Effects of Sulfonylurea Herbicides on Protein Content and Antioxidants Activity in Wheat in Semi-Arid Region

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Abstract— *Herbicides have been used in the crop field to increase the crop productivity and grain yield. But unfortunately since long back all research papers and articles showed the effect of this herbicide on the non-target organisms. The purpose of this study was to test the toxicity of two herbicides (Sekator and Zoom) on the biochemistry of hard wheat: Triticum durumDesf and common wheat: Triticumaestivum L. The herbicides were applied at tillering stage in the dose full recommendeddoses. After eight days of treatment leaves were collected to determine differentparametersof proteincontent and enzyme activity involved in the antioxidant system (ascorbate peroxidase: APX).The results validated by statistical analysis revealed that there was a highly significant decrease in proteincontent in plant samples treated with the herbicide Zoom in Hidhab variety. By contrast, exposure to the herbicide Sekator causes significant variation in APX activity. Peroxidase activity were significantly decreased in Waha variety but it was significantly increased in Hidhab (HD), and APX activity increased compared with controls in HD treated with Zoom herbicide.The decrease in protein content is an indication of reduction in the growth of the plants leading to a decrease in the yield, while increased level of peroxidase activity pointed to the occurrence of a scavenging mechanism.*

Keywords— effect, herbicides, oxidative stress, protein content, peroxidase, hard wheat, common wheat.

I. INTRODUCTION

Cereals have great importance in agricultural research programs worldwide [1].While wheat and barley are the main cereal crops in Algeria, durum wheat is by far the most cultivated grain. However, wheat crop often confront abiotic stresses such as drought, salinity, which are among the most important strength-limiting factors of wheat production particularly in arid and semi-arid areas [2]. Bread wheat (*Triticumaestivum* L.) is an important staple food in Algeria. This crop ranks third after durum wheat (*Triticum durum*Desf.) and barley (*Hordeumvulgare* L.), with a yearly cropped area of 0.8 million hectares, representing 24.2% of the 3.3 million hectares devoted to small grain cereals. *Triticum durum* L represents 6–8% of the total worldwide wheat production and its major use is the manufacturing of pasta, couscous and traditional dishes, such as burgul[3]. The presence of weeds within the crop may adversely affect production in a number of ways. Weeds are unwanted plant species growing in the domesticated crops. The competition of weeds for nutrients may results in such obvious responses as dwarfing in plant size, nutrient starved conditions, wilting and actual dying out of plants [4]. Weeds are notorious yield reducers that are, in many situations, economically more important than insects, fungi or other pest organisms [5]. They may compete with the cash crop for light interception [6]. Competition for soil nitrogen may also appear with some high nitrogen-competitive weed species (e.g. *Amaranthusretroflexus* L.) [7]. Moreover, weeds are often more competitive than cereals for nitrogen nutrition coming from the soil [8]. In some cases, the allelopathic effect of weeds can be harmful to cereal crops as shown by [9] with wild barley (*Hordeumspontaneum* Koch) to the detriment of *Triticumaestivum* L.

Although herbicides have improved the viability of farmers and helped to reduce the risk of soil erosion, the weed control with cultivation is less expensive than herbicides, which may be seen as a potential ecological hazard [10].The evaluation of herbicides used in wheat fields depends not only on their efficiency of controlling weeds, but also on their effect on growth and yield of wheat plants [11].

Chlorsulfuron, and metsulfuron methyl plus sulfosulfuron are post-emergence sulfonylurea herbicides. These herbicides act through inhibition of acetolactate synthase and affecting the meristemic tissues. Growth ceases soon after treatment and chlorosis and the necrosis of these tissues soon follow [12]. It has been reported that these

herbicides are able to control weeds like *Sinapisarvensis* L. (wild mustard), *Capsella bursa-pastoris* (L.) Medicus (shepherds purse), *Chenopodium album* L. (common lambsquarters), *Polygonum convolvulus* L. (black bindweed), *Loliumrigidum*Gaudin (ryegrass), and *Phalaris minor* Retz. (canarygrass) [13].

Herbicides drastically influence all aspects of primary and secondary metabolisms in crops when given to control undesired weeds. Their applications in fields limit harmful effects of weeds. However, they can have certain effects on changes in physiological process of crops [14]. A few studies have, however, shown that like other abiotic stresses, herbicide also induces oxidative stress in plants. One of the biochemical changes occurring when plants are subjected to biotic or abiotic stresses is the production of reactive oxygen species (ROS) [15]. Over expression of ROS affects negatively plant and cell metabolism as a mechanisms of herbicide detoxification [16, 17]. To resist oxidative damage, plants have developed antioxidant protective mechanisms that enable them to counteract the production of ROS. These protective mechanisms include changes in the lipid composition, changes in the antioxidant enzyme activity, increased sugar or amino acid contents, and changes in the level of soluble proteins and gene expression [18, 19]. The peroxidase enzyme is reported in wide range of biological functions such as indole acetic acid (IAA) catabolism, pathogen defense and scavenging of toxic peroxides by oxidation [20].

The objective of this study was to elucidate the effects of two herbicides on protein content and enzymatic (peroxidase) antioxidants in *Triticum* seedlings.

II. MATERIALS AND METHODS

1.1. Study area and site characteristics

The field experiments of wheat were conducted at two locations in Tebessa (semi-arid region in Algeria) during 2012/2013. The sowing was conducted with an on-line leading seed drill solla, at the rate of 300 seeds /m ². The crop was fertilized with100 $kgha^{-1}$of urea 46% and sprayed by Sekator (150ml /ha) and Zoom (120 g/ ha) in March, these herbicides used in wheat fields to control weeds. Leaves of varieties Waha and HD were collected from the plot treated with herbicidesSekatorand Zoom, while all other samples (controls) of both varieties were removed from thecultivated site at the plot untreated.

1.2. Herbicides used

Two herbicides were applied at tillering stage in the dose full recommended doses to treat weeds of wheat:

Sekator: belongs to the sulfonylurea as, they exercise the iractionthroughblocking cell division at them eristem, by inhibiting the acetolactate synthase enzyme (ALS), which is crucial in the biosynthesis of essential branched-chainami no acids: valine, leucineandisoleucine[21, 22].

Zoom:Is selective herbicide against annual and someperennialweeds dicotyledonousinwheat. It is absorbed by the roots and leaves.It actsprimarilyby inhibitingthe growth of weeds which become necroticanddie within a few weeks[23].

1.3. Determination of Soluble Proteins

The method of Bradford [24] was used to determine the concentration of soluble proteins in wheat leaves with BSA as standard. Absorbance was recorded at 595 nm. Soluble proteins were expressed as µg/g FW.

1.4. Enzyme Assays

After 7 days of treatment, the fresh leaves (1g) were ground in a cold mortar with 5 ml of phosphate buffer (80mm, pH7.5). The homogenate is then filtered through a cloth properly before centrifugation at 12000g for 20min Cold (centrifuge Sigma 3-16K). The supernatant obtained was used as enzyme extract for determination of different enzyme activities.

1.5. Determination of the Activity-ascorbate peroxidase (APX)

The spectrophotometric determination ofascorbate peroxidase activity is carried out following theprocedure adopted by [25].The final reaction volume of 3ml contains: 100µl of enzyme extract, 50µl of 0.3% H_2O_2 and 2850µl phosphate buffer NaK-Ascorbate (50 mMNaK, 0.5 mMascorbate, pH7.2). The calibration of the device is in the absence of the enzyme extract. Lecture is performed at 290 nm (spectrophotometer GeneSys 8) for 1min and for a linear molar extinction coefficient = 2800 M^{-1} .cm-^{1}. APX activity is expressed as nmol/min/mg of protein.

1.6. Statistical analysis

All values reported in this study are the mean of at least three replicates. For each parameter, data were subjected to a one-way ANOVA analysis (MINITAB software version 16.0). The standard deviation was plotted in all graphs.

II. RESULTS AND DISCUSSION

The effect of Sekator and Zoom on the content of protein (µg/g of Fw) illustrated in figure 1 and the variance analysis for each herbicides and variety of wheat (Table 1), showed highly significant differences ($p \leq 0.05$) for HD variety treated with Zoom and non-significant differences ($p > 0.05$) for variety Waha and HD weeded by Sekator.

Table. 1:Analysis of variance results comparing protein content obtained for each herbicide applied to the two varieties of Triticum.

Herbicides	Varieties	Statistical parameters			
		ddl	Mean Square	F_{obs}	P
Sekator	**Waha**	1	10,00	0,74	**0,438 NS**
	HD	1	04,05	3,77	**0,124 NS**
Zoom	**Waha**	1	00,02	0,00	**0,960 NS**
	HD	1	11,98	23,45	**0,008****

*: Significant differences ($p \leq \alpha = 0.05$).
**: highly significant differences ((p <_α = 0,01)
NS: non-significantdifferences (p> 0.05)

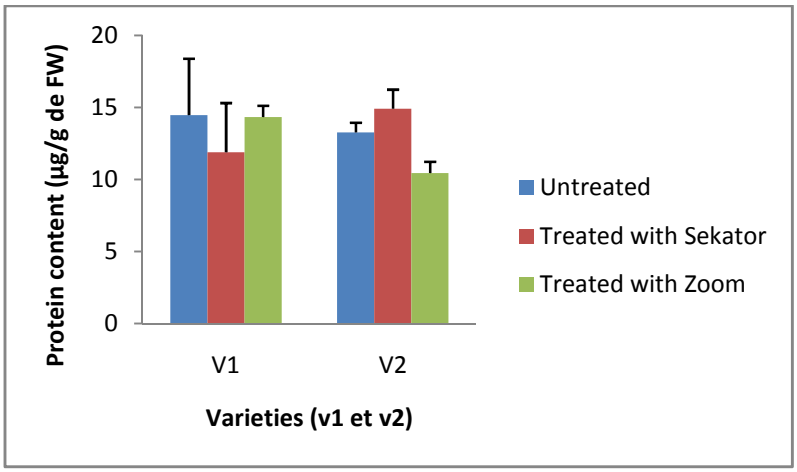

Fig.1:Effect of Sekator and Zoom on protein content (µg/g de FW) in two wheat varieties Waha (V1) and HD (V2).

The induction of APX activity, an enzyme important in defense system is a response to oxidative stress in plants. The APX protects the cell against oxidative damage by H_2O_2toxicity. From Figure 2 we observed that treatment with herbicides Sekator and Zoom stimulates the activity of APX enzyme in HD variety. Whereas this activity was inhibited for concentration of Sekator in Waha variety. This was confirmed by analysis of variance showed highly significant differences (**) APX activity (Table 2)as a function of concentration of the herbicide Sekator. Enhancement in the activity of peroxidase suggests that this enzyme serves as an intrinsic defense tool to resist herbicide induced oxidative damage in plants.

Table 2:Analysis of variance results comparing peroxidase activity obtained for each herbicide applied to the two varieties of Triticum.

Herbicides	Varieties	Statistical parameters			
		df	Mean Square	F_{obs}	P
Sekator	**Waha**	1	1666,7	26,33	**0,007 ****
	HD	1	1442,4	48,53	**0,002 ****
Zoom	**Waha**	1	309	1,73	**0,258 NS**
	HD	1	474, 2	10, 32	**0,033 ***

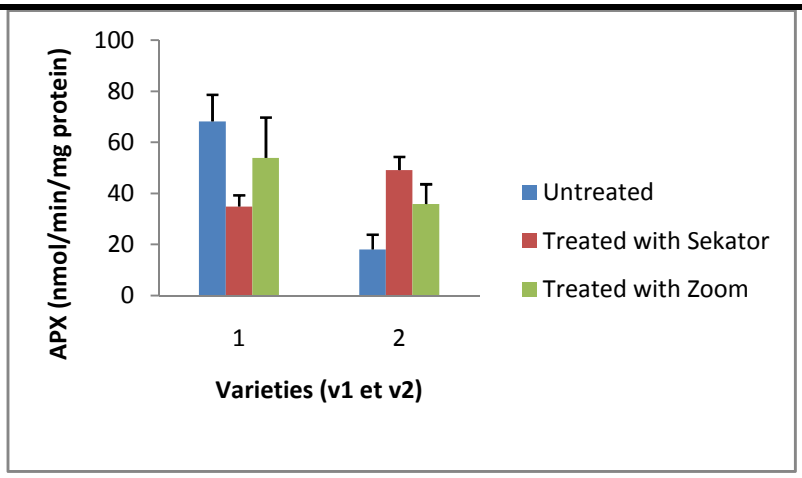

Fig.2: Effect of Sekator and Zoom on peroxidase activity (nmol /min/ mg protein) in two wheat varieties Waha (V1) and HD (V2).

Herbicides are used for weed control in the agricultural fields. Since these compounds can be carried away from the fields they are applied, they cause environmental hazards in wide areas [26]. When herbicides are applied in an uncontrolled manner, not only are weeds damaged but the metabolic and physiologic processes of non-targeted plants would also be affected [27, 28, 29]. Plants have developed various strategies in order to cope with negative effects of herbicides. Changes in antioxidant enzyme level determine herbicide tolerance of plants [26, 30]. In this study, we observed altered biochemical parameters in wheat leaves as a result of the herbicide treatment.The reduction in the amount of protein could be due to decrease in protein synthesis or an increase in the rate of protein degradation [31].

Reduction in protein content of plants may be due to the enhanced rate of protein denaturation and break down of existing protein to amino acid or reduced *denovo synthesis* of protein which is also supported by the findings of [32, 33, 34, 35].

Peroxidases which constitute a wide variety of heme-containing enzyme act in a wide range of normal and stress related physiological processes in plants [36].

Ascorbate–glutathione (AsA–GSH) pathway plays significant role in protecting cells against ROS in plants. APX and GR are the key enzymes of the AsA–GSH pathway [37]. APX utilizes AsA as the electron donor and functions by scavenging H_2O_2. APX and GR activities have been shown to increase in various plant species under different stress conditions [38, 39]. [28] previously reported that herbicides, particularly at high concentrations, cause the accumulation of H_2O_2 and $O^{\cdot}{}_2$ at high amounts, which can inhibit the activities of enzymes including, in particular, CAT and group III peroxidases. In this study, we observed that under the influence of the herbicide, the activity of APX in cereal leaves disks was lowered or increased. The lower APX activity was noted in maize disks incubated in the lowest TOPIK concentration and after 1 h of treatment. The highest activation (5.4–5.8-fold) was observed in wheat and rye, less so in maize. It seems likely that APX and other peroxidases play a substantial role in the elimination of cell damage induced by xenobiotics[40]. Changes in activities of antioxidant enzymes at various herbicide concentrations and exposure times are directed to suppressing oxidative stress by eliminating ROS produced during all stages of stress. Differences in enzyme activities in different plant species are related to plant physiology and functioning of antioxidant systems in cells [41].

III. CONCLUSIONS

From the observed results, it is concluded that the activity of peroxidase increased in response to the herbicide concentration from recommended doses in HD variety of common wheat. The increased peroxidase activity indicates the scavenging mechanism of the plants against the ROS produced in response to the herbicide stress. The defense mechanism of the plants against stress was thus observed with the increased production of peroxidase.From the present study it can be concluded that HD variety of *Triticum*was most sensitive among the selected varieties as maximum reduction in protein content was observed in it along with maximum increase in peroxidase activity.

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