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Investigation of the Effects of Salicylic Acid on Some Biochemical Parameters in *Zea mays* to Glyphosate Herbicide

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Abstract

In this study, investigated the possible mediatory role of salicylic acid (SA) in protecting Zea mays L. "Martha F1" seedlings from glyphosate toxicity. 0.5 mM SA was treated as preemergence and 17-145 mM glyphosate herbicide was treated postemergence to same groups. The effects upon Peroxidase (POD), Ascorbate Peroxidase (APX), Superoxide Dismutase (SOD), Catalase (CAT) reduced glutathione (GSH), Glutathione Reductase (GR), Glutathione S Transferase (GST), lipid peroxidation, total chlorophyll and total soluble carbohydrate content of this herbicide were investigated on the 1st, 5th and 10th days following the treatment.

Keywords: Glyphosate; Salicylic acid; Antioxidant; Lipid peroxidation; Total chlorophyll; Total soluble carbohydrate

Introduction

Zea mays L. is the most important cereal crop in the World after wheat and rice. While in western countries maize production is highly mechanized, in many other -mainly developing countries - the crop is still grown by smallholders and medium-scale farmers, using traditional and low-input cultivation techniques. Yields under those circumstances are much lower. Besides, maize is an important staple food in developing countries, and a basic ingredient for local drinks and food products. It is also and outstanding feed for livestock, high in energy, low in fiber and easily digestible. As a source of starch, it is major ingredient in industrialized food products [1].

Pesticides are the chemical species that cause death and avoid or reduce growth of plants or animals that are considered as pests. Herbicides are a class of pesticides that are used to kill weeds and other undesirable life forms in agricultural crops [2-4].

Glyphosate is the most extensively used herbicide in the agriculture. Weed management programs in glyphosate resistant field crops have provided highly effective weed control, simplified management decisions, and given cleaner harvested products. However, this systemic herbicide can have extensive unintended effects on nutrient efficiency and disease severity, thereby threatening its agricultural sustainability [5].

Glyphosate acts as a non-selective total herbicide by inhibiting the shikimate pathway responsible for the biosynthesis of aromatic amino acids and phenolic compounds [6], thereby causing impairment of general metabolic processes, such as protein synthesis and photosynthesis [7-9].

When plants are sprayed in crop fields and sub lethal doses of herbicides reach non-target plant species in adjacent habitats through drift, runoff and/or volatilization, resultant effects on sensitive species can be observed in any of four ways: a) Plants at the seedling stage during spray will have their vegetative parts affected, b) the same plants could express the effect through negative impacts on seed production at later stages, c) plants at the reproductive phase during spray have their seed production impacted or d) the vegetative parts of the F1 generation are affected. Therefore, it appears that seedlings and plant species at late vegetative and reproductive stages may be affected differently, and this is most likely influenced in turn by the type of herbicide applied [10].

SA is a common plant-produced phenolic compound and a potential endogenous plant hormone that plays an important role in plant growth and development [11,12]. The role of SA is intensively studied in plant responses to biotic stress. In recent years, the involvement of SA in the response to abiotic stresses has come into light [13]. It has been suggested that SA has great agronomic potential to improve the stress tolerance of agriculturally important crops [14,15]. Besides providing disease resistance to the plants, SA could regulate the activities of antioxidant enzymes and increase plant tolerance to abiotic stresses [16,17]. Recent evidence also suggests that SA is an important regulator of photosynthesis because it affects leaf and chloroplast structure [18,19].

In indirect stress perception ROS are components frequently used as signalling molecules. However, ROS themselves can be subject to direct or indirect perception mechanisms [20]. Under normal growth conditions, ROS are inevitably generated in cellular compartments during oxygen metabolism, but antioxidative systems control the level of ROS. Efficient defense system enzymatic antioxidant: POD, APX, SOD, CAT, GR and GST and also non-enzymatic antioxidants: ascorbate, GSH etc. may regulate ROS level directly or indirectly and thus, the antioxidants are an indicative of level of tolerance in plants [21]. In stress condition, the balance between the productions of ROS and antioxidants get disturbed and thus, level of ROS is enhanced to an extent that causes severe damage to the biomolecules [22,23]. ROS directly react with biomolecules cause lipid peroxidation, protein oxidation and DNA mutation [24,25].

This work was to show the changes of the antioxidant system in response to glyphosate herbicide and the effect of SA pretreatment on maize. The antioxidant status was investigated through analyzing changes in POD, APX, SOD, CAT, GSH, GR, GST changes and

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determining the lipid peroxidation level. Besides, in this study, total chlorophyll and total carbohydrate content in *Z. mays* were determined. In addition, this work was to provide evidence for SA protective interference action and regulation of oxidative stress caused by glyphosate toxicity in maize.

Materials and Methods

Preparation of the plant samples

In the present study, the glyphosate herbicide was provided from Sygenta Company and *Z. mays* L. cv. "Martha F1" seeds were provided from May Seed Company. The samples were grown in perlite-containing pots by using Hoagland's solution [26]. The tests were conducted in a climate room with a temperature of 23 ± 2 ºC and a humidity of 60%. Samples were planted after a portion of the plants was kept for six hours in distillated water and another portion was kept for six hours in 0.5 mM SA solution. On the 21st day of the growth, post-emergence glyphosate was applied to corn plants of appropriate size by spraying in doses of 17, 23, 30, 39, 51, 66, 85, 111 and 145 mM. The leaf samples were extracted from the treatment groups on the 1st, 5th and 10th days and subjected to analyses.

In the preliminary trials performed with solutions in different concentrations prepared by taking the application dose of glyphosate to the terrain into consideration, the toxic doses were determined for corn and the upper and lower concentrations of this dose was applied to corn by considering the possible residue in the soil depending on the half life of herbicide. In the evaluation after preliminary trials it was observed that SA response is better in 0.5 mM concentration concerning stress response.

Determination of POD

POD activity was performed by following the methods of Peters et al. [27]. Enzyme activity was measured at 436 nm according to Mac Adam et al. [28].

Determination of APX

APX activity was performed by following the methods of Nakano and Asada [29] and Cakmak [30]. The enzyme activity was defined as the alteration in absorbance per minute at 290 nm. APX activity was calculated by using the extinction coefficient of 2.8 mM-1 cm-1.

Determination of SOD

SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of Nitro Blue Tetrazolium (NBT) according to the method of McCord and Fridovich [31]. One unit of the enzyme activity was defined as the amount of enzyme required to result in a 50% inhibition of the reduction rate of NBT under assay conditions.

Determination of CAT

CAT activity was measured according to the method of Luck by measuring the decrease of absorbance at 240 nm because of H_2O_2 decomposition. One unit of enzyme activity was defined as the amount of the enzyme that decreased 1 µmol H_2O_2 min^{-1} [32].

Determination of GST

GST activity was assayed according to the method of Habig et al. [33] with 1-Chloro-2,4-DiNitroBenzene (CDNB) as substrate. Enzyme activity was determined by monitoring changes in absorbance at 340 nm, which is related to the rate of CDNB conjugation with GSH.

Determination of GR

GR activity was assayed by the method of Cribb et al. [34]. The reaction was initiated by the addition of the GSSG to the cuvette, and the decrease in absorbance at 405 nm was examined at 30 °C for 1 min with UV spectrophotometry. A unit of GR activity is defined as the amount of the enzyme catalyzing the reduction of 1 µM of NADPH per min.

Determination of GSH

Glutathione amount was measured according to the method by Akerboom and Sies [35]. GSH concentration was estimated from a standart curve and reported as µmol GSH/mg protein.

Determination of Lipid peroxidation

The method was performed by following Heath and Packer [36]. Absorbance of the supernatant was measured at 532 nm and 600 nm and MDA content was calculated using an extinction coefficient of 155 mM-1 cm -1 by subtracting the absorbance at 532 nm from that at 600 nm.

Determination of total chlorophyll

De Kok and Graham's method [37] was employed in pigment extraction. Absorbance values of the centrifuged samples were read according to Lichtenthaler and Welburn [38] at 662, 645 and 470 nm.

Determination of soluble carbohydrate content

The content of total soluble carbohydrate was measured according to the method recommended by Rosenberg using glucose as a standard at 620 nm [39].

Determination of total soluble protein

We determined the total soluble protein content as previously described by Bradford [40] using BSA as a standard. We spectrophotometrically measured reactions at 290 nm.

Statistical analysis

Statistical analysis was performed using SPSS 15.0 software. Duncan's test [41] was used for significance control ($p<0.05$) following variance analysis.

Results

Enyzme activities

POD activity was highest on the 1st day in 66 mM glyphosate applied group, on the 5th day and 10th day in 111 mM glyphosate applied group. The lowest POD activity was measured in control group on the 1st, 5th and 10th days. POD activity increased on the 5th and 10th days depending on days. These changes were statistically significant ($p<0.05$) (Table 1).

It was determined that the lowest APX and SOD activity were observed in control group on the 1st, 5th and 10th days. APX and SOD activity increased as the number of days increases (Tables 2 and 3). We statistically determined that CAT activity increased on the 5th and reduced on the 10th days together with concentration increase (Table 4).

The lowest GSH content on the 1st day was determined in control group. There was an increase in GSH content together with increasing glyphosate concentration. GSH content increased on the 5th and 10th days in 17-66 mM glyphosate applied groups and decreased on the 10th

day in 85-145 mM glyphosate applied groups (Table 5). The GR activity increased on the 5th day while decreased on the 10th day. The highest GR activity was determined on the 5th day in 145 mM glyphosate applied group as 0.492 μ/mg protein (Table 6). The highest activity of GST was determined on the 10th day in 145 mM glyphosate applied group. These changes were statistically significant (Table 7).

MDA content

The MDA content increased compared to control group. MDA content also increased on the 5th and 10th days compared to 1st day in the SA-treated plants. The highest MDA content was determined as 7.00 μmol MDA/g FW in 66 mM glyphosate applied group on the 1st day, 9.58 μmol MDA/g FW in 85 mM glyphosate applied group on the 5th day and 14.31 μmol MDA/g FW in 145 M glyphosate applied group on the 10th day (Table 8).

Total chlorophyll

The highest total chlorophyll content was determined in control group on the 1st, 5th and 10th days. The lowest total chlorophyll content was determined as 11.41 μg/g in 145 mM glyphosate applied group on the 1st day, 9.73 μg/g in 66 mM glyphosate applied group on the 5th day and 9.62 μg/g in 145 mM glyphosate applied group on the 10th day. We statistically determined that total chlorophyll content reduced on the 5th and 10th days (Table 9).

Total soluble carbohydrate

The highest total soluble carbohydrate content was determined in control group on the 1st, 5th and 10th days. The total soluble carbohydrate content decreased depending to increasing concentrations on the 5th and 10th days. The lowest total soluble carbohydrate content was determined as 0.43 μg/g in 145 mM glyphosate applied group on the 1st day, 0.24 μg/g in 111 mM glyphosate applied group on the 5th day and 0.18 μg/g in 145 mM glyphosate applied group on the 10th day. These changes were statistically significant ($p<0.05$) (Table 10).

Discussion

Glyphosate is commonly used in agriculture, forestry, and nurseries for the control or destruction of herbaceous plants [42]. Plants have evolved various protective strategies to minimize the herbicide toxicity. One of the protective mechanisms is the antioxidant system [43]. SA is used for regulation of oxidative stress in plants subjected to unfavorable environmental conditions [44]. The present study explores the effect of SA on *Z. mays* under glyphosate stress.

Adverse effects after coffee exposure to glyphosate have been shown both as damage [45,46] and as a reduction in plant nutrient concentration [47] after a glyphosate spray drift simulation [48].

POD activity in plant tissues has been used as a biomarker for various contaminant stresses [49-51]. POD upregulation after herbicide exposure has been demonstrated in wheat [52], tobacco [53] and many other plant species. Basantani et al. reported that CAT activity found to increase after glyphosate treatment in the two *V. radiata* varieties. There was 2.7 fold increase in activity at 4 mM as compared to control in PDM11, and 1.7-fold in PDM54 [54] In other researchs related to SA determined that SA, a signal molecule, modified the antioxidative system by inhibiting CAT and stimulating POD enzymes [44,55]. It has been shown that exogenous SA application resulted in the alleviation of Cd-induced ROS overproduction in *Arabidopsis thaliana* [56] and maize seedlings [44]. Belkadhi et al. reported that the Cd-treated plantlets presoaked with SA exhibited less lipid and protein oxidation and membrane alteration, as well as a high level of total antioxidant capacities and increased activities of antioxidant enzymes except of CAT. They suggested that SA plays an important role in triggering the root antioxidant system, thereby preventing membrane damage as well as the denaturation of its components [57]. In this study, we found that in SA-treated plants, POD activity was increased in all treatment groups but CAT activity was decreased on the 10th day (Tables 1 and 4). In the SA-pre-treated plants, the reason of the increase in POD activity may be related to the induction of stress resistance by SA. In the SA-pre-treated plants the decrease in CAT activity may be related to the SA-mediated mechanism underlying the accumulation of H_2O_2.

APX appears to play an essential role in the scavenging process when they coordinate with SOD [58]. Jiang and Yang (2009) reported that APX activity increased during the exposure to prometryne [22]. After treated with silicon, there was an increase of APX activity in salt-stressed cucumbers [59]. These results were supported our data, which indicate that APX activity increase during the exposure to glyphosate (Table 2). In this study the SOD activity increased in the treatment groups compared to control groups (Table 3). The reason of this increase in the APX and SOD activity may be related to the antioxidant characteristics of SA.

POD (U/mg protein)			
0.5 mM SA+ Glyphosate (mM)			
	1st day	**5th day**	**10th day**
Control	A3.95±0.03e	A3.95±0.02f	A3.98±0.01h
17	C4.16±0.03de	B4.71±0.07e	A5.35±0.17g
23	C4.16±0.05de	B5.20±0.07d	A6.45±0.21f
30	C4.39±0.03cd	B4.74±0.08e	A6.92±0.01e
39	C4.37±0.09cd	B5.71±0,13c	A7.21±0.05e
51	C5.03±0.25a	B6.33±0.23b	A8.70±0.36d
66	C5.17±0.03a	B7.04±0.04a	A10.08±0.14c
85	C4.95±0.03a	B6.95±0.02a	A11.32±0.09b
111	C4.84±0.04ab	B7.15±0.06a	A11.82±0.1a
145	C4.53±0.16bc	B6.19±0.09b	A11.35±0.07b

Table 1: Changes in POD activity in *Zea mays* leaves. The different lower case letters indicate significant differences ($p<0.05$) among the different concentrations of glyphosate according to Duncan's tests. The different upper case letters indicate significant differences ($p<0.05$) for each concentration of glyphosate according to independent samples *t* tests

	APX (U/mg protein)		
0.5 mM SA+ Glyphosate (mM)			
	1st day	**5th day**	**10th day**
Control	A0.85±0.01d	A0.91±0.01e	A0.89±0.01d
17	C0.94±0.02bc	B1.14±0.01d	A1.58±0.01c
23	C0.99±0.01b	B1.34±0.01c	A2.08±0.04b
30	C0.97±0.01bc	B1.14±0.05d	A2.60±0.23a
39	C0.92±0.03cd	B1.57±0.06a	A2.11±0.11b
51	C0.90±0.01cd	B1.50±0.04ab	A1.94±0.03b
66	C0.95±0.04bc	B1.31±0.04c	A2.00±0.06b
85	C1.10±0.03a	B1.49±0.04ab	A1.88±0.01b
111	C1.10±0.01a	B1.38±0.09bc	A2.62±0.09a
145	C1.11±0.02a	B1.32±0.01c	A2.88±0.06a

Table 2: Changes in APX activity in *Z. mays* leaves. The different lower case letters indicate significant differences ($p<0.05$) among the different concentrations of glyphosate according to Duncan's tests. The different upper case letters indicate significant differences ($p<0.05$) for each concentration of glyphosate according to independent samples *t* tests

	SOD (U/mg protein)		
0.5 mM SA+ Glyphosate (mM)			
	1st day	**5th day**	**10th day**
Control	A3.17±0.01g	A3.16±0.03i	A3.17±0.01j
17	B3.25±0.02fg	A3.74±0.01h	A3.70±0.01i
23	C3.28±0.01f	B3.91±0.01g	A4.13±0.02h
30	C3.47±0.02e	B4.15±0.02f	A4.63±0.0g
39	C3.82±0.07d	B4.32±0.01e	A5.12±0.01f
51	C3.86±0.01d	B4.42±0.01d	A5.29±0.01e
66	C4.05±0.03b	B4.68±0.01c	A5.68±0.01d
85	C3.96±0.01c	B4.71±0.04c	A6.07±0.03c
111	C4.13±0.01ab	B5.21±0.01b	A6.49±0.02b
145	C4.19±0.01b	B5.30±0.01a	A7.11±0.01a

Table 3: Changes in SOD activity in *Z. mays* leaves. The different lower case letters indicate significant differences ($p<0.05$) among the different concentrations of glyphosate according to Duncan's tests. The different upper case letters indicate significant differences ($p<0.05$) for each concentration of glyphosate according to independent samples *t* tests

	CAT (U/mg protein)		
0.5 mM SA+ Glyphosate (mM)			
	1st day	**5th day**	**10th day**
Control	A3.50±0.03e	A3.52±0.01g	A3.50±0.01f
17	C3.64±0.02bc	A3.92±0.01f	B3.29±0.03e
23	C3.59±0.01cd	A4.13±0.01e	B381±0.03d
30	C3.51±0.02e	A4.30±0.01d	B4.09±0.04c
39	C3.44±0.01f	A4.28±0.01d	B4.08±0.01c
51	C3.53±0.01de	A4.56±0.01c	B4.02±0.01c
66	C3.53±0.01de	A4.82±0.03b	B4.01±0.03c
85	C3.68±0.01b	A4.90±0.01a	B4.21±0.04b
111	C3.81±0.03a	A4.89±0.01a	B4.24±0.02b
145	C3.78±0.02a	A4.91±0.01a	B4.47±0.01a

Table 4: Changes in CAT activity in *Z. mays* leaves. The different lower case letters indicate significant differences ($p<0.05$) among the different concentrations of glyphosate according to Duncan's tests. The different upper case letters indicate significant differences ($p<0.05$) for each concentration of glyphosate according to independent samples *t* tests

GSH (U/mg protein)			
0.5 mM SA+ Glyphosate (mM)			
	1st day	**5th day**	**10th day**
Control	A1.91±0.01e	A1.90±0.01i	A1.89±0.01f
17	C1.96±0.03e	B2.29±0.05h	A2.98±0.01e
23	C1.99±0.01e	B2.79±0.19g	A3.79±0.01d
30	C2.17±0.09d	B3.23±0.10f	A3.96±0.03d
39	C2.30±0.01bc	B3.67±0.17e	A4.37±0.35c
51	C2.27±0.02cd	B4.10±0.02d	A5.10±0.05b
66	C2.38±0.03abc	B4.72±0.19c	A5.25±0.10ab
85	C2.41±0.02ab	A6.21±0.06b	B5.52±0.07a
111	C2.45±0.02a	A6.95±0.01a	B5.31±0.02ab
145	C2.31±0.01bc	A6.86±0.03a	B4.56±0.06c

Table 5: Changes in GSH content in *Z. mays* leaves. The different lower case letters indicate significant differences ($p<0.05$) among the different concentrations of glyphosate according to Duncan's tests. The different upper case letters indicate significant differences ($p<0.05$) for each concentration of glyphosate according to independent samples *t* tests

GR (U/mg protein)			
0.5 mM SA+ Glyphosate (mM)			
	1st day	**5th day**	**10th day**
Control	A0.095±0.0018d	A0.093±0.0006f	A0.093±0.0003f
17	C0.105±0.0025c	A0.369±0.0129e	B0.218±0.0040e
23	C0.115±0.0023b	A0.427±0.0157d	B0.251±0.0191cd
30	C0.120±0.0018b	A0.412±0.0055d	B0.263±0.0068bc
39	C0.115±0.0040b	A0.475±0.0071ab	B0.293±0.0051a
51	C0.122±0.0016b	A0.483±0.0036a	B0.275±0.0009ab
66	C0.120±0.0010b	A0.456±0.0121bc	B0.235±0.004de
85	C0.131±0.0006b	A0.437±0.0087cd	B0.243±0.0015cd
111	C0.128±0.0015a	A0.491±0.0007a	B0.263±0.0003bc
145	C0.117±0.0003a	A0.492±0.0012a	B0.261±0.0072bc

Table 6: Changes in GR activity in *Z. mays* leaves. The different lower case letters indicate significant differences ($p<0.05$) among the different concentrations of glyphosate according to Duncan's tests. The different upper case letters indicate significant differences ($p<0.05$) for each concentration of glyphosate according to independent samples *t* tests

GST (U/mg protein)			
0.5 mM SA+ Glyphosate (mM)			
	1st day	**5th day**	**10th day**
Control	A0.088±0.01d	A0.090±0.01h	A0.090±0.01f
17	B0.098±0.01bc	B0.100±0.01g	A0.133±0.01e
23	C0.118±0.08a	B0.147±0.01f	A0.283±0.03d
30	C0.105±0.01b	B0.153±0.01e	A0.392±0.01bc
39	C0.093±0.01cd	B0.175±0.01c	A0.396±0.03bc
51	C0.096±0.01bcd	B0.169±0.01d	A0.397±0.01bc
66	C0.103±0.01bc	B0.168±0.01d	A0.380±0.03c
85	C0.096±0.01bcd	B0.167±0.01d	A0.418±0.03b
111	C0.114±0.02a	B0.320±0.02b	A0.472±0.03a
145	C0.115±0.01a	B0.346±0.01a	A0.458±0.01a

Table 7: Changes in GST activity in *Z. mays* leaves. The different lower case letters indicate significant differences ($p<0.05$) among the different concentrations of glyphosate according to Duncan's tests. The different upper case letters indicate significant differences ($p<0.05$) for each concentration of glyphosate according to independent samples *t* tests

MDA (µmol MDA/ g fresh weight)			
0.5 mM SA+ Glyphosate (mM)			
	1st day	**5th day**	**10th day**
Control	A5.82±0.04f	A5.83±0.03h	A5.81±0.03i
17	C5.83±0.03f	B6.02±0.01h	A7.21±0.01h
23	C6.11±0.06e	B7.02±0.01g	A7.58±0.22g
30	C6.13±0.02e	B7.51±0.03f	A10.00±0.07f
39	C6.33±0.06d	B7.76±0.10e	A10.45±0.06e
51	C6.96±0.04ab	B8.57±0.01d	A11.38±0.01d
66	C7.00±0.01a	B9.18±0.02b	A12.29±0.03c
85	C6.84±0.08b	B9.58±0.17a	A12.70±0.04b
111	C6.67±0.04c	B8.91±0.07c	A12.90±0.01b
145	C6.41±0.06d	B8.84±0.07c	A14.31±0.13a

Table 8: Changes in MDA content in *Z. mays* leaves. The different lower case letters indicate significant differences ($p<0.05$) among the different concentrations of glyphosate according to Duncan's tests. The different upper case letters indicate significant differences ($p<0.05$) for each concentration of glyphosate according to independent samples *t* tests

Total Chlorophyll (µg/g)			
0.5 mM SA+ Glyphosate (mM)			
	1st day	**5th day**	**10th day**
Control	A13.07±0.04bc	A13.01±0.04a	A13.07±0.04a
17	A12.98±0.05cd	B11.86±0.07bc	B11.83±0.02b
23	A13.28±0.02a	B11.99±0.04b	C11.84±0.02b
30	A13.23±0.07ab	B11.78±0.01c	B11.74±0.02b
39	A12.87±0.10d	B11.39±0.08d	B11.44±0.03c
51	A13.06±0.06bcd	B11.50±0.03d	C11.45±0.01c
66	A12.69±0.11e	C9.73±0.07g	B10.19±0.06d
85	A11.69±0.04f	C9.92±0.06ef	B9.91±0.05e
111	A11.50±0.01g	C10.01±0.03e	C9.92±0.08e
145	A11.41±0.02g	B9.79±0.10fg	C9.62±0.13f

Table 9: Changes in total chlorophyll in *Z. mays* leaves. The different lower case letters indicate significant differences ($p<0.05$) among the different concentrations of glyphosate according to Duncan's tests. The different upper case letters indicate significant differences ($p<0.05$) for each concentration of glyphosate according to independent samples *t* tests

Total Carbohydrate (µg/g)			
0.5 mM SA+ Glyphosate (mM)			
	1st day	**5th day**	**10th day**
Control	A0.50a	A0.51a	A0.49a
17	A0.53a	B0.47b	C0.40b
23	A0.47b	B0.40c	B0.37c
30	A0.51a	B0.43bc	C0.36c
39	A0.49b	B0.39c	C0.31c
51	A0.51a	B0.40c	C0.29d
66	A0.52a	B0.39c	C0.26d
85	A0.50a	B0.36c	C0.24d
111	A0.49a	B0.24d	C0.18e
145	A0.43b	B0.30d	C0.21b

Table 10: Changes in total carbohydrate in *Z. mays* leaves. The different lower case letters indicate significant differences ($p<0.05$) among the different concentrations of glyphosate according to Duncan's tests. The different upper case letters indicate significant differences ($p<0.05$) for each concentration of glyphosate according to independent samples *t* tests

A number of studies showed that exogenous application of SA influence the antioxidant capacity of plant. At the same time, since adaptation to oxidative stress includes not only the regulation of the synthesis and repair of proteins but also increased andioxidant activity [60]. Belkadhi et al. reported that antioxidant activity effect was improved by SA in Cd-stressed plantlets [57].

GST is a phase II enzyme that aids conjugating pollutants or/and their metabolites with glutathione favoring their further excretion [61-63]. High activities of GST are usually associated with the presence of organic pollutants or pro-oxidant conditions [62]. GR is one of the potential enzymes of the enzymatic antioxidant system, which sustains the reduced status of GSH via ascorbate–glutathione pathway and plays a vital role in maintenance of sulfhydryl group and acts as a substrate for GST [64]. In our research, in the SA-treated plants GST and GR enzyme activities and total GSH content increased considerably compared to the control (Tables 5-7). This may be expressed by the fact that more ROS is occured in the plants applicated with higher dosages of the herbicide and GSH, GR and GST are formed being used as an antioxidant during the detoxification reactions with the produced ROS.

There are reports showing that MDA content increased in various plants with the effect of herbicide implementation [65,66]. Singh et al. reported that the oxidative damage markers lipid peroxidation (MDA) and protein oxidation products increased with doses of D2, UV-B1 and UV-B2 [23]. Lipid peroxidation was partially increased by applying SA to glyphosate maize plant (Table 8). The reason of this increase may be related to the induction of stress resistance by SA.

Chlorophyll is a natural pigment that absorbs light energy for photosynthesis. A greater understanding about contents of chlorophyll pigments, would be expected to yield improved methods of evaluating plant responses to the environmental stresses [67,68]. Baninasab and Baghbanha reported that the application of SA improved chlorophyll fluorescence ratio of cucumber (*Cucumis sativus* L.) seedlings exposed to salt stress [69]. In this research, we found decrease in the total chlorophyll content compared to the control associated by applying SA to glyphosate in maize plant. The decrease may be due to the formation of proteolytic enzymes such as chlorophyllase, which is responsible for the chlorophyll degradation [70]. Probably, in our findings, decrease in the total chlorophyll may be correlated to chlorophyllase enzyme.

Carbohydrates are the direct products of photosynthetic activity and constitute a source of energy and metabolites as well as structural building blocks [71,72]. It was determined in our study that, in SA-treated plants, total soluble carbohydrate decreased considerably in *Z. mays* exposed to glyphosate (Table 10). Besides this, related to decrease in the total chlorophyll content.

Conclusions

In this study, it was detected that glyphosate caused toxic effect for culture plant *Z. mays* and that stress effects may be reduced by SA against the damage that may be caused by glyphosate. Besides this, POD, APX, SOD and GST, were activated by SA treatment, while others like GR, GSH, CAT were found to be inhibited. This is linked to the SA-increased level of POD, APX, SOD and GST activities under glyphosate stress. It was also determined that glyphosate affected on the MDA level, total chlorophyll and total soluble carbohydrate.

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