslia paniculata
	Centaurea depressa

The present study was conducted to assess the effects of different concentrations of tribenuron-methyl treatment on GSH content and GST activities in the roots and shoots of the cultivars, *Triticum aestivum* L. cv. Izgi-2001, *Triticum aestivum* L. cv. Alpu-2001, *Hordeum vulgare* L. cv. Bilgi-91, and *Hordeum vulgare* L. cv. Kalayci-97, as they are commonly planted and used for beer and bread making.

MATERIALS AND METHODS

Chemicals

Tribenuron-methyl {methyl 2-[4-methoxy-6-methyl-1,3,5-triazin-2-yl(methyl)carbamoylsulfamoyl]benzoate} was purchased from Dupont, USA.

1-chloro-2,4-dinitrobenzene (CDNB), reduced GSH, PVP-K30, ethylenediaminetetraacetic acid (EDTA), hydroxymethyl aminomethane (Tris), Igepal CA-630, methanol, bovine serum albumin (BSA), ethanol absolute (99.8 %), Folin-Ciaceltau phenol reagent, cupper sulfate ($CuSO_4 \cdot 5H_2O$), potassium sodium tartrate, sodium hydroxide, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, sodium carbonate, 2-mercaptoethanol, pepstatin A, hydrochloric acid and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and 1,4-dithioerythritol (DTE) were purchased from Sigma (St. Louis, MO). All other chemicals were analytical grade and were obtained from commercial sources at the highest purity available.

Plant materials

The plants selected for this study belong to wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) species. The cultivars used were *Triticum aestivum* L. cv. Izgi-2001, *Triticum aestivum* L. cv. Alpu-2001, *Hordeum vulgare* L. cv. Bilgi-91 and *Hordeum vulgare* L. cv. Kalayci-97, as they are commonly planted in the area of Central Anatolia. Selected plant seeds were washed and immersed in tap water

for 2 h, then in distilled water for a further 2 h. The seeds were then planted in 10-cm-deep plastic vials containing soil. While the control groups (group 1) were watered only with distilled water, groups 2–5 were treated with 0.5, 1.0, 1.5, and 2.0 M concentrations of tribenuron-methyl, respectively. All groups were then watered only with distilled water for 12 days following planting. To create a closed system, the trays were covered with cellophane bags and the planted seeds were kept in growth chambers at 25 °C. After 12 days, the plants were harvested. In order to determine GST activity, total thiol group and protein contents from different tissues, the shoots and roots were separated. The seeds used in these experiments were obtained from Anatolia Agricultural Research Institute (ATAEM), Eskisehir, Turkey.

GSH determination

Fresh roots and shoots of the 12-day-old seedlings were washed and homogenized, in a ratio of 1:4 w/v, with 5 % w/v TCA by using UltraTurrax at 13 500 rpm for 90 s. The homogenate was centrifuged at 4 °C, 12 000 rpm for 15 min and the pH of the supernatant was adjusted to 4.0–5.0 with 1 M NaOH. The content of GSH in crude extract was determined using the Ellmann (DTNB) procedure [29,30], in which DTNB is reduced by SH groups to form 1 mol of 2-nitro-5-mercaptobenzoic acid per mol of SH. The nitromercaptobenzoic acid anion has an intense yellow color that can be used to quantify SH groups by measurement at its maximum absorbance at 412 nm [31].

The reaction mixture comprised the sample (0.1 ml), 100 mM pH 8.4 Tris HCl buffer (2 ml) and Ellmann reagent (0.1 ml; 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 GSH concentration in the samples was calculated from the standard curve using 2.1–21 μg/ml of GSH. Data are expressed as μg/g fresh weight of tissues. All results were of at least three replicates.

Preparation of cytosolic extracts from fresh plant material

The shoot and root tissues from all groups (group 1: control and groups 2–5: herbicide treated) were rinsed with distilled water. Fresh plant material (2.5 g of shoots or roots) was first pulverized in liquid N_2 in precooled porcelain mortars, then extracted, in a ratio of 1:3 w/v, with 100 mM pH 7.0 phosphate buffer, including 0.05 mM DTE, 1 mM EDTA and 3.5 % (w/v) PVPP at 4 °C. The mixture was then homogenized for 4 × 30 s. periods by Ultra-Turrax T25 at 13 500 rpm on ice. The crude homogenate was centrifuged at 15 000 rpm for 30 min. at 4 °C. The pellet was discarded, and the supernatant fraction was passed through a layer of filter paper. Usually, this filtrate (referred to as cytosol) was immediately subjected to enzyme activity measurement without further storage. Otherwise, the cytosol was stored at –80 °C as aliquots (1 ml), for later protein assays.

Determination of cytosolic GST activities toward CDNB

GST activities against the substrate CDNB were determined spectrophotometrically by monitoring the formation of the conjugation product at 340 nm according to the method of Habig et al. [32] as modified previously [33]. The wheat and barley cytosolic fractions were used as the enzyme source. All enzyme activity measurements were carried out at 25 °C using a spectrophotometer equipped with thermoregulated cell holder.

A typical reaction mixture included 100 mM phosphate buffer, pH 6.5, 1 mM CDNB, 1 mM GSH, and 20 μg wheat or barley cytosolic protein in a final volume of 1 ml.

The activity measurements were performed using T80 PG Instruments double-beam spectrophotometer at 340 nm. The reactions were followed for 3 min, which is in the linear period of the reaction. The reactions were started by the addition of cytosol. Incubation mixtures without the enzyme source were used as blanks (nonenzymatic reactions), and concentrations of the formed conjugation products were determined from the slopes of initial reaction rates. The reaction rate was calculated using the ε values of CDNB as 0.0096 μM^{-1} cm^{-1} [34]. The GST activities were expressed as unit/mg protein. One

unit of enzyme activity is defined as the amount of enzyme that forms one nmole of product per minute under defined assay conditions.

Protein contents were determined by the method of Lowry et al. [35] using BSA as a protein standard.

Data presented are the averages of at least three independent experiments, each of them in three replications.

RESULTS AND DISCUSSION

The results are summarized in Figs. 2–4. The data in Fig. 2 show that tribenuron-methyl treatment influenced the GST activities in all plants. The shoots and roots of the four plants exhibited different levels of enzyme activity upon exposure to various herbicide concentrations. However, the highest GST activity was observed in the roots, especially for the 1.5 M tribenuron-methyl treated Izgi-2001 and Alpu-2001 roots (192 and 180 % of control, respectively). No significant increase in the enzyme activity was observed in Kalayci-97 roots. In contrast to the roots, the Kalayci-97 shoots treated with 2.0 M herbicide has some GST activity (as 117 % of control), again Izgi-2001 and Alpu-2001 shoots treated with 1.5 M herbicide had higher GST activities (165 and 131 % of control, respectively). But, there is no activity in Bilgi-91 shoots compared to their roots.

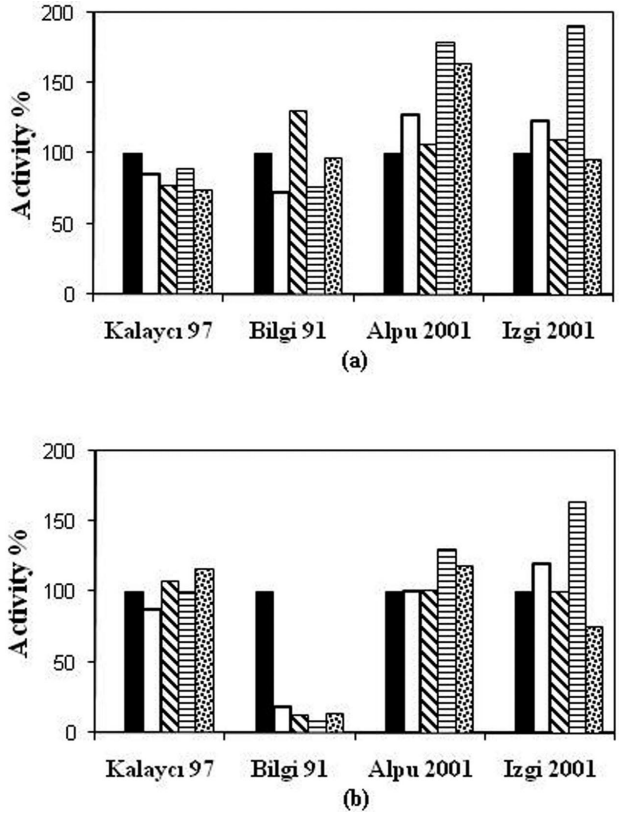

Fig. 2 GST activity in roots (a) and shoots (b) of different plants in control conditions and after treatment with herbicide. Columns represent the different concentrations of herbicide, from left to right; full columns: control, plain columns: 0.5 M, diagonal columns: 1.0 M, horizontal columns: 1.5 M and dotted columns: 2.0 M. (Activity per gram tissue of GST of the herbicide treated/activity per gram tissue of control × 100 %. 1 unit GST activity: nmole/min/mg protein).

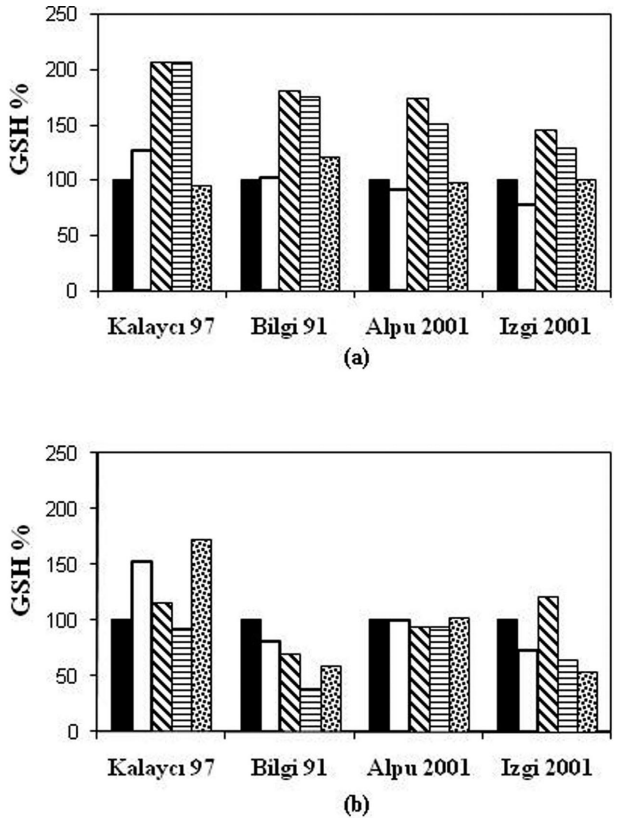

Fig. 3 GSH content of roots (a) and shoots (b) of different plants in control conditions and after treatment with herbicide. Columns represent the different concentrations of herbicide, from left to right; full columns: control, plain columns: 0.5 M, diagonal columns: 1.0 M, horizontal columns: 1.5 M and dotted columns: 2.0 M. (μg g^{-1} tissue treated/μg g^{-1} control × 100 %).

In Fig. 3, the results of individual treatments of the different concentrations of herbicide on the GSH content of Izgi-2001, Alpu-2001, Kalayci-97, and Bilgi-91 roots and shoots are shown. Results similar to those for GST activities were observed. In general, as a fold increases, the roots of the plants have better GSH levels, differing in range according to the concentrations of herbicide treatment and the plant itself. As seen in Fig 3, the GSH levels in all plant roots were increased with both 1.0 and 1.5 M tribenuron-methyl treatment (Kalayci-97, 205 % with both; Bilgi-91, 180 and 175 %; Alpu-2001, 173 and 150 %, Izgi-2001, 145 and 129 % of control, respectively). In the case of plant shoots, even Kalayci-97 shoots had lower GSH levels then their own roots, they were still having highest GSH levels with 0.5 and 2.0 M herbicide treatment (154 and 173 % of control, respectively) by comparing to the other plant shoots (the third highest GSH levels in shoots was observed as Izgi-2001, 122 % of control). 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. In the literaure, the increase in enzyme activity was also reported for different plants with various herbicide induction [36,37]. Even the reason of that mechanism is still not very well known, by considering Figs. 2 and 3 together, it is possible to suggest that the reason could be either by a direct activation of existent GST isoenzyme(s) or possibly by the induction of newly produced isoenzyme(s), due to herbicide treatment, at the protein level.

Figure 4 shows the protein contents of all plants induced by herbicides by comparing to control group. The protein contents of the plants were found to differ with herbicide treatments, except Alpu-2001 roots for all concentrations of herbicide treatment and Kalayci-97 roots treated with 1.5 M herbi-

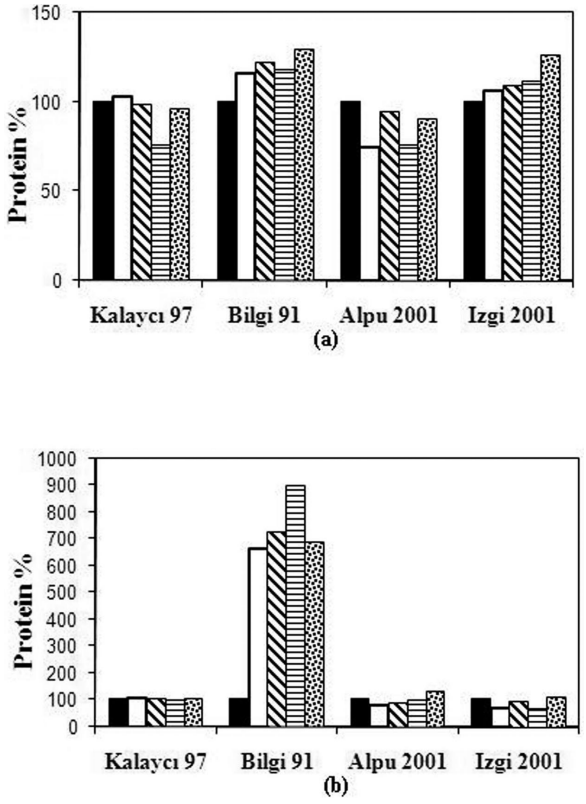

Fig. 4 Protein content in roots (a) and shoots (b) of different plants in control conditions and after treatment with herbicide. Columns represent the different concentrations of herbicide, from left to right; full columns: control, plain columns: 0.5 M, diagonal columns: 1.0 M, horizontal columns: 1.5 M, and dotted columns: 2.0 M.

cide showed a decrease in protein concentration (75 % of control). In contrast, Bilgi-91 shoots induced with herbicide had the highest protein content (900 % of control). However, as previously noted, there is no activity in Bilgi-91 shoots, therefore, these results are not direct evidence for the control of GST activity level. The induction of selected plants with this herbicide may cause differentiation in the other GSH-related enzyme levels, which are also recruited in the detoxification system, like glutathione peroxidases (GPOXs). It should also be noted that, since it is used for the demonstration of multiple forms of GSTs in all biological organisms from animals to plants, CDNB is known as the universal substrate for nearly all GSTs. But, it can by no means be regarded as proof of the presence of that specific class of GSTs. However, the absence of any detectable CDNB activity does not provide proof of the absence of GSTs, as some GST isoenzymes have very little activity toward CDNB, but very high activity toward other substrates [38]. It is also known from the literature that GSTs show both specific and overlapping substrate preferences [39,40]. The model GST substrates that display selectivity for particular subunits are often used in a "diagnostic" sense to identify isoenzymes. For example, 1,2-dichloro-4-nitrobenzene (DCNB) is selective for M class GSTs and used as a selective marker for this class. The reason behind this selectivity is probably because of the transcription of distinct types of GST genes which are differentially regulated and encode the enzyme subunits that show different substrate specifities. Further research may be designed with other substrates and herbicides to determine GST activity response of these plants. However, in agreement with previous literature [41,42], the outcomes of this study support the idea that herbicides stimulate the GST activities in various ranges depending on the origin of the plant, the herbicide, and its treatment conditions.

When all the plants were analyzed, the GSH content was found to be rather high and seemed to be induced by herbicide treatment. In addition to their known role in intracellular detoxification of xenobiotics (including herbicides), GSH may also function in other stress-related conditions, such as scavenging of H_2O_2 [43,44], and phytochelatin synthesis [45–47]. The presence of high GSH content in our results indicates a general adaptability to stress conditions. However, the GST activity increase in wheat varieties (Alpu-2001 and Izgi-2001) were found to be higher than that of the barley varieties (Kalayci-97 and Bilgi-91). Even though all organisms belong to monocotyledonous species, probably the translocation in barley varieties was faster than in the wheat varieties. Therefore, wheat and barley varieties reflect a difference in the rate of metabolism with regard to tribenuron-methyl.

CONCLUSION

The results in this research illustrate that the induction of selected wheat and barley plants with tribenuron-methyl may intensify the metabolic detoxification of this herbicide through stimulation of the direct synthesis of both GST and GSH. *Triticum aestivum* L. cv. Izgi-2001 treated with a triazinylsulfonylurea herbicide, tribenuron-methyl, was found to be the most induced by comparing to other varieties. Because plant GSTs have been shown to accumulate in different stress situations, the individual GST isoenzymes might have specific functions that are possibly related to the inducing stress stimulus, like herbicides. Therefore, further studies are needed to elucidate the presence of specific tribenuron-methyl GST isozymes in this plant and the ability of tribenuron-methyl to enhance selectively the activity of such GST isozyme(s) should be pointed.

However, the result of this small-scale study would probably be a good starting point for the determination of optimum herbicide rates for large-scale applications on the agricultural lands. Those appropriate and reduced herbicide rates will still protect the crops, but will reduce the payment for herbicides in use. Besides this mentioned economical potential, the outcomes of this study can be used to construct the parameters for the production of herbicide-resistant wheat and barley varieties through transgenic studies. Therefore, not only the economical benefits, but complemented systematic studies about this issue bring more understanding to related topics in the literature.

ACKNOWLEDGMENT

The author thanks the Anatolia Agricultural Research Institute/ATAEM (Eskisehir, Turkey) for the plant samples.

REFERENCES

1. J. D. Hayes, D. J. Pulford. *CRC Crit. Rev. Biochem. Mol. Biol.* **30**, 445 (1995).
2. E. Boyland, L. F. Chasseaud. *Adv. Enzymol. Rel. Areas Mol. Biol.* **32**, 173 (1969).
3. J. O. D. Coleman, M. M. A. Blake-Kalff, T. G. E. Davies. *Trends Plant Sci.* **2**, 144 (1997).
4. P. A. Rea. *J. Exp. Bot.* **50**, 895 (1999).
5. R. H. Shimabukuro, H. R. Swanson, W. C. Walsh. *Plant Physiol.* **46**, 103 (1970).
6. K. P. Timmerman. *Physiol. Plantarum* **77**, 465 (1989).
7. J. V. Dean, J. W. Gronwald, C. V. Eberlein. *Plant Physiol.* **92**, 467 (1990).
8. C. J. Andrews, M. Skipsey, J. K. Townson, C. Morris, I. Jepson, R. Edwards. *Pestic. Sci.* **51**, 213 (1997).
9. R. Edwards, D. P. Dixon, V. Walbot. *Trends Plant Sci.* **5**, 193 (2000).
10. H. M. Brown, S. M. Neighbors. *Pestic. Biochem. Physiol.* **29**, 112 (1987).
11. V. A. Wittenbach, M. K. Koeppe, F. T. Lichtner, W. T. Zimmerman, R. W. Reiser. *Pestic. Biochem. Physiol.* **49**, 72 (1994).

12. M. K. Koeppe, A. C. Barefoot, C. D. Cotterman, W. T. Zimmerman, D. C. Leep. *Pestic. Biochem. Physiol.* **59**, 105 (1998).
13. R. H. Shimabukuro, D. S. Frear, H. R. Swanson, W. C. Walsh. *Plant Physiol.* **47**, 10 (1971).
14. C. K. Cottingham, K. K. Hatzios. *Weed Sci.* **40**, 359 (1992).
15. C. K. Cottingham, K. K. Hatzios, S. A. Meredith. *Weed Res.* **33**, 161 (1993).
16. D. S. Frear, H. R. Swanson. *Pestic. Biochem.* **3**, 473 (1973).
17. D. Bartling, R. Radzio, U. Steiner, E. W. Weiler. *Eur. J. Biochem.* **216**, 579 (1993).
18. I. Cummins, D. J. Cole, R. Edwards. *Plant J.* **18**, 285 (1999).
19. V. P. Roxas, R. K. Smith, E. R. Allen, R. D. Allen. *Nat. Biotechnol.* **15**, 988 (1997).
20. D. P. Dixon, D. J. Cole, R. Edwards. *Arch. Biochem. Biophys.* **384**, 407 (2000).
21. R. Thom, D. P. Dixon, R. Edwards, D. J. Cole, A. J. Lapthorn. *Mol. Biol.* **308**, 949 (2001).
22. L. A. Mueller, C. D. Goodman, R. A. Silady, V. Walbot. *Plant Physiol.* **123**, 1561 (2000).
23. L. Loyall, K. Uchida, S. Braun, M. Furuya, H. Frohnmeyer. *Plant Cell* **12**, 1939 (2000).
24. S. C. Kampranis, R. Damianova, M. Atallah, G. Toby, G. Kondi, P. N. Tsichlis, A. M. Makris. *J. Biol. Chem.* **275**, 29207 (2000).
25. <http://www2.dupont.com/Agriculture/tr_TR/assets/downloads/2.5.1.11.Granstar.tr.pdf>. Accessed 22 May 2009.
26. M. A. Baghestani, E. Zand, S. Soufizadeh, M. Jamali, F. Maighany. *Crop. Prot.* **26**, 1385 (2007).
27. M. A. Baghestani, E. Zand, S. Soufizadeh, N. Bagherani, R. Deihimfard. *Crop Prot.* **26**, 1759 (2007).
28. E. Zand, M. A. Baghestani, S. Soufizadeh, A. Eskandari, R. PourAzar, M. Veysi, K. Mousavi, A. Barjasteh. *Crop Prot.* **26**, 1349 (2007).
29. G. L. Ellman. *Arch. Biochem. Biophys.* **74**, 443 (1958).
30. G. L. Ellman. *Arch. Biochem. Biophys.* **82**, 70 (1959).
31. J. Sedlak, R. H. Lindsay. *Anal Biochem.* **25**, 192 (1968).
32. W. H. Habig, M. J. Pabst, W. B. Jakoby. *J. Biol. Chem.* **249**, 7130 (1974).
33. E. Oztetik. *Purification of Glutathione S-Transferases and genetic characterization of Zeta isozyme from Pinus Brutia*, *Ten*., Ph.D. Thesis, Middle East Technical University, Ankara (2005).
34. W. H. Habig, W. B. Jakoby. *Methods Enzymol.* **77**, 398 (1981).
35. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randal. *J. Biol. Chem.* **248**, 265 (1951).
36. P. J. Hatton, I. Cummins, D. J. Cole, R. Edwards. *Physiol. Plant.* **105**, 9 (1999).
37. A. Zajc, T. Neuefeind, L. Prade, P. Reinemer, R. Huber, B. Bieseler. *Pestic. Sci.* **55**, 248 (1999).
38. E. Oztetik. *Bot. Rev.* **74**, 419 (2008).
39. B. Mannervik, H. Danielson. *CRC Crit. Rev. Biochem.* **23**, 283 (1988).
40. P. G. Boar, M. Coggan, P. Johnston, V. Ross, T. Suzuki, G. Webb. *Pharmacol. Ther.* **48**, 357 (1990).
41. F. Mauch, R. Dudler. *Plant Physiol.* **102**, 1193 (1993).
42. N. Soltani, C. Shropshire, P. T. Sikkema. *Crop Prot.* **25**, 346 (2006).
43. C. H. Foyer, B. Halliwell. *Planta* **133**, 21 (1976).
44. M. A. Hossain, K. Asada. *Ind. J. Biochem. Biophys.* **24**, 52 (1987).
45. E. Grill, E.-L. Winnacker, M. H. Zenk. In *Sulfur Nutrition and Sulfur Assimilation in Higher Plants*, H. Rennenberg, C. Brunold, L. J. De Kok, I. Stulen (Eds.), pp. 89–95, SPB Academic Publications, The Hague (1990).
46. W. E. Rauser. *Annu. Rev. Biochem.* **59**, 61 (1990).
47. G. Noctor, L. Gomez, H. Vanacker, C. H. Foyer. *J. Exp. Bot.* **53**, 1283 (2002).

