Organ-dependent GST activation in *Ocimum basilicum* by endosulfan exposure

Ramírez Sandoval Maricela y Velázquez Fernández Jesús Bernardino[a]*

[a]*Posgrado en Ciencias Biológicas Agropecuarias y Pesqueras, Universidad Autónoma de Nayarit. Tepic, Nayarit, México, Tel.: 01-311-2118800 ext. 8965; Fax: 01-311-2118816*
**E-mail: jesusbvf@gmail.com*

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Abstract
Ocimum basilicum L., Basil, has shown to be a bioremediator candidate for the organochlorine pesticide endosulfan. Nevertheless, the mechanism of phytoremediation of the pesticide is still unknown. Glutathione-S-Transferase (GST) is an enzyme involved in plant defense against pollutants and oxidative damage. Because of this, GST activity changes would be expected after an exposition to endosulfan. Thus, to evaluate the response of basil to endosulfan exposure, we measured GST activity in leaves, roots, stems and rhizosphere. Plants were exposed to 0, 10, 100, 1000 mg endosulfan/Kg of soil. The GST activity increased after endosulfan exposure depends on organ and endosulfan concentration. In leaves, GST activity increased at 10 mg/Kg, but decreased at 1000 mg/Kg. Root GST activity was increased at 10 mg/Kg, but became normal at higher concentrations of endosulfan. GST activity from stem and rhizosphere showed no significant variation with the endosulfan concentration.

Keywords: Phytoremediation, Endosulfan, Ocimum basilicum, plant GST.

1. Introduction

Phytoremediation has been proved to be a good strategy for pollutant removal or elimination of detrimental effects of pollution (EPA, 2001). Recently, we have demonstrated that Basil (*Ocimum basilicum* L.) is able to enhance natural attenuation of endosulfan in soil (Ramírez-Sandoval *et al*., 2011). However, knowledge on the underlying mechanism is lacking. As a first approach, we aimed to study glutathione-S-transferase. In plants, this enzyme has been showed to be involved in some phytoremediation activities such as biotransformation (Dixon *et al*., 2002), translocation into vacuoles (Marrs *et al*., 1995), or detoxification (Yadav, 2010).

2. Experimental Section

Experimental exposition to endosulfan was carried out as described previously (Ramirez-Sandoval 2011). After 15 days of exposition, samples of leaves, roots, stems and soil were taken. Soil attached to roots was sampled to measure rhizosphere GST activity. GST extraction was evaluated as described elsewhere (Scarponi, Quagliarini & del Buono, 2006). Briefly, plant samples were frozen with liquid nitrogen and pulverized in a mortar. Powder was suspended in 30 mL of buffer (Tris 100 mM pH 7.5, EDTA 2 mM, DTT 1 mM and polyvinylpyrrolidone PVP 15 g/L) in a 1:5 w/v ratio. After filtering through muslin, homogenate was centrifuged during 50 min at 7,000 rpm. Supernatant was assayed for GST activity. GST activity was determined as described by Habig & Jakoby (1981). Protein content was measured by Lowry method.

Statistical analysis was carried out by ANOVA with a post-hoc Bonferroni test setting $p < 0.05$ for significance. For comparison between groups of different concentration or different parts of the plant, Mann-Whitney's U was used.

3. Results and Discussion

Each part of the plants show characteristic GST activity values (Table 1) and behaviour as endosulfan concentration is changed. Soil was experimentally spiked with endosulfan at different concentrations: 0, 10, 100 or 1,000 mg/Kg soil. No effect of endosulfan exposition was observed in rhizosphere GST. In roots, a two-fold increase is observed at 10 mg endosulfan/Kg soil; at higher concentrations, values close to normal are observed. In stems, a 60 % increase in GST activity is observed at 100 mg endosulfan/Kg soil; also, a sligthly increment is observed at 10 mg/Kg but was not statistically significant. In leaves, 10 mg endosulfan/Kg soil caused a 90 % increase is observed, this effects decrease to a 50 % above normal at 100 mg endosulfan/Kg soil; and at 1,000 mg endosulfan/Kg soil, the leaf GST activity is 50

Table 1 GST activity in *Ocimum basilicum* L. and its rhizosphere.

Part of the plant	GST activity (nmoles/min*mg protein) at different concentrations of endosulfan in soil (mg endosulfan/Kg of soil) (n=3)			
	0	10	100	1,000
Rhizosphere	1.859 (0.303)	1.780 (0.425) [b]	1.654 (0.412) [c]	1.600 (0.315)
Roots	1.750 (0.583)	4.098 (0.679)*,[a,c]	1.558 (0.412)	1.760 (0.641)
Stems	1.598 (0.505)	2.125 (0.623) [b]	2.618 (0.627)*,[a]	1.565 (0.523)
Leaves	1.006 (0.039) [a,b]	1.909 (0.548)*,[a,b]	1.536 (0.305)*,[b,c]	0.529 (0.089)*,[a,b,c]

*p < 0.05 respect to 0 mg endosulfan/Kg soil. Mean values (SD) are showed.
[a] p < 0.05 respect to rhizosphere.
[b] p < 0.05 respect to roots.
[c] p < 0.05 respect to stems.

% below the value in absence of endosulfan. These results strongly suggest that plant is responding to endosulfan concentration and that this is organ-dependent.

Leaf GST activity is severely compromised within the response mechanism, since an increment is observed at 10 mg endosulfan/Kg soil, but GST activity decreases as the endosulfan concentration increases. Leaf is a high-metabolic organ, thus, it is not surprising that leaves are involved in the plant response to stress. GST plays an important role in dealing with xenobiotics (Dixon, Lapthorn & Edwards 2002). Hatton et al. (1999) have shown the leaf GST function in detoxifying *Setaria faberi* from herbicides of different groups. Our results also show the main role of leaf GST in response mechanism to pollutants. Indeed, the leaf GST activity shows the highest variations depending on the concentration of endosulfan.

A boost in GST activity was observed in roots at 10 mg endosulfan/Kg soil (Table 1). This increase was two-fold the normal value in roots. The role of roots in detoxifying compounds has been shown. In facts, it has been suggested as one of the first organs responding to pollutants. Schröder et al. (2007) have suggested that xenobiotics are glutathionylated in roots and subsequently expelled out of the plant. As a consequence of glutathionylation, root tissue recognizes GSH-X conjugate (glutathionylated xenobiotic) and the complex is selectively non-absorbed into the roots. Because of this, endosulfan might be less environmentally bioavailable. In our results, the GST increased in roots is 130 % from normal, while in leaves was only 90 %. This suggests a more important role of root GST than leaf GST, and of GST from both organs than those from stems or rhizosphere. Interestingly, the stem GST also increased but in a modest way. At 100 mg endosulfan/Kg soil, the stem has the highest GST activity among the organs evaluated: the root GST activity almost decreased back to normal and leaf GST activity diminishes. Since this was evaluated in different experiments but at the same time, the stems response is not caused by time; it consists of a not-so-high but steady increase of GST activity. Still, is worth to mention that GST activity in stems is higher than in leaves in normal and in the most situations, except at 10 mg endosulfan/Kg soil.

4. Conclusions

GST activity has been described as a part of the plant response to pollutants or acclimation. Nevertheless, our results strongly suggest that this response involves all parts of the plant but in a different degree and with a different behavior as a response to the pollutant. Leaf GST shows a biphasic response, increasing at low endosulfan concentration (10 mg endosulfan/Kg soil) and decreasing at a higher endosulfan concentration (1,000 mg endosulfan/Kg soil). Roots GST activity increased two-fold at 10 mg endosulfan/Kg soil and got normal values at higher concentrations. Stem GST activity show a low but consistent increase. GST is not the only enzyme involved in plant response mechanisms to pollutant. Nowadays, we are studying other enzymes involved in the plant response to endosulfan.

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