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Salicylic Acid and Cytokinin Protects Maize Plant against Glyphosate Action

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THE MITIGATING effects of salicylic acid (SA) and cytokinin (CK) against glyphosate stress on young maize plants were investigated. The application of glyphosate has harm effects on maize plants, *i.e*. plants became shorter, chlorosis, leaves turned to yellow more or less dry in some parts then died. The morphological changes associated with decrease in the chlorophyll content; meanwhile the levels of protein, proline and the activity of ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) were increased. Available data suggest that the protective effect of SA and CK was accompanied with increase in chlorophyll content and reduction in proline and the activity of APX, CAT, POD and SOD in comparison with glyphosate treatment. The combination between SA and CK not alleviate the harm effects of glyphosate on maize. The present results gave an insight about the use of SA and CK alone in alleviated in some extent the detrimental effects due to the glyphosate action.

Keywords: Glyphosate, Maize, Salicylic acid, Cytokinin, Protein, Proline, Antioxidative enzymes.

Application of herbicides considered one of the important factors affecting crop production. It was reported that, excess herbicides damage the weeds and crops through changes the metabolic and physiological processes within the plant cells (Song *et al*., 2008; Yin *et al.*, 2008 and Jiang & Yang, 2009). Glyphosate [(N-phosphonomethyl) glycine] is a highly effective broad-spectrum, non-selective, systemic, post-emergence herbicide used extensively worldwide (Tan *et al.*, 2006 and Vila-Aiub *et al.*, 2008). Glyphosate degradation appears to be very slow or does not take place in higher plants (Vila-Aiub *et al*., 2007). Most herbicides inhibit plant metabolic pathways or physiological processes by interacting with specific proteins (Dayan *et al*., 2010). The target site of glyphosate action is the enzyme 5-enoylpyruvylshikimate-3-phosphate synthase (EPSPS) of the shikimate pathway (Ge *et al.*, 2010). Inhibition of EPSPS leads to reduced feedback inhibition of the pathway, resulting in massive carbon flow to shikimate-3-phosphate, which is converted into high levels of shikimate (Stephen & Stephen, 2008). Salbego *et al*. (2010) showed that the inhibition of this enzyme causes an accumulation of shikimic acid and a consequent reduction in the biosynthesis of aromatic amino acids, auxins and vitamins as well as a number of key metabolites produced via the shikimate pathway. This leads to a suspension of plant growth, and in turn to plant death. Glyphosate also disrupts chloroplasts, membranes, cell walls; alters protein, nucleic acid

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synthesis, photosynthesis, respiration; reduces chlorophyll, and other porphyrin compound synthesis (Singh *et al*., 2012). A lower amount of leaf chlorophyll is a distinguishing characteristic in plants exposed to sublethal concentrations of glyphosate (Tan *et al.*, 2006).

Glyphosate leads to oxidative stress in plants which is most probably a secondary effect of the blocked shikimate pathway (Sergiev *et al*., 2006). Excessive herbicides and other toxic organic substances are able to induce intracellular over-production of reactive oxygen species (ROS) and damage the plant cells (Yin *et al.*, 2008). Plants utilize a well-organized antioxidative defense system comprising enzymatic system to scavenge ROS (Liu *et al.*, 2009). The cooperative function of these antioxidants such as SOD, APX and CAT plays an important role in scavenging ROS and maintaining the physiological redox status of organisms (Cho & Seo, 2005).

Salicylic acid (SA) is an endogenous, phenolic growth regulator which can act as a non-enzymatic antioxidant. It is considered to be a plant growth regulator, which plays an important role in regulating a number of plant physiological processes including photosynthesis (Arfan *et al*., 2007 and Hayat *et al*., 2010). SA provides protection against biotic and abiotic stresses of plants (Singh *et al.*, 2012). Exogenous SA has been shown to ameliorate the damaging effects of heavy metals in rice (El-Khallal *et al*., 2009), salinity in wheat (Eraslan *et al*., 2007) drought in maize (Noreen *et al*., 2009) and herbicides in barley (Ananieva *et al.*, 2004). In addition, the important protective action of SA probably reflects its ability to induce the expression of genes coding not only for PR-proteins, but also, for genes encoding extension in *Arabidopsis* plants (Merkouropouls *et al.*, 1999). Burkhanova *et al*. (1999) reported that SA induced synthesis of heat shock proteins in tobacco plants and fast activation of the 48 kDa protein kinase, which was identified as SIPK (salicylic acid induced protein kinase).

Cytokinin promotes cell division and is implicated in plant growth and development. It can not only enhance plant growth but also can improve stress tolerance (Schmidt & Zhang, 1997). Treatment with kinetin protects creeping bent grass subjected to drought (Zhang & Schmidt, 2000), and use of zeatin riboside alleviates heat stress injury (Liua *et al.*, 2002).

The objective of this work was to characterize several biochemical parameters that are responsive to salicylic acid and cytokinin. In addition to investigate whether these were associated with partial alleviation of glyphosate toxicity through regulation of the antioxidant status of maize plants.

Materials and Methods

Plant materials and treatments

As a model system, maize plants (*Zea mays* L. cv. Kneza-640) were used. Grains of maize were washed with running tap water and then soaked for 30 sec

in 70% ethanol followed by disinfection with 20% (v/v) sodium hypochlorite for 20 min and rinsed three times with sterile distilled water. Grains were soaked in tap water for 24 h, and put on moistened filter paper for germination in darkness (at 25 °C). Two-day old seedlings were swoon in each pot containing sandy soil (85% sand, 10% silt and 5% clay) at a relative humidity of 50 ± 4%, day/night temperature 30/20 ±2°C, and light intensity of 350.67 ± 4.16 Lux. in the greenhouse at the Botany Department, Faculty of Science, Zagazig University, during April 2013. The pots were irrigated with a half-strength Hoagland's solution with trace microelements. The plants with fully developed second leaves were divided into five groups treated (sprayed, approximately 3 ml per plant) with:

C= Distilled H_2O (control).
G = Aqueous solutions of glyphosate (10 mM).
GS=Aqueous solutions of glyphosate (10 mM) and SA (0.5 mM).
GC=Aqueous solutions of glyphosate (10 mM) and CK (0.1 mM Benzylaminopurine).
GSC=Aqueous solutions of glyphosate (10 mM), SA (0.5mM) and CK (0.1 mM benzyl adenine).

The parameters investigated were measured after 10 days of the treatment due to the half-life of glyphosate on foliage being estimated at 10.4 to 26.6 days (Tu, 1994). Morphological appearance was recoded by imaging with a digital video camera interfaced to a computer running software from the same manufacturer (SPOT 4.6). The second fully developed leaf was used as a source material for biochemical analyses.

Photosynthetic pigments

The contents of the photosynthetic pigments chlorophyll *a* (chl *a*), chlorophyll *b* (chl *b*) and Carotenoides in fresh leaves were determined using the spectrophotometric method recommended by Metzner *et al.* (1965) and described by Hassanein *et al*. (2009). The concentration of each pigment (µg/ml) was calculated using the following equations:

Chl *a* = $10.3\ E_{663} - 0.918\ E_{644}$
Chl *b* = $19.7\ E_{644} - 3.87\ E_{663}$
Carotenoides= $4.2\ E_{452} - (0.0264$ chl *a* + 0.4260 chl *b*)
Finally, the pigment contents were expressed as mg g^{-1} fresh weight (FW).

Total proteins

Total protein was determined according to the method described by Bradford (1976) with bovine serum albumin as a standard. An amount of 1gm of sample was ground in a mortar with 5ml of phosphate buffer (pH 7.6) and was then centrifuged at 8000 rpm for 20 min. 30µl of different samples were mixed with 70µl of distilled water then 2.9 ml of Coomassi Brillaint Blue solution was added and mixed thoroughly. The tubes were incubated for 5 min at room temperature and absorbance at 600 nm was recorded against the reagent blank. A standard curve of Absorbance (600 nm) versus concentration (ug) of protein was calculated.

Proline

1gm of sample was extracted with phosphate buffer (pH 7.6) and then centrifuged at 8000 rpm for 20 min. Proline was assayed according to the method described by Bates *et al*. (1973). 2ml of extract, 2ml of acid ninhydrin and 2ml of glacial acetic acid were added and incubated for 1 h in a boiling water bath followed by an ice bath. The absorbance was measured at 520 nm using Spekol Spectrocololourimeter VEB Carl Zeiss. A standard curve was obtained using a known concentration of authentic proline.

Protein extraction for SDS-PAGE

For SDS-PAGE, seedling tissue of each sample was ground to powder under liquid nitrogen and melted in ice-cold extraction buffer (50 mM Tris-HCl, pH 8, 10 mM NaCl, 1% SDS, 5% 2-mercaptoethanol, 0.1 mM PMSF, 0.1 mM DTT), followed by centrifugation at 10000 rpm at 4 ^{o}C for 15 min. Proteins of the clear supernatants obtained after centrifugation were stored at -20 ^{o}C until used.

One-dimensional SDS-PAGE

Proteins, 30 ug of each sample, were separated by SDS-PAGE according to the method of Laemmli (1970). The separation was performed with a 10% separating gel and a 4% stacking gel using a protein vertical electrophoresis unit (Hoefer Scientific Instruments). Electrophoresis was started at 10 mA constant current until the tracking dye entered the separating gel and continued at 25 mA until the tracking dye reached the end of the gel. Protein subunit bands were stained with coomassie blue R-250 by standard techniques. The molecular weights of standard proteins added in a comparison ladder (in kDa as follows: 30, 40, 50, 60, 80, 120 and 220 kDa, 7 bands) were used to determine molecular weight of the added proteins.

Antioxidative enzymes

Extraction of enzymes

Fresh leaf tissue (0.5 g) was harvested and homogenized under ice-cold conditions in 5.0 ml of extraction buffer, containing 50 mM phosphate buffer (pH 7.5), 1% polyvinyl pyrrolidone (PVP), 0.5% Triton X100, and 1 mM EDTA. The homogenate was centrifuged at 10,000 rpm for 20 min at 4 ^{o}C. The supernatant was used to measure the activities of SOD, APX and GR.

Ascorbate peroxidase (APX)

APX activity was measured by the method of Nakano & Asada (1987). The reaction mixture (1 ml) contained 100 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.3 mM H_2O_2 and enzyme extract. The oxidation of ascorbic acid was measured by the decrease in absorbance at 290 nm for 3 min using UV-vis spectrophotometer (Model Ultrospec 3000, Pharmacia Biotech, USA). The enzyme activity was calculated using the extinction coefficient 2.8 mM^{-1} cm^{-1} and expressed in enzyme units (100 g FW)$^{-1}$. One unit of enzyme is the amount necessary to decompose 1 µmol of substrate per min at 25 ^{o}C.

Catalase (CAT)

CAT activity was assayed by measuring the initial rate of hydrogen peroxide disappearance using the method of Velikova *et al.* (2000). One milliliter of

catalase assay reaction mixture contained 10 mM potassium phosphate buffer, pH 7.0 with an appropriate aliquot of enzyme extract and 33 mM hydrogen peroxide. The decrease in hydrogen peroxide was followed as a decline in optical density at 240 nm and the activity was calculated using the extinction coefficient of 40 mM cm^{-1} for hydrogen peroxide.

Peroxidase (POD)

POD was measured and determined by Kokkinakis & Brooks (1979) method. Activity of peroxidase was determined based on the appearance of brown colors resulting from guaiacol oxidation in the presence of hydrogen peroxide. Reaction mixture consisted of 50µL sample extract, 2.6 mL of 0.1 M sodium phosphate buffer at pH 6.1 and 0.3mL of 1% guaiacol was added into the solution. A total of 0.3mL of 30% H_2O_2 was added prior to reaction. Changes in absorbance at 420 nm were followed for three min using a spectrophotometer (Bio-Rad smartspec plus, USA). Peroxidase activity was calculated using the formula below and expressed as unit/mg protein:

$$\text{Specific activity of peroxidase} = \frac{\text{Total activates of the sample}}{\text{Protein content of the sample}}$$

$$\text{Total activities} = \frac{\text{Abs. x dilution factor} \times 1000}{\text{Volume of enzyme used in the assay}}$$

Superoxide dismutase (SOD)

SOD activity was measured according to the method of Beauchamp & Fridovich (1971). The reaction mixture (1.5 ml) contained 100 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 2.25 mM nitroblue tetrazolium (NBT), 60 mM riboflavin and enzyme extract. After mixing, the contents in the cuvet were illuminated for 10 min. A tube with enzyme extract kept in the dark served as a blank, while the control tube which contained no enzyme extract was kept in the light. The absorbance was measured at 560 nm against a blank using a UV-vis spectrophotometer (Model Ultrospec 3000, Pharmacia Biotech). NBT reduction in the light was measured in the presence and absence of enzyme extract. SOD activity is presented as absorbance of control minus absorbance of sample, giving the total inhibition. One unit of activity is the amount of enzyme required for 50% reduction in color and was expressed in units of the enzyme $100g^{-1}$ FW h^{-1}.

Statistical analysis

All data were reported as mean ± standard deviation (±SE) for the three independent samples (n=3) and subjected to one-way analysis of variance (ANOVA) using Analyst software. The LSD values were then computed at 5% level of probability for comparison of the difference between means.

Results and Discussion

Effect of glyphosate, SA and CK applied alone and in combination on morphological appearance, growth and pigments of maize leaves

Ten days after treatment with 10 mM glyphosate, maize plants showed severe changes in morphological appearance and plants became shorter than the control. It caused leaf-tip chlorosis; leaves turned yellow and become more or less dry in some parts with lower water contents, then died (Fig.1&2). Glyphosate is among the amino acid synthesis inhibitors herbicides which are readily absorbed through plant foliage and translocated in the phloem. Efficient translocation and a lack metabolism of glyphosate in most plant species (Franz

et al., 2000) allows to accumulate in and inhibit apical meristems. This inhibition encourages sprouting of axillary buds (Gilreath *et al*., 2001), which results in a witch's broom' appearance of affected plants. Glyphosate treatment of corn and cotton seedlings inhibited polar auxin transport (Gilreath *et al*., 2001) and was associated with enhanced rates of auxin metabolism in seedlings.

Fig.1. Glyphosate, cytokinin and SA treatments effects on the growth and morphology of *Zea mays* plants.
C= Distilled H_2O, G= Aqueous solutions of glyphosate (10 mM), GS= Aqueous solutions of glyphosate (10 mM) and 0.5 mM of SA, GC= Aqueous solutions of glyphosate (10 mM) and 0.1 mM of CK, GSC= Aqueous solutions of glyphosate (10 mM) and 0.5mM of the SA and 0.1 mM of the CK.

Fig.2. *Zea mays* leaves turned to yellow and become drier with glyphosate treatment (upper) compared with the control (lower).

Glyphosate inhibit synthesis of the aromatic amino acids tyrosine, tryptophan, and phenylalanine (Singh *et al*., 2012). Glyphosate symptoms are not apparent until three to five days after treatment and include stunting, foliage

discoloration, and slow plant death. This injury could be due to oxidative stress leading to death of some leaf parts (Fig. 1&2). Glyphosate application could rise the superoxide levels and cause ROS accumulation which accompanied by lipid peroxidation (Radwan, 2012). Accumulation of ROS could not only cause lipid peroxidation but also induce membrane damage and cause severe disturbances in the physiological metabolism of plants leading to cell death and accelerated plant senescence (Xu *et al.,* 2006 and Ogweno *et al.,* 2009). Many assume that insufficient aromatic amino acid production to maintain necessary protein synthesis is the primary effect of glyphosate, and this is consistent with the slow development of symptoms (Stephen & Stephen, 2008).

In comparison with the glyphosate treatment, plants treated with glyphosate plus CK and SA showed a healthier appearance. Morphologically, using CK and SA alone appeared to be better than using a combination of them for protecting maize plants against glyphosate injuries (Fig.1&2). SA is known to regulate the antioxidant status through inhibition or activation of the antioxidant enzymatic system scavenging the generated ROS (Apel & Hirt, 2004). Salicylic acid also uncouples oxidative phosphorylation which leads to increased ADP: ATP and AMP: ATP ratios in the cell (Hawley *et al.,* 2012). Singh *et al.* (2012) showed that SA application may be responsible for activation of defense genes. Meanwhile the pretreatment with benzyladenine (CK) reduce herbicide toxicity and treated plants with CK might become more tolerant against oxidative stress (Kiadó, 2005). Cytokinin could act as a protector by activation of the antioxidant defense systems and caused an increase in glutathione (GSH) which participate in the regulation of the cell antioxidant systems (Sergiev *et al.,* 2006).

The combination of SA and CK reduced the improving effect of each alone on glyphosate stress. This result is in agreement with Argueso *et al.* (2012) who demonstrated that SA negatively regulates cytokinin signaling. They added that the negative regulation of SA-dependent defense responses and that a complete phosphorelay cascade, initiated at the level of cytokinin receptors and culminating in type-A ARR phosphorylation and activation. This type-A ARR function is promoted by cytokinin and occurs downstream of SA; in the absence of functional type-A ARRs, defense gene expression.

Pigment contents and particularly chlorophyll parameters were used to elucidate the mode of action of herbicides on plant physiology (Conrad *et al.,* 1993). Reduction in Chl.*a* and Chl.*b* in maize leaves as a result of application of glyphosate was found (Fig.3). Chlorophyll degradation that leads to chlorosis might be occurred in response to ROS generation in glyphosate sprayed leaves (Mittler *et al*., 2004 and Sergiev *et al.*, 2006). Reports for maize, barley, tobacco and sunflower (Mateos-Naranjo *et al.,* 2009) showed a high sensitivity of chlorophyll to glyphosate action. Nandula *et al.* (2007) verified that the chlorophyll content decreased at 7 days after glyphosate treatment. The decrease of chlorophyll content may be due to an increase of chlorophyll degradation or to a decrease of chlorophyll synthesis (Santos, 2004). Moreover herbicide stress

induced a reduction in the number of chloroplasts (Cakmak *et al.,* 2009). Glyphosate prevent chlorophyll synthesis by inhibiting the formation of the porphyrin precursor d-amino levulinic acid (Zaidi *et al*., 2005).

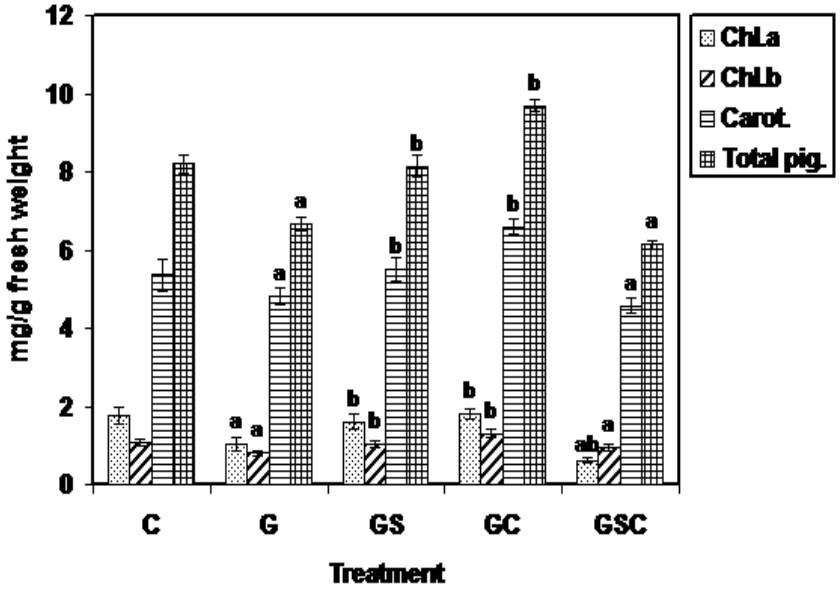

Fig.3. Effect of glyphosate, SA and CK applied alone and in combination on the pigment contents (mg/g fresh weight) of maize leaves.

Chl.*a* = Chlorophyll *a*, Chl.*b* = Chlorophyll *b*, Carot. = Carotenoides,
Means within a column followed by a, b are significantly different from the C (control) and G (glyphosate) treated samples, respectively.

It was found that SA and CK alone with glyphosate increased the pigment contents, and in combination with glyphosate significantly retarded the pigment contents of maize plants (Fig.3). Khodary (2004) observed a significant increase in growth characteristics, pigment contents and photosynthetic rate in maize, sprayed with SA. CK increase plant tolerance (Iqbal *et al.*, 2006a) and considered as ABA antagonists and auxins antagonists/synergists in various processes in plants (Pospisilova, 2003). It was hypothesized that cytokinin could increase plants tolerance by interacting with other plant hormones, especially auxins and ABA (Iqbal *et al*., 2006b). CK also retard senescence, having effect on membrane permeability to mono and divalent ions, and localized induction of metabolic sinks (Letham, 1978).

The alteration in pigment content due to combination between CK, SA and glyphosate may be parts of the pathway that regulates plant's response in this situation. It is postulated that there is an antagonistic effect between CK and SA in plants at low temperature which may negatively effects on the plant growth via controlling the expression level of CYCD3;1 gene (Xia *et al.*, 2008).

Effect of glyphosate, SA and CK applied alone and in combination on protein and proline of maize leaves

A noticeable increase in the total proteins in glyphosate with or without CK and SA treated leaves compared with the control (Fig. 4). The increase in the proteins through treatment with glyphosate may be result of increase protein synthesis (Peixoto *et al.*, 2006) or less protein degradation (Çag *et al*., 2009). The stimulation of protein synthesis lead to protein accumulation may involve in sequences of enzymes activity as defense mechanism against herbicide stress (Peixoto *et al.*, 2006). Five CBF regulon genes encode proteins that have been predicted to have protease or protease inhibitor activity (Maruyama *et al.*, 2004 and Vogel *et al.*, 2005). A possible explanation for this is that these proteins could have a regulatory role during stress by modifying activity of a transcriptional regulator or component of a signal transduction pathway. In support of this notion, Levy *et al.* (2004) identified a mutant in the chloroplast proteolytic machinery which exhibited impaired photoprotection and photosynthesis upon stress. In agreement with our results, it was reported that SA results in pronounced increase in total protein contents and formation of new proteins in pea and sunflower leaves as tolerance tools (Çag *et al*., 2009). Meanwhile CK (Benzylaminopurine) stimulate RNA and protein biosynthesis, producing a number of growth-enhancing effects (Skinner *et al.*, 2009).

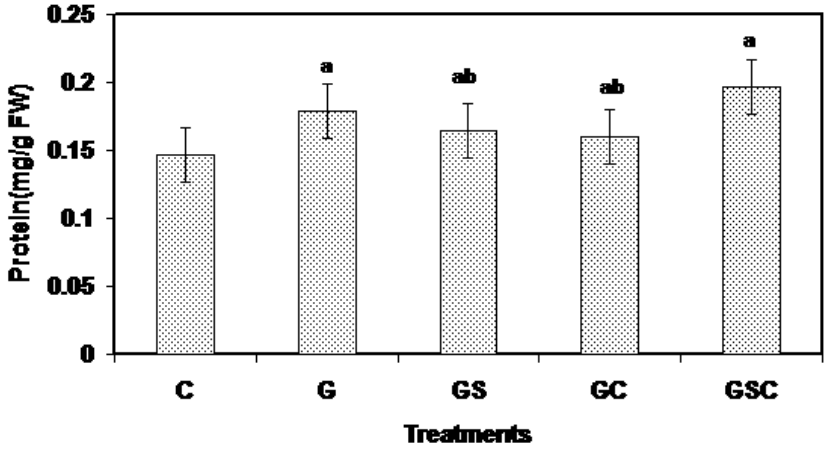

Fig.4. Effect of glyphosate, SA and CK applied alone and in combination on the protein contents of maize leaves.

Means within a column followed by a, b are significantly different from the C (control) and G (glyphosate) treated samples, respectively.

Our results showed a significant accumulation of proline content with glyphosate treatment (Fig.5), in agreement with El-Taybe & Zaki (2009). Proline is one of the most common compatible osmolytes in stressed plants (Mafakheri *et al*., 2010), help in protection of macromolecules during

dehydration (Yancey *et al*., 1982), and act as a hydroxyl radical scavenger (Zhang *et al*., 2001). Proline has multiple functions, such as osmoticum, scavenger of free radicals, protector role of cytoplasmic enzymes, source of nitrogen and carbon of post stress growth, stabilizer of membranes, machinery for energy to regulate redox potential (Ashraf & Foolad, 2007). SA and CK alone increase proline content compared with control and decreased the glyphosate-enhanced proline accumulation, *i.e.* increase plant tolerance to glyphosate. SA regulated the level of proline, total amino acids and soluble protein content in tobacco leaves (Wei *et al*., 2006). Peleg & Blumwald (2011) showed that elevated CK levels promoted survival under stress conditions, inhibited leaf senescence and induced increased proline levels.

The combination between CK, SA and glyphosate was not effect on glyphosate-enhanced proline accumulation compared with control. It is postulated that there is an antagonistic on the plant tolerance to glyphosate.

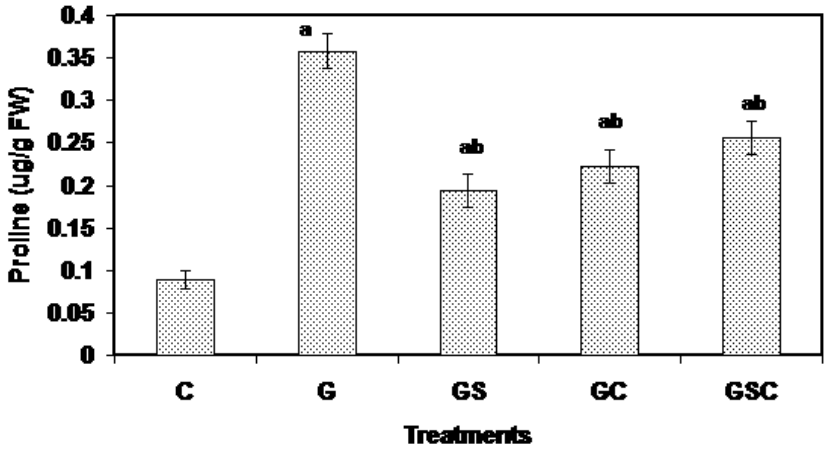

Fig.5. Effect of glyphosate, SA and CK applied alone and in combination on the proline contents of maize leaves.

Means within a column followed by a, b are significantly different from the C (control) and G (glyphosate) treated samples, respectively.

Effect of glyphosate, SA and CK applied alone and in combination on protein electrophoretic pattern of maize leaves.

The results showed that the protein profiles were represented by 5 major and common distinct bands with molecular weight of 40, 50, 60, 80 and 220 kDa (Fig.6). SDS-PAGE analysis of maize leaves revealed an almost identical protein pattern and band intensity variation for the different treatments with an exception of the 45 kDa molecular mass protein band (Fig. 6), which was strongly stimulated in the GC and GSC line, but was not dependent upon glyphosate treatment. Accumulation of proteins of molecular weights 45 which induced by CK had a possible role in adaptation to glyphosate stress. Moldes *et al.*

(2008) recorded that protein of 45 kDa and its mRNA accumulated in the soybean plants which are tolerant to glyphosate stress. Concerning protein band of molecular weight 26 kDa, results showed that intensity of this protein increased in response to stress with or without SA and CK. Induction of 26 kDa protein in maize plants has been peculated to represent osmotin that is involved in the rapid accumulation of proline and glucine betain during stress (Dell Aquila & Spada, 1992). In maize plants, Zorb *et al*. (2004) detected three groups of differentially regulated proteins in roots and shoots under salt stress: (A) Proteins which are involved in protein biosynthesis and protein modifications by Kinase, (B) Enzymes of the carbon metabolism and (C) Enzymes of the nitrogen metabolism. In addition, several protein kinase and phosphatase are involved in response to salt stress (Serrano & Gaxiola, 1994).

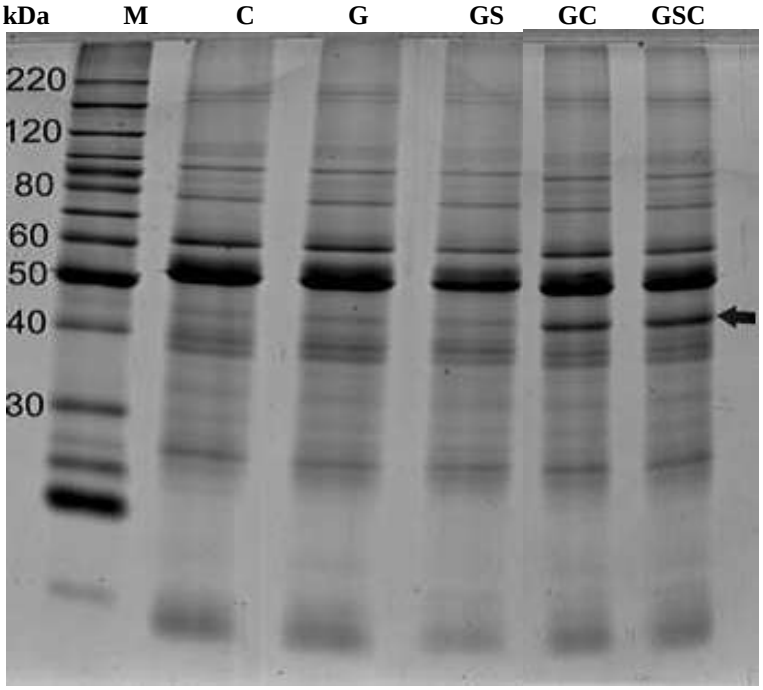

Fig.6. Effect of glyphosate, SA and CK applied alone and in combination on electrophoretic profiles of protein extracted from maize leaves.

Effect of glyphosate, SA and CK applied alone and in combination on the antioxidant enzyme activities of maize leaves

There are different enzymatic scavenging activities per a particular ROS can be found in the cell compartments. In this work, the activities of CAT, SOD and APX were slightly increased; meanwhile the activity of POD is highly induced in response to glyphosate (Fig.7). Moreover, SA treatment with glyphosate decrease the activities of the measured enzymes, meanwhile CK decrease the activities of CAT, SOD and POD but increase the activity of APX. Enzymatic

ROS-scavenging pathways are important antioxidant defense mechanisms in plants. Among the set of antioxidant enzymes, SOD acts as a first line of defense response which converting the superoxide radical into H_2O_2 (Wang *et al.*, 2004). Then H_2O_2 has to be further detoxified by CAT and/or POD or APX to water and oxygen (Shah *et al.*, 2001). Detoxification of H_2O_2 prevents the oxidation of biological molecules and destruction of the cells (Liochev & Fridovich, 1994). APX appears to play an essential role in the scavenging process when they coordinate with SOD (Wang *et al.*, 2004). It is the initial enzyme that detoxifies cellular H_2O_2 through the activity of the ascorbate–glutathione scavenging cycle and serves as catalyzer in the reaction of ascorbate with H_2O_2 (Asada, 1999). CAT is one of the major antioxidant enzymes that eliminate hydrogen peroxide by converting it into oxygen and water. However, in most cases, the CAT activities were suppressed under various herbicides exposure except for seedlings stage (Grata˜o *et al*., 2005).

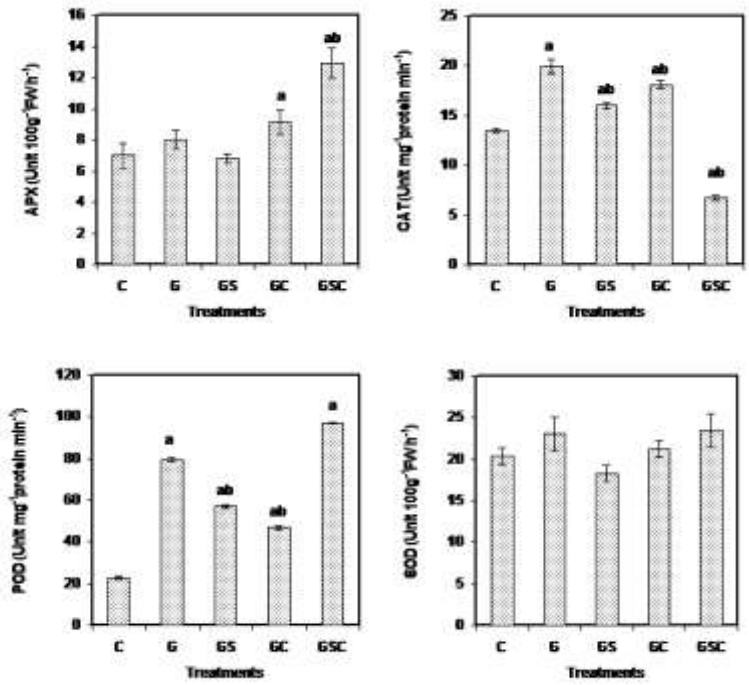

Fig.7. Effect of glyphosate, SA and CK applied alone and in combination on the antioxidant enzyme activities of maize leaves.

APX= Ascorbate peroxidase, CAT= Catalase, POD= Peroxidase, SOD= Superoxide dismutase.
Means within a column followed by a, b are significantly different from the C (control) and G (glyphosate) treated samples, respectively.

According to Cui *et al*. (2010), it was possible that SA and CK would decrease ROS production and oxidative damage to plasma membrane, which consequently improved cell membrane integrity and prevented herbicides from

entering the cells. The oxidative response in plants can be exacerbated by stressful conditions (Grata˜o *et al*., 2005). SA a signal molecule modifies the antioxidative system by inhibiting catalase and stimulating peroxidase enzymes (Rao *et al*., 1997). Obviously, the observed high activity of POD and low level of protein in SA treated maize plants discussed as a protective mechanism against glyphosate toxicity. Krantev *et al*. (2008) reported the exogenous application of SA enhanced the activities of antioxidant enzymes APX and SOD with a concomitant decline in the activity of CAT in maize plants. Inhibition of catalase by SA could serve as second messengers to induce the expression of plant defense related genes (Conarth *et al*., 1995).

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حماية نبات الذرة من الجليوفوسات باستخدام حمض السالسليك والسيتوكينين

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استهدفت هذه الدراسة استخدام حمض السالسيك والسيتوكينين لحماية نبات الذرة من أضرار المبيد العشبي جليوفوسات. زرعت حبوب الذرة في تربة رملية وتم ريها بمحلول هوجلند (MHS) وبعد الانبات وظهور الورقة الثانية قسمت النباتات إلى خمس مجموعات الأولى رشت بالماء (القياسية) والثانية رشت بمحلول الجليوفوسات (١٠ mM) والثالثة رشت بمحلول الجليوفوسات (١٠ mM) وحمض السالسيك (٠,٥ mM) والرابعة رشت بمحلول الجليوفوسات (١٠ mM) والسيتوكينين (٠,١ mM) اما الخامسة فقد رشت بمحلول الجليوفوسات (١٠ mM) وحمض السالسيك (٠,٥ mM) والسيتوكينين (٠,١ mM) .

أظهرت النتائج أن رش نباتات الذرة بمحلول الجليوفوسات أدى إلى نقصان في معدل النمو واصفرار الأوراق ثم الاحتراق التدريجى والموت متزامنا مع نقص ملحوظ في محتوى الأوراق من الكلورفيل. بينما زاد تراكم كل من البروتين والبرولين ونشاط انزيمات APX و CAT و POD و SOD. ومعاملة نباتات الذرة بحمض السالسليك أوالسيتوكينين خفف من التاثير الضار لمحلول الجليوفوسات وأدى إلى تحسن في الصفات المورفولوجية المصاحبه. ويعزى ذلك إلى دور حمض السالسليك اوالسيتوكينين في حماية الانسجة الحية من زيادة جهد التأكسد الناتج عن الجليوفوسات بالتفاعل مع الشقوق الحرة وحماية الاغشية البلازمية والحفاظ على وظائفها وتنظيم مواد ضد التأكسد الانزيمية والغير انزيمية. والنتائج تشير إلى ان لكل من حمض السالسيك والسيتوكينين دورا في استثارة مركز التحكم الايضية لتحمل نباتات الذرة للاجهاد الناتج عن الجليوفوسات. وأدى الجمع بين كل من حمض السالسليك والسيتوكينين إلى فقد دور كل منهما على حدة وقد يعزى ذلك لتاثير حمض السالسليك السلبي على مراكز الاثارة للسيتوكينين.