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The influence of different concentrations of herbicide systemic chevalier on physiological and biochemical parameters in spring wheat (*Triticum aestivum* L.)

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Abstract

This research aims at finding the effect of different concentrations of systemic herbicide Chevalier on three varieties of Spring Wheat (cv Salama, ARZ and Hidhab). Grains of wheat, seedlings were germinated in plastic pots 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 Hoagland solution. At the 3 leaf stage, plants were divided into two groups for each variety; one was treated with Chevalier herbicide (control +four increasing concentrations (0.6, 0.9, 1.2 and 1.5 mg /pot). After 2 days leaves were collected, physiological and biochemical parameters were analyzed to characterize the specific response of each cultivar. Data show that pigment chlorophylls contents were strongly modified but were similarly affected by treatment herbicide. Alterations caused by the herbicide, were presented in the form of disturbances affecting the growth and biochemical metabolism of cereal. The results reveal the existence of oxidative stress generated by xenobiotic on the varieties studied, proportional to the concentration used. A large genotypic variability was observed response variety and put in evidence, mostly affects the antioxidant system.

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Introduction

Most environmental stresses are affecting on the production of active oxygen species in plants, causing oxidative stress (Smirnoff, 1993; Hendry, 1994; Bartosz, 1997). Also, there is growing evidence that in plants subjected to environmental stress. The balance between the production of activated oxygen species and the quenching activity of antioxidant is upset, which often results in oxidative damage (Del Rio *et al.*, 1991; Del Vos *et al.,* 1992; Smirnoff, 1993; Karpinski *et al.,* 1997).

Environmental stress causes significant crop losses. The stresses are numerous and often crop- or location-specific. They include increased UV-B radiation, water, high salinity, temperature extremes, mineral nutrient deficiency, metal toxicity, herbicides, fungicides, air pollutants, light, temperature and topography.

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 (Kunert *et al.,* 1985; Peleg *et al.,* 1992).

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 (Edwards, 1996; Kuzniak, 2002; Anderson and Davis, 2004; Misra *et al.,* 2006; Nemat Allah and Hassan, 2006) Antioxidants are crucial for plant defense against oxidative stress. Removal of ROS are regulated by antioxidant enzymes such a catalase, peroxidase and various other endogenous antioxidants such as ascorbate, glutathione and the associated glutathione metabolism enzyme. Some herbicides produce oxidative stress.

In this regard, the objective of this study was to investigate the phytotoxic action of herbicide Chevalier on three varieties of spring wheat, we determined the increased level of ROS induced by herbicide action. Hydrogen peroxide and superoxide anion radical (SAR) and change in the activity of antioxidant enzymes: ascorbate peroxidase, guaïacol peroxidase and catalase.

Materials and methods

Plant materials and growth conditions

Grains of wheat (*Triticum aestivum* L.), cv Salama, ARZ and Hidhab 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 (Hoagland and Arnon 1950), continuously aerated containing: KNO_3 3mM, Ca $(NO_3)_2$ 1 mM, KH_2PO_4 2 mM, $MgSO_4$ 0.5mM, Fe-Ethylene diamine tetra acetic acid (EDTA) 32.9µM, and micronutrients: H_3BO_4 30 µM, $MnSO_4$ 5 µM, $CuSO_4$ 1 µM, $ZnSO_4$ 1 µM, and $(NH4)_6Mo_7O$ 1 µM for 10 days. At the 3 leaf stage, plants were divided into two groups for each variety; one was treated with Chevalier herbicide (control +four increasing concentrations for each herbicide/variety).The herbicides were applied only once as foliar sprays, doses of herbicides 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 were collected after 2days, rinsed with copious amounts of water and dried by plotting with paper towels before the subsequent analyses.

Chlorophyll and total protein assay

Chlorophyll was extracted in 80% acetone. Absorbance was measured at 663 and 645 nm by a spectrophotometer. Extinction coefficients and equations reported by Lichtenthaler and Wellburn (1983) were used to calculate the amounts of Chl a, Chl b and carotenoids. Measurements were done in triplicate.

Biochemical assays

Total protein

The total protein of leaves of wheat is made by the method of Bradford (1976) using Coomassie blue (G250, Merck) as reagent and bovine serum albumin (BSA, Sigma) as standard protein. The absorbance reading is carried out at the wavelength of 595 nm.

Determination of lipid peroxidation

Lipid peroxidation was determined by measuring malondialdehyde (MDA), a major thiobarbituric acid reactive species (TBARS), and product of lipid peroxidation (Heath and Packer, 1968). Samples (0.2 g) are ground in 3 mL of trichloro acetic acid (0.1%, w/v). The homogenate was centrifuged at 10000 ×g for 10 min and 1 mL of the supernatant fraction was mixed with 4 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA. The mixture was heated at 95°C for 30 min, chilled on ice, and then centrifuged at 10000 ×g for 5 min. The absorbance of the supernatant was measured at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The amount of MDA was calculated using the extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmol g^{-1} FW.

Enzyme assays

Fresh leaves were homogenized in 50 Mm potassium phosphate buffer pH 7.6, the homogenized samples were centrifuged at 12000 ×g for 20 min and the supernatant was used as crude enzyme extract in CAT, APX and GPX (Loggini *et al.,* 1999).Catalase (CAT) activity was determined as a decrease in absorbance at 240 nm for 3 min following decomposition of H_2O_2 (Cakmak and Horst, 1991).The reaction mixture 3 ml contained 50 mM phosphate buffer pH 7.2, 15 mM H_2O_2 and 100 µl of crude enzyme extract. The activity was calculated using the extinction coefficient 39400 $M^{-1}cm^{-1}$.

Ascorbate peroxidase (APX) activity was determined by following the decrease of ascorbate and measuring the change in absorbance at 290 nm for 3 min in 3 ml of a reaction mixture containing 50 Mm potassium phosphate buffer pH 7.2, 0.5 Mm ascorbic acid, H_2O_2 and 100 µl of crude enzyme extract (Nakano and Asada, 1981). The activity was calculated using the extinction coefficient 2800$M^{-1}cm^{-1}$.

For gaïacol peroxidase (GPX) activity, the reaction mixture consisted of 50 mM potassium phosphate, 9 mM gaïacol buffer pH 7.2, 50 µ H_2O_2 and 100 µl of crude enzyme extract. The enzyme activity was measured by monitoring the increase in absorbance at 470 nm extinction coefficient of 2470 M^{-1} per cm during polymerization of gaïacol (Hiner *et al.,* 2002).

Results and discussion

These studies were performed to analyze some cellular reactions, representative of oxidative damage and antioxidant response in three varieties of wheat treated with herbicide Chevalier. Data show that pigment chlorophylls contents were strongly modified but were similarly affected by treatment herbicide (Fig. 1). Chlorophyll a and b decreased with increased herbicide concentrations.

The percentage change to 72 %, 84 % respectively for Salama, 53%, 42 % for ARZ and 71 %, 84 % for HD1220, at the dose max as compared to the controls (Fig. 1A, 1B). The total carotenoids content in the leaves investigated decreasing as compared with controls for Salama; Chevalier induced no significant reduction in the contents of photosynthetic pigments in three varieties (Fig.1C). 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. Also, Nemat Allah *et al. (2008)* reported a decrease in chlorophyll content and Hill reaction (PSII) in broad bean and maize seedlings treated with the herbicide fluometuron.

The Fig. 2A, illustrate the effects of the Chevalier on the total protein content among the three varieties, we note a highly significant stimulation in total protein ($p \leq 0.005$) of treaties by contribution to the control, for the three genotypes. Protein in leaves is shown in increasing trend with increasing concentrations of herbicide (Fig. 2A). Thereby stimulating the rate of protein recorded at the plants,

reflects the existence of proteins synthesized during the stress of xenobiotic and/or heavy metals, possibly existing in the experimental water environments, as reported in work of Dixon *et al.* (2003).

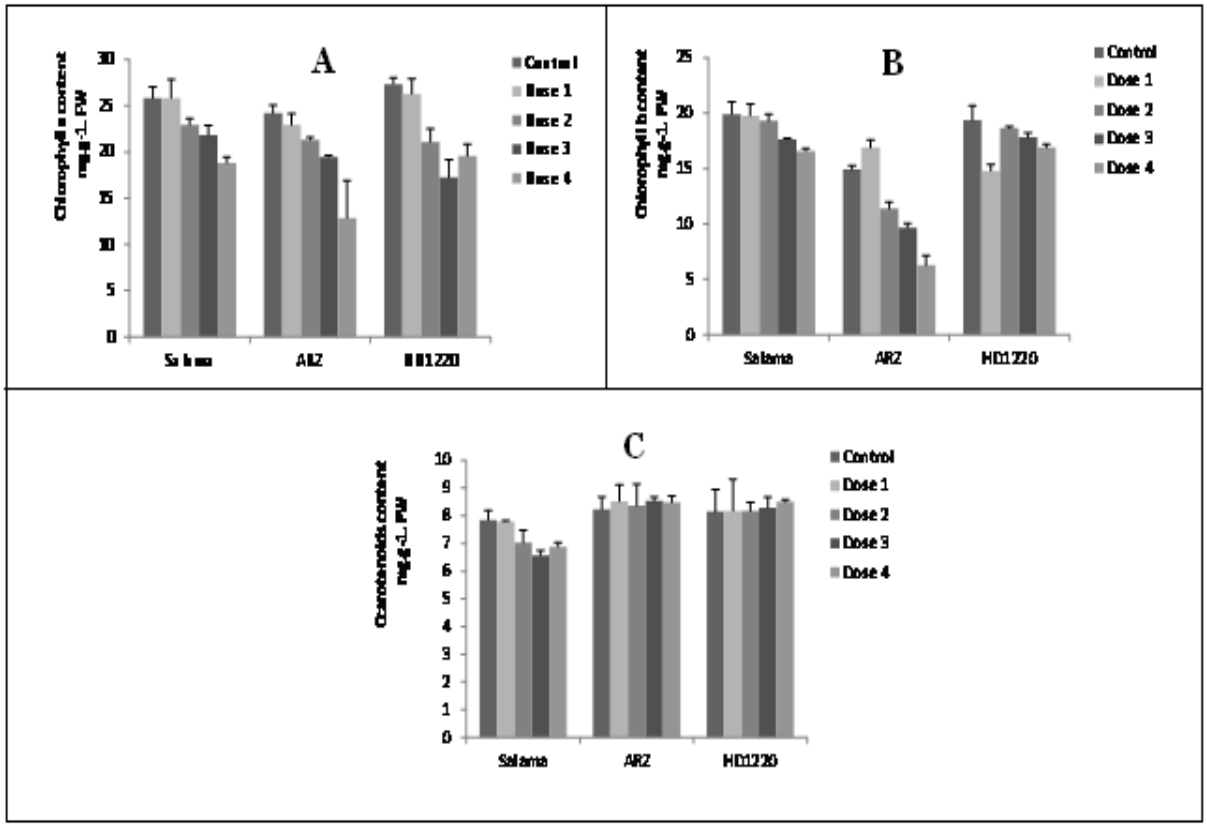

Fig. 1. Effects of chevalier on chlorophyll a (A), chlorophyll b (B) and carotenoids (C) contents in leaves of three varieties studied.

Lipid peroxidation (MDA) of the Salama leaves was 0,99 µmole.g-1 FW in control plant and it was significantly increased to 1,73 µmole.g^{-1} protein by 1.5 mg/pot concentrations of herbicide (Fig. 2B).

The content of MDA in leaves of ARZ was the lowest (0.73 µmole.g^{-1} protein in the control plant but significantly increased to around 1.63 µmole.g^{-1} protein levels by 1.5 mg/pot treatments.

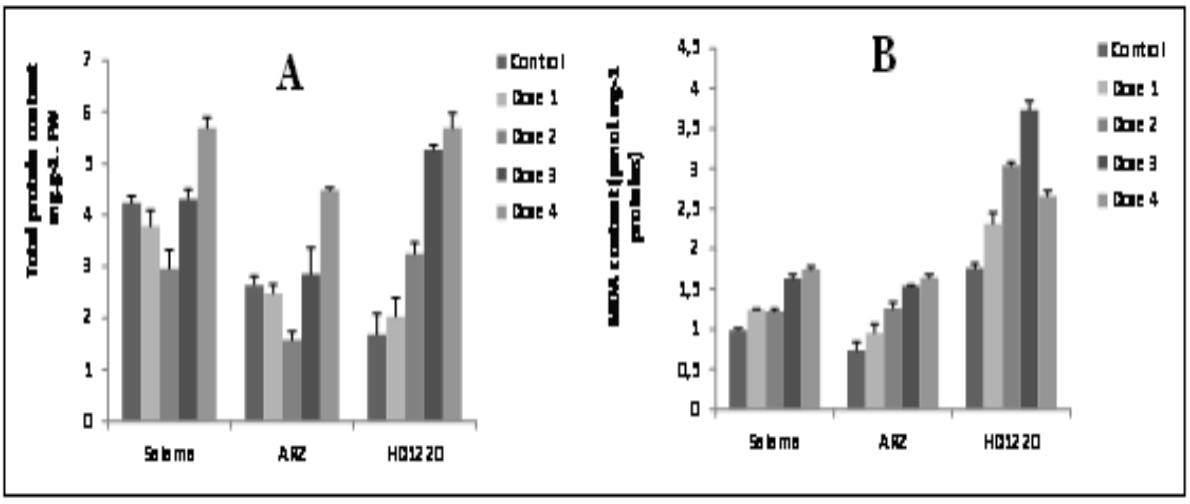

Fig. 2. Effects of chevalier on total protein (A) and MDA (B) contents in leaves of three varieties studied.

Therefore, the increases in lipid peroxides in the present study indicate that the chevalier-induced oxidative stress appeared obvious in spring wheat, a status that seemed consistent in HD1220 than in Salama and ARZ.

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 (Dixit *et al.,* 2001).

The effects of Chevalier on the catalase activity (CAT) are shown in Fig. 3A in leaves of wheat; reveal that the latter increases very significantly ($p \leq 0.005$) contribution by the control, for the three varieties. The analysis of variance to two criteria for classification, shows that the levels of catalase differ significantly from one variety to another and seem to be more low among the variety ARZ who, displays in the presence of the same four concentrations of Chevalier an increase of 36 per cent of the content of catalase to the max dose male by report to the control (Fig. 3A).

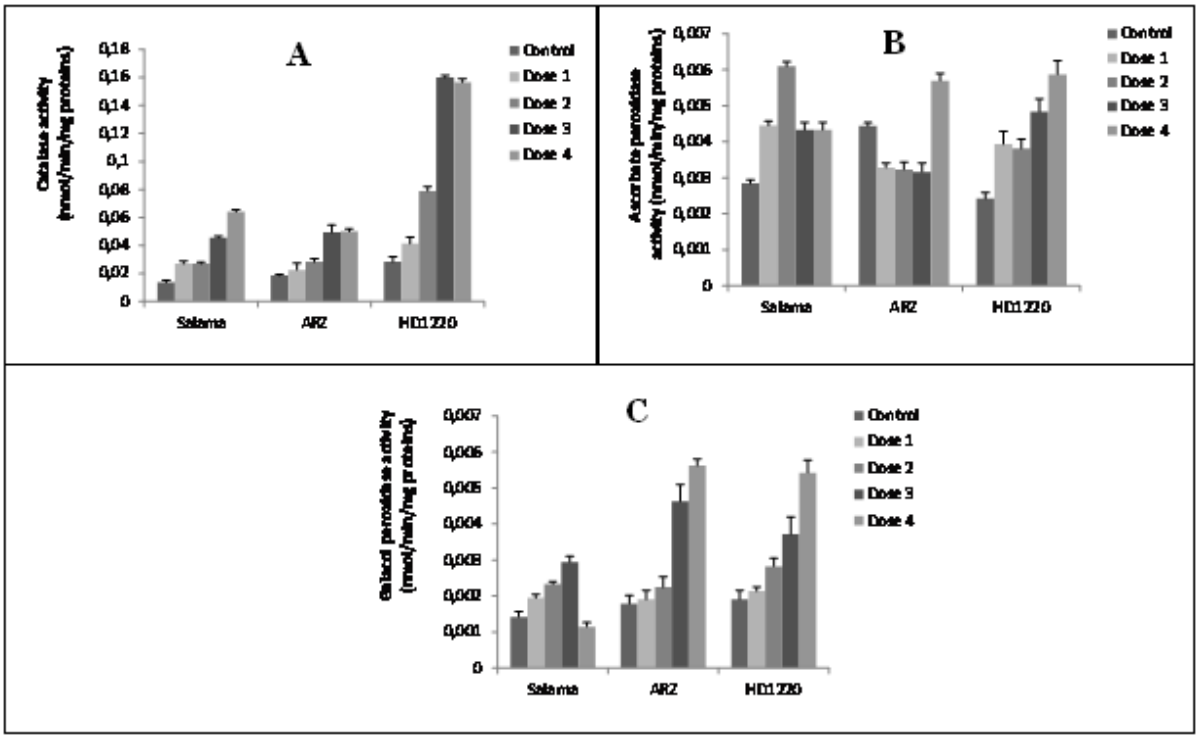

Fig. 3. Effects of chevalier on catalase (A), ascorbate peroxidase (B) and gaïacol peroxidase (C) activities in leaves of three varieties studied.

The activation of the APX determined by the different concentrations of herbicide is more important in HD1220 with a maximum of 0.006 nmol /min/mg protein at the dose max and a minimum of0.0038 nmol /min/mg protein at 0.9 mg/pot compared to the control. In ARZ, the activity of this enzyme decreases where as the concentration of the herbicide is growing under the effect of other concentrations (Fig. 3B). For what is the difference between the cultivars, the activity of the APX has been more important in HD1220, which suggests that this variety detoxify better than ARZ and Salama the hydrogen peroxide (Fig. 3B).

In the case of the gaïacol peroxidase and under treatment by Chevalier, we note a similar situation to the one reported in the case of the dynamics of catalase, because for the same concentrations, the activity increases as the dose increases and this increase has just corroborate our hypothesis of a oxidative damage caused by the two active substances of Chevalier on the three varieties studied (Fig. 3C).

The activity of the gaïacol peroxidase in the plants of spring wheat treated with different concentrations of Chevalier increases at the same time as the concentration of the herbicide increased in the genotypes studied with the exception under the ford dose or it becomes less than that of control in Salama. The activator effect exercised by Chevalier on this enzyme is more marked after treaty HD1220 and ARZ by dose max of the herbicide with three times when compared to untreated controls (Fig. 3C).

Conclusion

This comparative study which was designed to highlight the effect of systemic herbicide Chevalier on three varieties of spring wheat s, has shown that the response to this xenobiotic differs from a cultivar to another and depends on the dose herbicide applied. However, the changes observed in the total protein and the MDA indicate that the herbicides induce oxidative stress. Under the conditions employed, the increased activities of antioxidant enzymes could be suggested to play a role in wheat tolerance to herbicide.

The results of the response of each variety to xenobiotic present indicate that, Salama tolerated Chevalier by intense activity catalase. The response of ARZ is essentially based on an activity gaïacol peroxidase and catalase; it becomes tolerant to the Chevalier and H1220 exploit the three enzymes antioxidants, which allows you to classify them as sensitive to herbicide.

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