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Differential response to treatment with herbicide chevalier induced oxidative stress in leaves of wheat

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ABSTRACT

The present study has evaluated effects of chevalier concentrations used for controlling cereals growth, on selected metabolic and stress- related physiological parameters in wheat (*Triticum durum* Desf.) cultivar Wercenis and (*Triticum aestivum* L.) cultivar HD1220 plants after two days of treatment with herbicide. Our results show that the chevalier induced oxidative stress triggered significant changes in growing plants, although, both cultivars of wheat seemed to have most likely similar contents of biomass foliar and roots, chevalier caused differential reductions; greater in Wercenis than in HD1220. However, photosynthetic pigments content was significantly lower in plants grow, a linear drop in chlorophyll content was observed with an increase in the herbicide at all concentrations. Increasing concentrations of herbicide increase total protein accumulation, the percentage changes are more in both varieties at 2, 2.5 RFD, increase in protein is more pronounced in Wercenis than in HD1220. While malondialdehyde, an indicator of lipid peroxidation, and membrane permeability, were constitutively elevated; indicates that the chevalier-induced oxidative stress appeared obvious in wheat, a status that seemed consistent in HD1220 than in Wercenis. The increase in GSH content accompanied with great induction of GST activity in Wercenis, in the present results, confirm the chevalier- induced oxidative stress, the opposite pattern of response observed in HD1220 could support the ability of this variety to tolerate chevalier and to overcome its toxicity.

Key words: Herbicide, Wheat, Oxidative stress, Tolerance, Metabolite, GSH, GST.

INTRODUCTION

Plants are confronted with exposure to oxidative stresses, such as strong light drought salinity low or high temperature and various chemicals including some air pollutants and herbicides, throughout their lives [1]. Under optimal environmental conditions, the antioxidant system in plant calls effectively protects them from potentially deleterious effects of AOSs. Under oxidative stress, plants produce active oxygen species, which are harmful to plant growth due to their detrimental effects on the sub cellular components and metabolism of the plant [2]. Antioxidant enzymes and certain metabolites play an important role in adaptation and ultimate survival of plants during periods of stress. In fact, activities of antioxidative enzymes are inducible by oxidative stress [3] which reflects a general strategy required to overcome stress.

However, under environmental stress conditions, AOSs generation is enhanced, thus the cellular antioxidant capacity can be over-whelmed and oxidative stress occurs [4]. Excessive generation of ROS causes irreversible impairment of DNA and damage to membrane lipids leading to the production of Malondialdehyde (MDA) [5].

Herbicides play an important role in agriculture and demand for them is increasing. Herbicides are used extensively today to eliminate unwanted competing species of plants in the cultivated environment. Some herbicides produce

oxidative, several herbicides have been found to generate active oxygen species, either by direct involvement in radical production or by inhibition of biosynthetic pathways.

The generation of the hydrocarbon gas ethane, the production of malonaldehyde and changes in electrolytic conductivity has frequently been used as sensitive markers for herbicide action in plants. Herbicides which block photosynthesis causes increased excitation energy transfers from triplet chlorophyll to oxygen while those inhibit carotenoid biosynthesis eliminate important quenchers of the triplet chlorophyll and O₂.

Plant tolerance to herbicides might confer by differential antioxidative mechanism [6]. There are many studies regarding the effects of herbicides on photosynthesis, assimilatory pigments content and their biosynthesis [7]. GSH is regarded as a key component of antioxidant defenses in most aerobic organisms and moreover to be limiting for tolerance to herbicides [8].

However, some plant species can tolerate through an efficient defense mechanism to detoxify herbicides and to scavenge ROS through a number of metabolites and enzymes [9]. GSH participates in ROS scavenging through ascorbate-GSH cycle [10]; the enzymatic conjugation of herbicide with GSH is mediated by GSTs [11]. GSTs are enhanced under certain conditions to increase the plant defense against several biotic and abiotic agents [12]. In addition, glutathione-S-transferase (GSTs) which are mostly involved in the detoxicative conjugation of some xenobiotic having an electrophilic group with GSH can also act as peroxidase.

Therefore, the present work was aimed to relate the differential effects of herbicide, Chevalier, widely used in Algeria to control weeds in wheat fields, composed for two active ingredients (Mesosulfuron Methyl, Iodosulfuron Methyl Sodium) and phytoprotector, on some physiological parameters of oxidative stress in wheat (*Triticum durum* Desf.) cultivar Wercenis and (*Triticum aestivum* L.) cultivar HD1220, with response of oxidative stress indices.

MATERIALS AND METHODS

Plant materials and growth conditions

Grains of wheat (*Triticum durum* Desf. cv Wercenis) and (*Triticum aestivum* L. cv HD1220) seedlings were surface sterilized by immersing in 3% sodium hypochlorite solution for 10min, thoroughly washed, soaked for 8 h and germinated in sand/clay soil (3:1 v/v) in plastic pots (20cm diameter × 25cm height). The pots were kept at 12 h photoperiod, 75% relative humidity and 26/14 °C day/night regime.

When seedlings were 10 days old, irrigation water was substituted with one-fourth strength Hoagland solution. At the 3 leaf stage, plants were divided into three groups for each variety; one was left to serve as control and one for herbicide treatment the recommended field dose used in wheat fields (330g ha⁻¹, 0.6 mg/pot). Therefore the third group treated with 1.5, 2 2.5-fold RFD of chevalier (0.9, 1.2 and 1.5 mg/pot). The herbicide was applied only once as foliar sprays, doses of herbicide had been determined in previous experiments. The quantity was calculated in relation to the surface area per pot and mixed in a suitable amount of water, enough to spray the surface area of each pot. Leaves and Roots were collected after 2 days, rinsed with copious amounts of water and dried by plotting with paper towels before the subsequent analyses.

Determination of photosynthetic pigments

Contents of chlorophyll and carotenoid were estimated in the fresh tissues after extraction with 80 % acetone according to the spectrophotometric method described by [13], the absorbance de solution was determined at 645, 663 and 470 nm.

Determination of content of total protein, lipid peroxidation and membrane permeability

Protein content was determined spectrophotometrically by reaction with Coomassie Brilliant Blue G according to [14]. Lipid peroxidation (MDA) and membrane permeability (EC %) in the leaves were measured to assess the membrane damage. For the measurement of lipid peroxidation in the leaf tissues, the thiobarbituric acid (TBA) test, which determines MDA as an end product of lipid peroxidation [15], was used. For this, leaf samples (500 mg) were homogenized in 4ml of 1% TCA (trichloroacetic acid) solution and centrifuged at 10000×g for 10min. The supernatant was added to 1 ml 0.5 % TBA in 20 % TCA. The mixture was incubated in boiling water for 30 min, and the reaction was stopped placing the tubes in an ice bath. Then, the samples were centrifuged at 10000 ×g for 5 min, and the absorbance of supernatant was read at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The amount of MDA-TBA complex was calculated from the extinction coefficient 155 mM⁻¹cm⁻¹.

Membrane permeability (EC %) was estimated through the leakage of electrolytes, described by [16]. Fresh leaves were placed in tubes, containing 30 ml bidistilled water and kept for 2 h in water bath at 30 °C for measuring the

initial conductivity (EC_1). The final electrolyte conductivity (EC_2) was measured after boiling the plant samples for 15 min. The leakage percentage was calculated as $(EC_1/EC_2) \times 100\%$.

Determination of reduced glutathione (GSH) and glutathione-S-transferase (GST)

The glutathione was assayed by the method of [17], based on measuring the absorbance of the 2-nitro-5 mercapturic resulting from the reduction of the acid 5-5 thiol-bis-2- nitrobenzoic acid (DTNB) by the thiol groups (-SH) glutathione.

Glutathione-S- transferase GST was extracted in 100 mM phosphate buffer, pH 6.5, centrifuged at $9000 \times g$ for 30 min. GST was assayed in mélange CDNB (20mM) – GSH(100 mM) and 100 mM phosphate, PH 6.5. The absorbance at 340 nm was measured and the activity was calculated by the extinction coefficient $E = 9.6 \text{ mM}^{-1} \text{ cm}^{-1}$

Statistical analysis

The experiment was set up in a completely randomized design. Each pot contained 15 plants, and each treatment contained three replicate pots. All measurements were subjected to analyses of variance (ANOVA) to determine the least significant difference.

RESULTS AND DISCUSSION

Growth responses and contents of photosynthetic pigments

Lead effects on biomass leaves, biomass roots and photosynthetic pigments were presented on table 1.

The biomass produced under stress oxidative conditions caused by herbicide, fell significantly in leaves and roots compared to controls. A treatment of plants with chevalier, however, resulted in a biomass decrease of about 31.2 % in leaves, 19.2 % in roots of Wercenis and about 27 %, 29.2% respectively in leaves, roots of HD1220 relative to the controls.

Table 1: Effect of Chevalier on biomass fresh, photosynthetic pigments of Wercenis and HD1220 at different concentrations

	Concentration of Chevalier (mg/pot)	Biomass leaves (g)	Biomass roots (g)	Chlorophylls (a+b) (mg/g FW)	Carotenoid (mg/g FW)
Wercenis	0	4,55*± 0,38	4,79*± 0,63	2,12*± 0,02	0,31*± 0,01
	0.6	2,63* ± 0,12	1,61** ± 0,05	1,79*± 0,25	0,25*± 0,01
	0.9	2,87* ± 0,03	1,67** ± 0,08	1,68*± 0,08	0,27*± 0,02
	1.2	1,37** ± 0,03	1,51** ± 0,04	1,52*± 0,80	0,27*± 0,01
	1.5	1,42** ± 0,04	0,92** ± 0,15	1,23** ± 0,13	0,23*± 0,01
HD1220	0	3,58 *± 0,04 3	3,35*± 0,03	2,99*± 0,08	0,37*± 0,02
	0.6	35* ± 0,32	3,09*± 0,04	2,78*± 0,09	0,33*± 0,02
	0.9	2,24* ± 0,02	2,91* ± 0,04	1,48*± 0,10	0,21*± 0,03
	1.2	0,97** ± 0,03	1,16* ± 0,06	1,23*± 0,06	0,19** ± 0,02
	1.5	0,97** ± 0,14	0,98** ± 0,04	0,79** ± 0,04	0,15** ± 0,01

The values are means of three replicates ± standard deviation (S.D). ** Significant ($P < 0.01$), * significant ($P < 0.05$) compared to controls.

Data show that pigment chlorophylls and carotenoid contents were strongly modified but were similarly affected by lead treatment herbicide (Table 1).

Total chlorophyll including chlorophyll a and b decreased with increased herbicide concentrations (0.6, 0.9, 1.2 and 1.5 mg /pot). The percentage change to 58 %, 26.4 % respectively for Wercenis and HD1220 as compared to the controls. Chlorophyll content was height lower in plants grown in 0.6- 1.5 mg/pot concentrations in Wercenis but in 0.9-1.5 mg/pot in HD1220.

The total carotenoid content in the leaves investigated decreasing as compared with controls; Chevalier induced significant reduction in the contents of photosynthetic pigments in both varieties. However, pigment contents were inversely correlated with lipid peroxidation. This correlation indicates that herbicide action on photosynthetic pigments can be mediated by ROS.

Content of total protein, lipid peroxides and membrane permeability

The effects of herbicide Chevalier on total protein, lipid peroxides and membrane permeability of Wercenis and HD 1220 are shown in figure 2 and 3. As compared with controls, Chevalier induced significant augmentation in the content of total protein in both cultivars. Protein in leaves is shown in increasing trend with increasing concentrations of herbicide (figure 1).

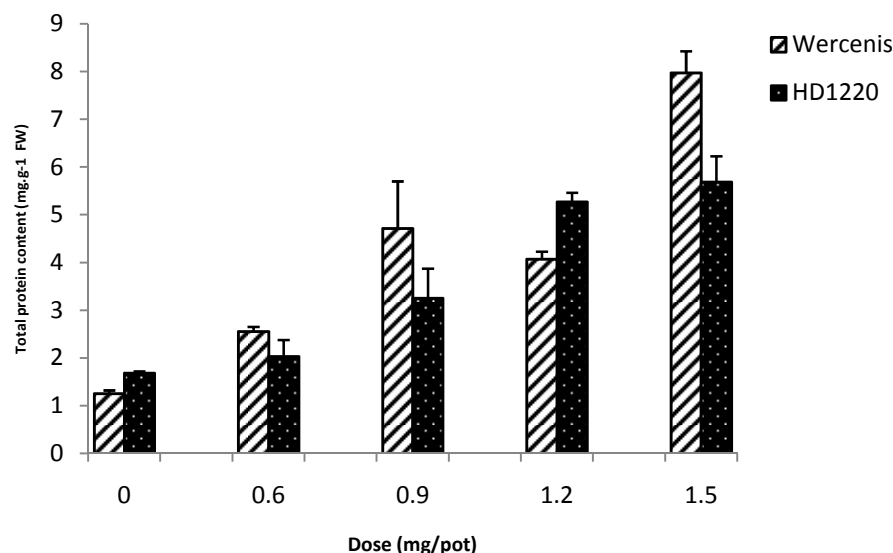


Figure 1. Effects of chevalier on total protein content in leaves of Wercenis and HD1220

At RFD of herbicide, content of protein was not significantly changed in HD1220, but was elevated (49 %) in Wercenis. At 0.9 g/pot concentration, the values changes for protein content in Wercenis and HD1220 were x3 and x2 per rapport to controls.

At 1.2 g/pot treatment, the values changes were x3 for both genotypes, and at 1.5g/pot the values changes were x6 and x3 per rapport to controls respectively for Wercenis and HD1220.

Levels of electrolyte leakage in both wheat cultivars were not influenced by RFD dose of herbicide (0.6 mg/pot), returned near controls levels while treating the plants with 0.9, 1.2 and 1.5 mg/pot of Chevalier provoked a strong increase in permeability membrane estimated in leaves of Wercenis and HD1220 wheat plants. Generally, Wercenis still showed high levels of electrolyte leakage.

The differences in membrane permeability of the wheat leaves under different chevalier concentrations were significant in both cultivars.

Lipid peroxidation (MDA) of the Wercenis leaves was 10.7 nmol.g⁻¹ FW in control plant and it was significantly increased to 29.4 and 38.2 nmol.g⁻¹ FW by the 1.2 and 1.5 mg/pot concentrations of herbicide, respectively (Figure 2). The content of MDA in leaves of HD1220 was the lowest (17.6 nmol.g⁻¹ FW) in the control plant but significantly increased to around 37.3, 46.6 nmole.g⁻¹ FW level by 1.2 and 1.5 mg/pot treatments permeability in leaves of Wercenis and HD1220.

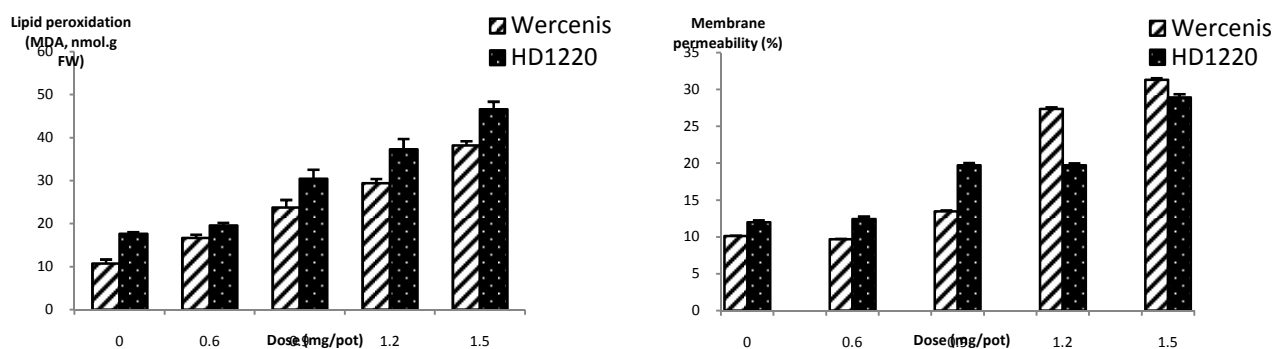


Figure 2. Effects of chevalier on content of lipid peroxides and membrane

Data for the lipid peroxidation in response to treatment by herbicide revealed quite similar results for the plasma membrane status of both genotypes. Chevalier induced increase in malondialdehyde content in leaves of wheat

plants (Figure 2). However, while the levels of MDA in HD1220 plants remained relatively stable at 0.6 mg/pot dose, a further increase was detected in both cultivars at 2, 2.5RFD treatments.

Content of GSH and GST

As shown in figure 4, chevalier high significantly increased GSH content of HD1220 at 0.9, 1.2 and 1.5 mg/pot concentrations then became of not significant effect at 0.6 mg/pot dose (RFD). On the contrary, the herbicide significantly affected these levels in Wercenis at different herbicide treatments. Therefore, the provoked effects of Chevalier GSH content appeared worse in Wercenis than in HD1220. At 1.2 and 1.5 mg/pot doses herbicide, GSH content of treated Wercenis and HD1220 contained much higher levels than controls.

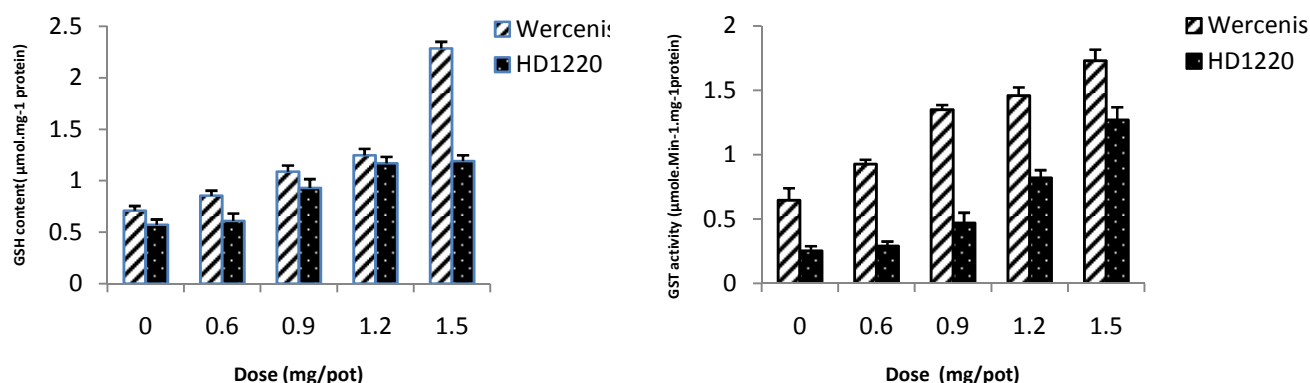


Figure 3. Effects of chevalier on content of GSH and GST activity in leaves of Wercenis and HD1220

As shown in figure 4, GST in Wercenis was greatly enhanced by chevalier at 0.9, 1.2 and 1.5g/pot treatment. On the other hand HD1220 GST activity was significantly elevated by chevalier at 1.5, 2, and 2.5RFD. The dose-dependent curve shows that 0.6 mg/pot of chevalier caused an inhibition in the enzyme activity in HD1220 variety. The 2.5RFD treatment resulted in more induction in the enzyme activity in Wercenis than HD1220.

Herbicide causes many morphological, physiological and biochemical changes in growing plants. The results, generally, showed a great reduction in fresh weight of wheat leaves and roots by herbicide, the reduction was most pronounced with chevalier. Decrease of the photosynthetic pigments including chlorophyll and carotenoid, is the primary indicator of chevalier toxicity, represents the changes in biomass leaves and roots of wheat varieties (Wercenis and HD1220) plants. As compared with controls, Chevalier induced significant reduction in fresh weight of both cultivars. However, the decrease in Wercenis was recovered at 0.6 mg/pot concentration of herbicide, but in HD1220 at 0.9 mg/pot dose of Chevalier.

Although, both cultivars of wheat seemed to have most likely similar contents of biomass foliar and roots, Chevalier caused differential reductions; greater in Wercenis than in HD1220. These results could suggest that HD1220 might be considered as more susceptible variety to Chevalier than Wercenis. The decrease in fresh weights might evidence a stress symptom due to stress induced by different routes, among which oxidative stress. The target site is easily attackable by the herbicide in the susceptible than in the tolerant variety.

However, chlorophyll content was significantly lower in plants grow. A linear drop in chlorophyll content was observed with an increase in the herbicide at all concentrations. This drop in chlorophyll pigment can result from herbicide toxicity and concomitant increased ROS production, which in turn resulted in the damage to the photosynthetic apparatus. Degradation of chlorophylls and carotenoid is a well-known aspect of lead toxicity [18].

Also, [6] reported a decrease in chlorophyll (a+b) content and Hill reaction (PSII) in broad bean and maize seedlings treated with the herbicide fluometuron. They suggested that ROS generation due to the disturbance in the electron transport in PSI and PSII might lead to the herbicide-induced degradation of the biosynthetic machinery of chlorophyll pigments.

Two possible mechanisms of herbicide toxicity on photosynthesis have been proposed to explain the decrease in chlorophyll pigments. Herbicide can alter both chlorophyll biosynthesis by inhibiting protochlorophyllide reductase and the photosynthetic electron transport by inhibiting the water-splitting enzyme located at the oxidizing site of photo system II [19].

In our study, the application of different herbicide concentrations resulted in induction of protein accumulation. Plant incubated with 2.5RFD showed almost a highly increase in protein content as compared to control in both wheat plants. However, an increase in protein content with increasing herbicide dosage could be due to the increased levels of ROS. Previously, the researchers have also reported the differences effects of herbicides on chlorophyll and protein contents in crops, such as rice and potato [20], reported that the functionality of proteins can be affected by ROSs either by oxidation of amino acid side chains or by secondary reactions with aldehydic products of lipid peroxidation.

In cereals the plasma membrane is the most susceptible membrane structure to stress damages, and the impaired integrity is connected with leakage of electrolytes and other solutes [21].

The negative impact of stress oxidant wheat was further supported by the data for lipid peroxidation where continuous accumulation of the lipid breakdown products during recovery period was under consideration.

The elevated MDA level in response to chevalier was in agreement with previously reported results for wheat plants [22]. Protection of cell membrane, thus, is critical for freezing survival and it is usually achieved by accumulation of compatible solutes in the cytoplasm and alterations in membrane lipid composition [21].

Clearly indicates that levels of lipid peroxides (malonaldehyde, MDA) and membrane permeability (MP) increased in both varieties following treatment with chevalier. The magnitude of increase was greater in HD1220 than in Wercenis. However, significant increases were observed with 2.5 RFD of Chevalier. Moreover, the dose-dependent curve indicates overproduction of lipid peroxides in HD1220 than Wercenis.

Therefore, the increases in lipid peroxides and permeability membrane, in the present study, indicate that the chevalier-induced oxidative stress appeared obvious in wheat, a status that seemed consistent in HD1220 than in Wercenis. This consistence might be due to the ability of plants to detoxify and moreover to remove ROS through an enhancement of non-enzymatic and enzymatic antioxidants. [20], reported that the functionality of proteins can be affected by ROSs either by oxidation of amino acid side chains or by secondary reactions with aldehydic products of lipid peroxidation.

The increased rate of MDA give an index of lipid peroxidation and protein oxidation and therefore, of oxidative stress. Increasing in TBA-reacting substances was observed in many plant species due to several factors [23]. Moreover, [24] confirmed that the peroxidation of cell membranes severely affects its functionality and integrity and can produce irreversible damage to the cell function.

[25], found that MDA content increased by fluazifop-p-butyl in bristly strabur seedlings. Therefore, the relative susceptibility of HD 1220 to herbicide, in the present results, is probably due to weak target site and/or less efficiency in the detoxification of ROS and herbicide. Chevalier blocks the flow of electrons to PSI leading to generation of ROS that would react with lipids, proteins and pigments causing lipid peroxidation and membrane damage.

GSH is a versatile antioxidant that can directly scavenge ROS and participate in the AsA- GSH cycle. Moreover, GSH was suggested to play an important role as co-substrate for the conjugation of electrophilic via GST enzymes limiting therefore plant tolerance to herbicides [26]. The increase in GSH content accompanied with great induction of lipid peroxides in wheat plants, in the present results, confirm the Chevalier- induced oxidative stress. The response observed in HD1220 could support the ability of this variety to tolerate chevalier and to overcome its toxicity. Therefore, the change in GSH levels might explain the relative susceptibility of wheat to Chevalier. These results, therefore, confirm that the Chevalier- caused oxidative stress status seemed to be overcome in HD1220 but appeared unrecoverable in Wercenis. This variety contained, moreover, high contents of GSH confirming deficiency in ROS detoxification.

Moreover, the activity of the GST enzyme was also greater in the wheat cultivars, suggesting that this enzyme may be important not only for the detoxification, but also for that of herbicide phytotoxicity, perhaps via the elimination of the lipid peroxidation induced by herbicide. Therefore, the increases in GSH and GST in the relatively tolerant HD1220 variety could conclude that the detoxification rate of Chevalier was more important in HD1220 than in Wercenis. As a consequent would be repaired and the plant thus became healthy enough to produce antioxidants for scavenging of ROS. GST is believed to play a role in antioxidant metabolism by mechanisms that probably aid in the reduction of secondary noxious products resulting from exposure to stress-induced ROS. The present results confirm an augmentation in the GSH-mediated detoxification of chevalier based on the increases in MDA, GSH and GST.

CONCLUSION

In conclusion, both Wercenis and HD1220 cultivars seemed very similar before chevalier treatments yet respond different to the herbicide treatment. HD1220 seemed more susceptible to chevalier than Wercenis.

Biomass foliar and roots were greatly reduced either with increasing herbicide dose. This susceptibility was related with more accumulation of protein and lipid peroxides and with great induction in contents of GSH as well as in activity of GST. The differential susceptibility to Chevalier might result from differences in the response of both varieties to herbicide treatment probably due to the deferential detoxification of both chevalier and ROS. In confirmation, genotype was suffering from chevalier toxicity pointing out to its failure in the protection against oxidative stress. Consequently, the more tolerant variety could easily detoxify Chevalier and subsequently makes more antioxidants for scavenging of ROS.

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