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
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 Syngenta 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 °C and a humidity of 60%. Samples were planted after a portion of the plants was kept for six hours in distilled 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 MacAdam 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$_2$O$_2$ decomposition. One unit of enzyme activity was defined as the amount of the enzyme that decreased 1 µmol H$_2$O$_2$ 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 standard 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**

**Enzyme 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 µg/g 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 M 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 M 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 researches 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 H2O2.

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             |                             |         |         |         |
| 0.5                  | 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±0.02a                  | B6.95±0.02a | A11.32±0.05b |
| 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) | 1st day | 5th day | 10th day |
|-------------------|---------|---------|---------|
| Control           | A0.85±0.01d | A0.91±0.01e | A0.99±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 | A2.80±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±0.11b |
| 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) | 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 | A4.83±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                | C3.98±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) | 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 | B3.81±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.
### 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

| 0.5 mM SA+ Glyphosate (mM) | 1st day | 5th day | 10th day |
|----------------------------|---------|---------|----------|
| Control                    | 0.91±0.01e | 0.90±0.01i | 0.89±0.01f |
| 17                         | 0.96±0.003e | 0.95±0.03i | 0.98±0.01e |
| 23                         | 0.99±0.001e | 0.97±0.01g | 0.97±0.01d |
| 30                         | 0.17±0.009d | 0.33±0.10f | 0.96±0.03d |
| 39                         | 0.3±0.01bc | 0.67±0.17e | 0.37±0.35c |
| 51                         | 0.27±0.02cd | 0.40±0.02d | 0.50±0.05b |
| 66                         | 2.38±0.03abc | 4.72±0.19c | 5.25±0.10ab |
| 85                         | 2.41±0.02ab | 6.21±0.06b | 5.52±0.07a |
| 111                        | 2.45±0.02a | 6.95±0.01a | 5.31±0.02ab |
| 145                        | 2.31±0.01bc | 8.66±0.03a | 5.68±0.06c |

### 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

| 0.5 mM SA+ Glyphosate (mM) | 1st day | 5th day | 10th day |
|----------------------------|---------|---------|----------|
| Control                    | 0.095±0.0018d | 0.093±0.0006f | 0.093±0.0003f |
| 17                         | 0.105±0.0025c | 0.369±0.0129e | 0.218±0.0040e |
| 23                         | 0.115±0.0023b | 0.427±0.0157d | 0.251±0.0191cd |
| 30                         | 0.120±0.0018b | 0.412±0.0055d | 0.263±0.0068bc |
| 39                         | 0.115±0.0040b | 0.475±0.0071ab | 0.293±0.0051a |
| 51                         | 0.122±0.0016b | 0.483±0.0036a | 0.275±0.0099ab |
| 66                         | 0.120±0.0010b | 0.456±0.0121bc | 0.235±0.004de |
| 85                         | 0.131±0.0086b | 0.437±0.0087cd | 0.243±0.0015cd |
| 111                        | 0.128±0.0015a | 0.491±0.007a | 0.263±0.0003bc |
| 145                        | 0.117±0.0033a | 0.492±0.0012a | 0.261±0.0072bc |

### 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

| 0.5 mM SA+ Glyphosate (mM) | 1st day | 5th day | 10th day |
|----------------------------|---------|---------|----------|
| Control                    | 0.088±0.01d | 0.090±0.01h | 0.090±0.01f |
| 17                         | 0.098±0.01bc | 0.100±0.01g | 0.133±0.01e |
| 23                         | 0.118±0.08a | 0.147±0.01f | 0.283±0.03d |
| 30                         | 0.105±0.01b | 0.153±0.01e | 0.392±0.01bc |
| 39                         | 0.093±0.01cd | 0.175±0.01c | 0.396±0.03bc |
| 51                         | 0.096±0.01bcd | 0.169±0.01d | 0.397±0.01bc |
| 66                         | 0.103±0.01bc | 0.168±0.01d | 0.380±0.03c |
| 85                         | 0.096±0.01bcd | 0.167±0.01d | 0.418±0.03b |
| 111                        | 0.114±0.02a | 0.320±0.02b | 0.472±0.03a |
| 145                        | 0.115±0.01a | 0.346±0.01a | 0.458±0.01a |
### 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±0.05e |
| 51                         | C6.96±0.04ab | B6.57±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±0.05d | 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±0.03cd | B11.45±0.01c |
| 66                         | A12.69±0.11e | C9.73±0.07g | B10.19±0.06d |
| 85                         | A11.69±0.04ef | 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±0.10g | 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 antioxidant 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 metabolizes 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 occurred in the plants applied 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]. Baninab 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 maize plant (Table 8). The reason of this increase may be related to the induction of stress resistance by SA.

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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