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Salicylic Acid Pretreatment Modulates Wheat Responses to Glyphosate

Elena Shopova [1], Liliana Brankova [1], Zornitsa Katerova [1], Ljudmila Dimitrova [1], Dessislava Todorova [1,*], Iskren Sergiev [1] and Neveen B. Talaat [2]

[1] Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Bldg. 21, 1113 Sofia, Bulgaria; kostei@abv.bg (E.S.); lbrankova@abv.bg (L.B.); zkaterova.landzhova@gmail.com (Z.K.); dim.lyudmila@gmail.com (L.D.); iskren@bio21.bas.bg (I.S.)

[2] Department of Plant Physiology, Faculty of Agriculture, Cairo University, Giza 12613, Egypt; neveenbt@yahoo.com

* Correspondence: dessita@bio21.bas.bg

Abstract: Glyphosate is an extensively used herbicide because of its non-selective action for weed control. Salicylic acid (SA) is a phenolic compound that has the potential to increase plant tolerance to diverse stresses. To test SA ability to modulate plant responses to glyphosate we used young wheat (*Triticum aestivum* L.) seedlings grown as a water culture. Plants were sprayed with 1 mM SA, and 24 h later with 0.5 mM glyphosate. All measurements were performed 14 days after herbicide treatment. Wheat growth was reduced by glyphosate. Stress markers (proline and malondialdehyde) were significantly increased by glyphosate showing oxidative damages. Incapacity of wheat to cope with the oxidative stress was evidenced by reduction in thiols and phenolics content, accompanied by slight induction of superoxide dismutase and catalase activities. Enhanced activities of peroxidase, glutathione reductase and glutathione-S-transferase were expected to participate in glyphosate detoxification. SA applied alone had no important effects on measured parameters. SA pretreatment decreased stress markers and caused additional amplification of antioxidant defense systems in glyphosate-treated plants. Growth was partially restored in combine-treated plants due to SA application. SA probably triggered antioxidant defense to cope with the herbicide stress.

Keywords: antioxidants; growth; herbicide; plant growth regulator; stress markers; *Triticum aestivum* L.

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1. Introduction

Due to their low cost and high efficiency, glyphosate-based herbicides are applied worldwide. Glyphosate (*N*-(phosphonomethyl) glycine) is one of the most extensively used herbicide substances in modern agriculture because of its broad spectrum of weed control [1–4]. It is rapidly absorbed through leaves and transported systemically to regions of active growth within the plant, where it inhibits the biosynthesis of aromatic amino acids and phenolic compounds by blocking the shikimic acid pathway, thereby disrupting major metabolic processes such as photosynthesis and protein biosynthesis [5]. Glyphosate is a non-selective herbicide and it affects not only weeds but crop plants as well. A number of articles documented that it substantially altered germination and physiological responses of different crops such as pea [4,6–9], faba bean and common bean [10], soybean [11], tomato [12,13], maize [14,15], sorghum [10], wheat [6,7], etc. That is why the modulation of the herbicide action by application of ecologically safe plant growth regulators, which are capable to reduce the negative effects of the herbicide on non-target plants, gives rise to interest of fundamental and applied outlook.

Salicylic acid (SA) is an endogenous phenolic compound, which plays an important role in plant growth and development. It has been shown that SA is associated with the signaling networks and plant resistance to biotic and abiotic stress [16–19]. Increasing numbers of evidence was announced concerning the cross-talk interaction of SA with

other phytohormones or with diverse signaling molecules under either normal or stress conditions [17–19]. Exogenously applied SA has potential to increase the stress tolerance of economically important crops. SA could regulate, directly or indirectly, the activities of the enzymes of antioxidant defense system and modulate plant responses to various stresses [17–19], including those caused by herbicide application [12,20,21]. It can also inhibit the accumulation in plants of different plant protection products such as insecticides, fungicides and herbicides [22–25].

Wheat is a staple crop that is of fundamental importance to human civilization. Presently it is the most world-wide cultivated crop ensuring feeding of the human population [26]. It had been reported earlier that SA could modulate the glyphosate impact on seed germination and physiological traits of tomato [12], faba bean [27], barley [28] and maize [29]. To test the ability SA to regulate plant responses to glyphosate action we have run laboratory experiments with young wheat (*Triticum aestivum* L.) seedlings, grown under controlled conditions.

2. Materials and Methods

Wheat (*Triticum aestivum* L. cv. Sadovo-1) seeds were obtained from the Institute of Plant Genetic Resources "Konstantin Malkov" (Sadovo, Bulgaria). This particular variety of the common winter wheat is characterized with good tolerance to drought and low temperatures, relative resistance to lodging and high productivity. Thirteen-day old wheat seedlings grown as a water culture under controlled conditions (16/8 h day/night photoperiod, 150 μmol s^{-1} m^{-2} photon flux density, 24/18 °C), were leaf sprayed with 1 mM salicylic acid (dissolved in distilled water supplied with 1% (*v*/*v*) tween 80 as surfactant) and 24 h later sprayed with 0.5 mM glyphosate solution. All growth and biochemical measurements were performed 14 days after glyphosate treatment i.e., when wheat plants were 28 days old.

Fresh leaf material (approx. 250 mg) was grinded in 4 mL 0.1% trichloroacetic acid and centrifuged for 30 min at 15,000× *g*. The resulted supernatant was used to measure the content of stress markers. All spectrophotometrical analyses were performed on Multiskan Spectrum (Thermo Electron Corporation, Vantaa, Finland) UV/VIS spectrophotometer with plate reader. The content of free proline was determined after derivatization of 0.5 mL supernatant in 2.5 mL ninhydrin reagent for 1 h at 100 °C. After cooling the reaction mixture in ice bath, the optical density was measured at 520 nm. The proline amount was calculated by a standard curve following the method of Bates et al. [30]. Malondialdehyde was measured after incubation of 0.5 mL supernatant with 1.0 mL thiobarbituric acid for 45 min at 100 °C. The reaction was stopped in ice bath and the optical density was measured at 532 and 600 nm. The quantity of MDA was calculated on the basis of 155 mM cm^{-1} extinction coefficient following the method of Kramer et al. [31]. The method of Alexieva et al. [32] was used to determine the amount of hydrogen peroxide 75 μL supernatant, which was incubated with 75 μL 1 M KI for 1 h and the absorbance was read at 390 nm. The content of H_2O_2 was calculated by a standard curve. The total phenolics content was measured following the procedure of Swain and Goldstein [33]—the reaction mixture (20 μL supernatant, 130 μL dH_2O, 50 μL Folin–Ciocalteu reagent, and 50 μL Na_2CO_3) was incubated for 2 h at room temperature and the optical density was measured at 725 nm. The total phenolics content was calculated by a standard curve prepared with known amounts of gallic acid. The quantity of free thiol groups containing compounds was measured according to Ellman [34]—40 μL supernatant was incubated with 150 μL Ellman's reagent for 10 min and the absorbance was read at 412 nm.

The enzyme activities were determined in supernatant obtained by grinding of approximately 200 mg fresh leaves in 3 mL 100 mM potassium phosphate buffer (pH 7.0, supplemented with 1 mM EDTA and 1% PVP). The homogenate was centrifuged for 30 min at 15,000× *g* (4 °C). The activity of superoxide dismutase (SOD) was measured according to Beauchamp and Fridovich [35]. The reaction was followed at 560 nm and as one unit of SOD was defined the enzyme sufficient to cause 50% inhibition of the photochemical reduction of

nitroblue tetrazolium. The activity of catalase (CAT) was determined by monitoring the rate of decomposition of 6% H_2O_2 at 240 nm using the method of Aebi [36]. Guaiacol peroxidase activity was measured according to Dias and Costa [37] using 1% guaiacol as electron donor and 15% H_2O_2 as substrate. The reaction was followed at 470 nm. The activity of glutathione reductase (GR) was estimated by measuring the rate of reduction of GSSG at 412 nm following the method of Smith et al. [38]. Glutathione-S-transferase activity was measured according to Gronwald et al. [39]. The reaction was monitored at 340 nm and 1-chloro-2,4-dinitrobenzene was used as a substrate. All enzyme kinetics were measured on Shimadzu UV-1601 (Shimadzu, Kyoto, Japan) UV/VIS spectrophotometer. The homogenates were centrifuged in refrigerated centrifuge Sigma 2-16K (SciQuip, Wem, UK).

All experiments were performed three times in three replicates. The data presented are mean values ± SE. One way ANOVA and Duncan's post-hoc multiple range test were applied to distinguish the significant differences between treatments at $p < 0.05$.

3. Results

3.1. Effect of SA and Glyphosate Treatments on Growth Traits

Wheat growth was significantly inhibited by glyphosate as compared to the control (Figure 1). Herbicide application caused decrease in fresh weight of shoots (Figure 1A) by 34% and roots (Figure 1B) by 44%. Similarly, length of shoots (Figure 1C) and roots (Figure 1D) was inhibited by 14 and by 41%, respectively. Alone SA treatment did not provoke significant alteration in wheat growth. Plant growth was partially restored in combine-treated seedlings due to SA application, as compared to glyphosate-treated plants (Figure 1).

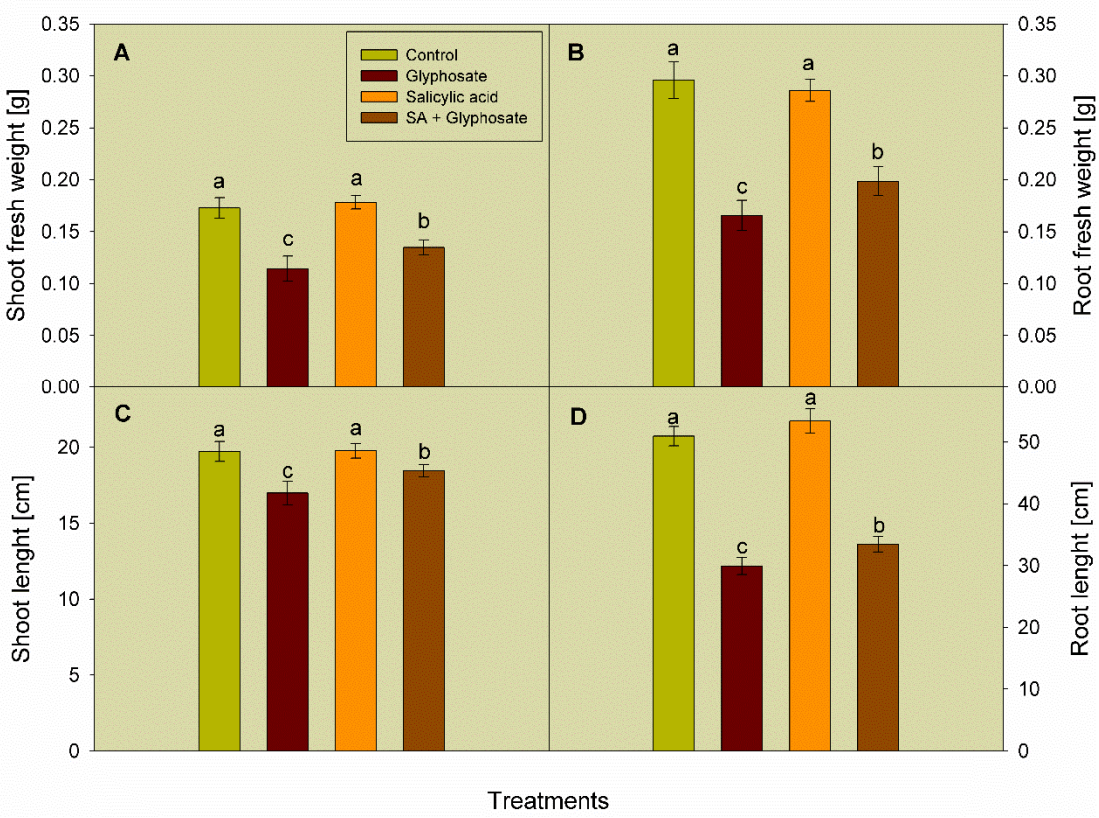

Figure 1. Fresh weight of shoots (**A**) and roots (**B**), length of shoots (**C**) and roots (**D**) of 28-day-old wheat plants 14 days after treatment with 0.5 mM glyphosate and pretreatment with 1 mM salicylic acid (SA). Bars represent mean values ± SE. Different letters within panels represent significant differences at $p < 0.05$.

3.2. Effect of SA and Glyphosate Treatments on Stress Biomarkers Content

The content of stress biomarkers proline (Figure 2A) and malondialdehyde, MDA (Figure 2B) was significantly increased by 82 and by 47%, respectively after glyphosate treatment. SA treatment by itself did not alter these parameters, while it led to a significant reduction in stress markers level in combine-treated wheat, as compared to those in glyphosate-treated seedlings.

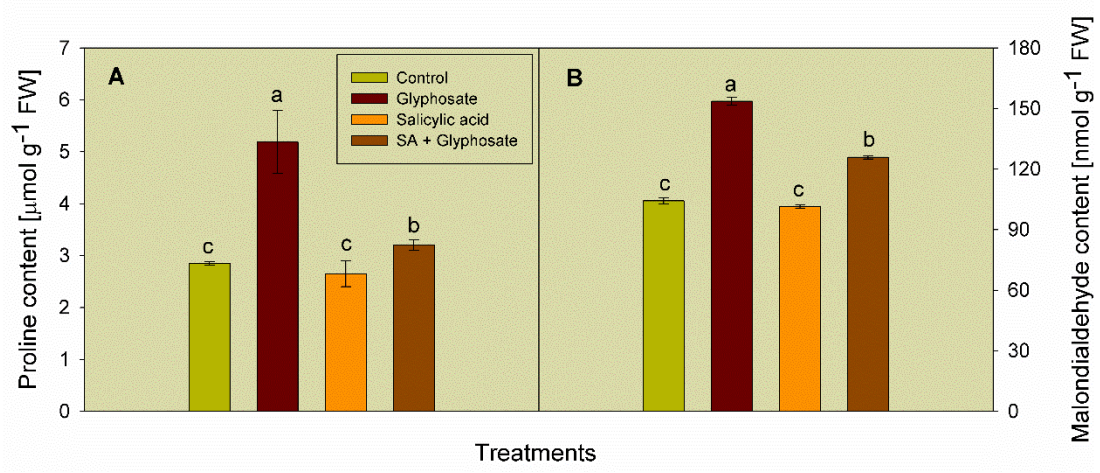

Figure 2. Content of free proline (**A**) and malondialdehyde (**B**) in leaves of 28-day-old wheat plants, 14 days after treatment with 0.5 mM glyphosate and pretreatment with 1 mM salicylic acid (SA). Bars represent mean values ± SE. Different letters within panels represent significant differences at $p < 0.05$.

3.3. Effect of SA and Glyphosate Treatments on Non-Enzymatic Antioxidant Content

Glyphosate treatments decreased plant phenolics by 12% (Figure 3A) and total thiol-containing compounds by 19% (Figure 3B). SA alone treatment caused a slight increase in non-enzymatic antioxidant, but it substantially augmented (by 17% over the respective controls) contents of plant phenolics and total thiols in combine-treated plants.

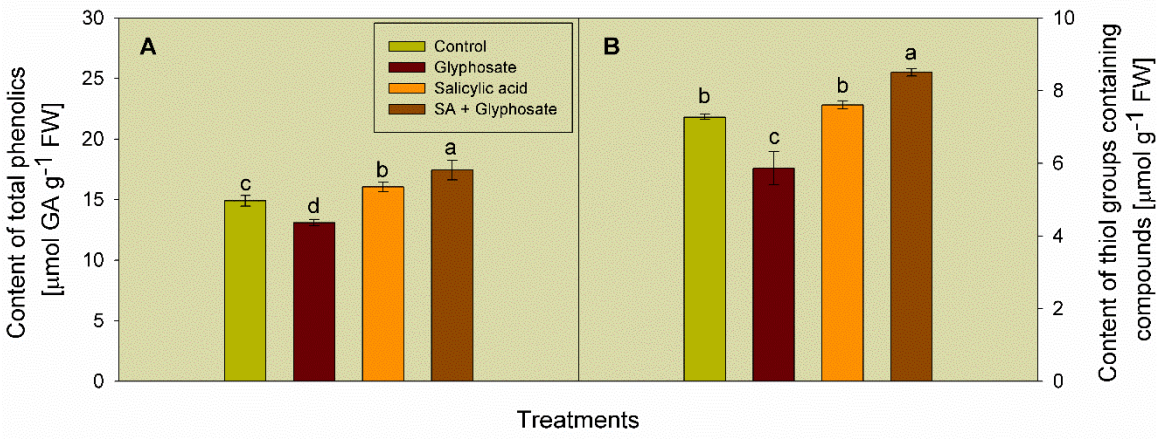

Figure 3. Content of total phenolics (**A**) and thiol groups containing compounds (**B**) in leaves of 28-day-old wheat plants, 14 days after treatment with 0.5 mM glyphosate and pretreatment with 1 mM salicylic acid (SA). Bars represent mean values ± SE. Different letters within panels represent significant differences at $p < 0.05$.

3.4. Effect of SA and Glyphosate Treatments on the Activity of Some Antioxidant Enzymes, and the Content of Hydrogen Peroxide

Hydrogen peroxide content (Figure 4A) was not significantly changed by glyphosate application. A slight induction of the activity of superoxide dismutase (SOD) by 21% (Figure 4B)

and catalase (CAT) by 17% (Figure 4C) was detected in glyphosate-treated seedlings. The activities of peroxidase (POX) (Figure 4D), glutathione reductase (GR) (Figure 4E), and glutathione-S-transferase (GST) (Figure 4F) were substantially increased by 269, 67 and 27%, respectively, due to glyphosate treatment as compared to the control levels.

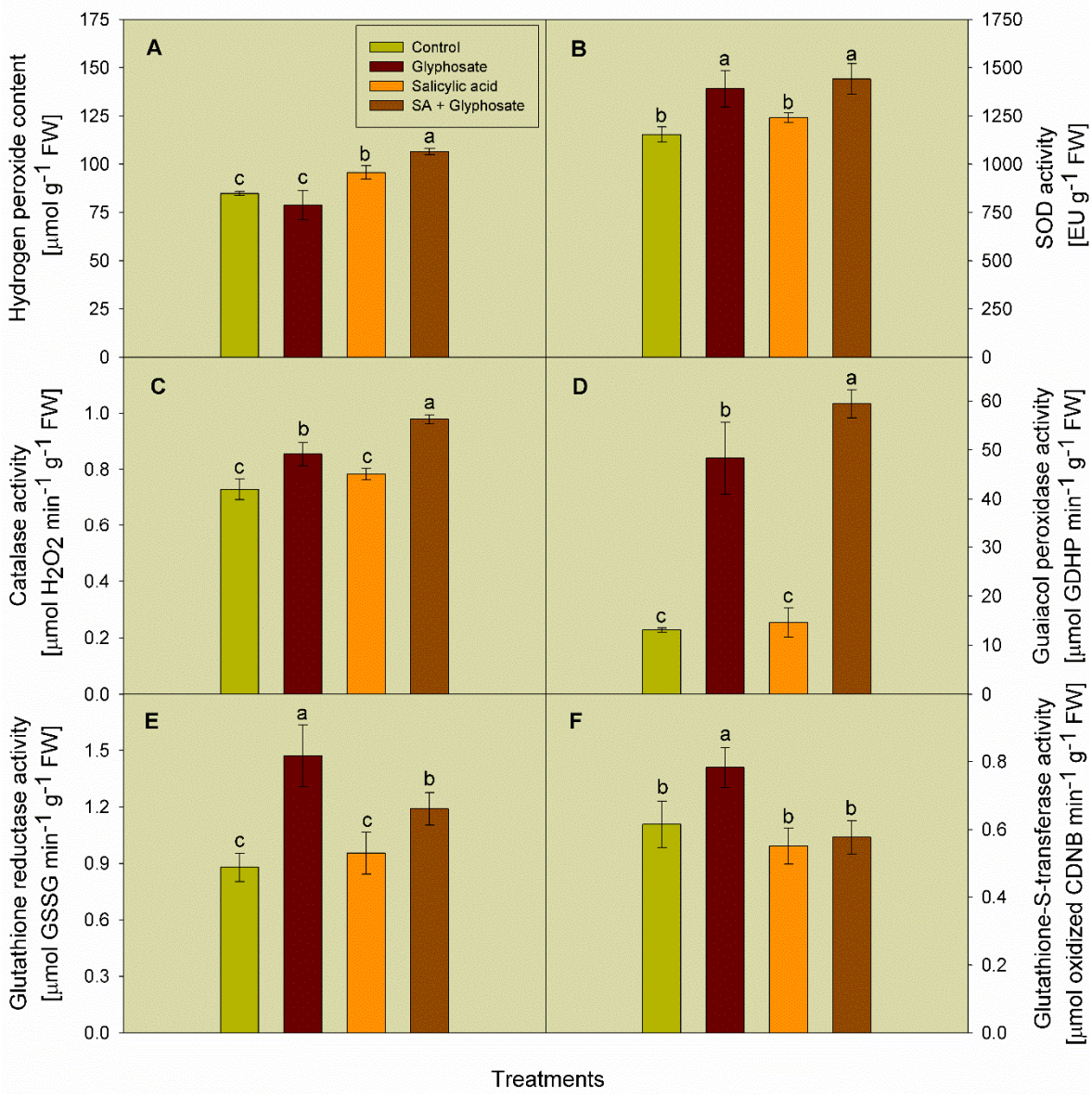

Figure 4. Content of hydrogen peroxide (**A**) and activity of SOD (**B**), catalase (**C**), guaiacol peroxidase (**D**), glutathione reductase (**E**) and glutathione-S-transferase (**F**) in leaves of 28-day-old wheat plants, 14 days after treatment with 0.5 mM glyphosate and pretreatment with 1 mM salicylic acid (SA). Bars represent mean values ± SE. Different letters within panels represent significant differences at $p < 0.05$.

Individually applied SA caused increase by 13% in H_2O_2 amount (Figure 4A), while it did not alter activities of antioxidant and herbicide-detoxifying enzymes. SA pretreatment substantially reduced the activities of GR (Figure 4E) and GST (Figure 4F) in combine-treated plants as compared to those detected in glyphosate-treated wheat. However additional amplification of the activity of antioxidant enzymes SOD, CAT and POX was observed after combined treatment and it reached up to 25, 34 and 353% respectively as compared to the control levels. Hydrogen peroxide content was also increased by the combine treatment up to 26% than the control.

4. Discussion

Herbicide treatment, among other stress factors, disrupts weeds metabolic processes resulting in death. However, usage of non-selective herbicides also induces stressful circumstances and causes injury to the non-target plants. Plants treated with herbicides undergo an increased generation of reactive oxygen species—ROS [40,41]. Over-accumulation of ROS triggers chain oxidation reactions that cause adverse effects on plant metabolism, leading to retarded crop growth and reduced yield and quality of agricultural production. Generally, measurement of the quantity of stress biomarkers gives valuable assessment for induced stress damages. To survive the destructive consequences of the unfavorable environmental factors, plants have evolved an effective ROS-scavenging defense system which includes enzymatic and non-enzymatic antioxidants that assist plants to continued growth under harsh environment and contribute to stress tolerance [1,2,40]. The antioxidant machinery includes enzymatic (SOD, CAT, POX APX, glutathione peroxidase, GR, MDHAR, DHAR and GST) and non-enzymatic antioxidant systems (ascorbic acid, glutathione, tocopherol, etc.) that work coordinately to regulate ROS [42]. The protective capacity of plants could be increased due to application of different plant growth regulating substances, including SA [40]. In this connection, the objective of our study was to assess the potential of SA to mitigate glyphosate-induced stress injuries in wheat through measurement of basic cellular and biochemical traits that participate in the antioxidant network.

Glyphosate caused typical adverse alterations in the growth of wheat (Figure 1) expressed by obvious retardation of fresh biomass accumulation, and plant organs elongation, which is in accordance with earlier studies on different glyphosate-treated crops [4,9,12,14,28,43]. Glyphosate-suppressed plant growth is also a consequence of accumulation of ROS, which harm cellular biomembranes as is evidenced by the increased amount of MDA (Figure 2B). MDA is a final product of the peroxidation of unsaturated fatty acids, ingredients of the cellular biomembranes, and its accumulation is a typical indicator for oxidative stress occurring in plants. Our results support earlier reports of [4,12,14,27,28] who also found an increased amount of MDA after glyphosate application. Besides MDA, the increase in free proline in plants treated with diverse stress factors is a frequent event [44]. We found that the application of glyphosate increased greatly proline content in wheat, and a similar increase was also detected in other glyphosate-treated crops as pea [4], maize [14], barley [28] and tomato [12,43]. Glyphosate altered antioxidant defense and provoked decrease in the level of the non-enzymatic antioxidants, thiols and phenolics (Figure 3), significantly decreasing wheat's ROS scavenger capacity while it increased the activity of antioxidant and herbicide-detoxifying enzymes (Figure 4). Decrease in non-enzymatic antioxidant due to glyphosate application was observed also by [12,28], along with an increase in enzymatic activities [12] showing the attempt of plants to cope with herbicide stress.

Pretreatment of plants with diverse chemicals at vegetative stages could improve plant tolerance to succeeding exposure of various abiotic stress factors through enhanced internal plant defense capacity (both enzymatic and non-enzymatic antioxidants) as compared to that of single stressed plants [42]. We found that single SA treatment did not change growth and biochemical traits, except for slightly increased phenolics (Figure 3) and H_2O_2 (Figure 4A) in wheat seedlings. However, when applied before herbicide it had important positive effect on wheat's morphometric (Figure 1) and biochemical (Figures 2–4) traits. Pretreatment with SA improved partially the growth of herbicide-treated plants and increased plant biomass, while it reduced oxidative stress injuries as evidenced by lowered MDA and proline amounts as compared to those of glyphosate-treated seedlings. Nowadays it is accepted that H_2O_2 may participate not only in oxidative stress induced injuries as ROS but can serve as signaling molecule activating various acclimation mechanisms, including molecular, phytohormone related, metabolic etc. [45,46]. Slightly induced H_2O_2 by SA application probably triggers antioxidant defense to cope with the herbicide stress—The activities of catalase and peroxidase were increased to a greater extent in comparison with those of glyphosate-treated only plants. The reduction in oxidative stress generated by glyphosate in SA + glyphosate-treated plants due to SA application was reported by [27]

and by [28] who also noted positive modulation in POX and CAT activities concomitant with substantial decrease in MDA and proline. Along with the antioxidant enzymes, non-enzymatic antioxidant contents (Figure 3) also were shifted up by SA pretreatment as compared to those in herbicide-treated only plants, suggesting boosted antioxidant defense to cope with oxidative stress. SA-induced non-enzymatic antioxidants (low molecular thiols and total phenolics) seem to have role of ROS scavengers and probably take part in lowering lipid oxidation in glyphosate-treated plants. Our findings are in accordance with earlier studies reported by [12,27,28] who provided useful information about the ability of SA to mitigate, at least in part, the plant stress induced by glyphosate via triggering the antioxidant defense. It is known that SA could activate antioxidant enzymes involved into glutathione-ascorbate cycle such as GR, MDHAR, DHAR, etc., as well as xenobiotic detoxifying enzyme GST [17,19]. Surprisingly we did not find additional induction of GR and GST activities in SA + glyphosate-treated plants in comparison with herbicide-treated. Controversially these activities were lower than those in glyphosate-treated only plants and were even near to control levels (Figure 4E,F). Probably in our experiment SA activated predominantly typical antioxidant enzymes such as CAT and POX, while it had little effect on glutathione-related enzymes and completely eliminated the induction effect of glyphosate on GST activity.

The alleviation of oxidative stress resulting in less ROS accumulation after pretreatment is related either to the enhanced antioxidant capacity or to the direct ROS scavenging by the primers themselves [42]. Further SA can participate straightforwardly as ROS scavenger (as of its phenolic nature) and activate directly or indirectly non-enzymatic and enzymatic defense of herbicide-treated plants to mitigate stress injuries [19]. In our study we confirmed that SA is able to modulate plant responses to glyphosate action influencing antioxidant defense.

5. Conclusions

Glyphosate substantially suppressed wheat growth and non-enzymatic antioxidant defense, while significantly increasing stress markers (proline and malondialdehyde) content. Its application slightly induced superoxide dismutase and catalase activities, while it importantly enhanced peroxidase, glutathione reductase and glutathione-S-transferase activities that were expected to participate in glyphosate detoxification. SA decreased stress markers and caused additional amplification of antioxidant defense systems in glyphosate-treated plants, while it had slight or no effect on glutathione-related enzymes GR and GST. SA probably triggered antioxidant defense to mitigate (at least in part) the herbicide stress consequences, which allowed combine-treated plants to restore partially their growth.

Author Contributions: D.T. and N.B.T.—Conceptualized and coordinated the research; L.D., Z.K., and D.T.—grew and treated the plants; I.S., D.T., E.S., L.B., L.D., and Z.K.—performed the laboratory analyses, collected and interpreted the data; L.B.—prepared figures; D.T. and Z.K.—prepared the original draft of the manuscript; I.S. and D.T.—reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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References

1. Gomes, M.; Smedbol, É.; Chalifour, A.; Hénault-Ethier, L.; Labrecque, M.; Lepage, L.; Lucotte, M.; Juneau, P. Alteration of plant physiology by glyphosate and its by-product aminomethylphosphonic acid: An overview. *J. Exp. Bot.* **2014**, *65*, 4691–4703. [CrossRef] [PubMed]
2. De Freitas-Silva, L.; Rodríguez-Ruiz, M.; Houmani, H.; da Silva, L.C.; Palma, J.M.; Corpas, F.J. Glyphosate-induced oxidative stress in Arabidopsis thaliana affecting peroxisomal metabolism and triggers activity in the oxidative phase of the pentose phosphate pathway (OxPPP) involved in NADPH generation. *J. Plant Physiol.* **2017**, *218*, 196–205. [CrossRef]
3. Ferreira, M.F.; Torres, C.; Bracamonte, E.; Galetto, L. Effects of the herbicide glyphosate on non-target plant native species from Chaco forest (Argentina). *Ecotoxicol. Environ. Saf.* **2017**, *144*, 360–368.
4. Sergiev, I.; Todorova, D.; Shopova, E.; Brankova, L.; Jankauskienė, J.; Jurkonienė, S.; Gavelienė, V.; Mockevičiūtė, R. Assessment of synthetic auxin type compounds as potential modulators of herbicide action in *Pisum sativum* L. *Biologia* **2020**, *75*, 1845–1853. [CrossRef]
5. Cobb, A.H.; Reade, J.P. *Herbicides and Plant Physiology*; John Wiley & Sons, Inc.: West Sussex, UK, 2010; p. 296.
6. Miteva, L.; Ivanov, S.; Alexieva, V.; Karanov, E. Effect of herbicide glyphosate on glutathione levels, glutathi-one-S-transferase and glutathione reductase activities in two plant species. *Compt. Rend. Acad. Bulg. Sci.* **2003**, *56*, 79–84.
7. Miteva, L.; Tsoneva, J.; Ivanov, S.; Alexieva, V. Alterations of the content of hydrogen peroxide and malondialdehyde and the activity of some antioxidant enzymes in the roots and leaves of pea and wheat plants exposed to glyphosate. *Compt. Rend. Acad. Bulg. Sci.* **2005**, *58*, 733–738.
8. Miteva, L.P.-E.; Ivanov, S.V.; Alexieva, V.S. Alterations in glutathione pool and some related enzymes in leaves and roots of pea plants treated with the herbicide glyphosate. *Russ. J. Plant Physiol.* **2010**, *57*, 131–136. [CrossRef]
9. Mondal, S.; Kumar, M.; Haque, S.; Kundu, D. Phytotoxicity of glyphosate in the germination of Pisum sativum and its effect on germinated seedlings. *Environ. Health Toxicol.* **2017**, *32*, e2017011. [CrossRef]
10. Maldani, M.; Aliyat, F.Z.; Cappello, S.; Morabito, M.; Giarratana, F.; Nassiri, L.; Ibijbijen, J. Effect of glyphosate and paraquat on seed germination, amino acids, photosynthetic pigments and plant morphology of Vicia faba, Phaseolus vulgaris and Sorghum bicolor. *Environ. Sustain.* **2021**, 1–11. [CrossRef]
11. Jiang, L.-X.; Jin, L.-G.; Guo, Y.; Tao, B.; Qiu, L.-J. Glyphosate effects on the gene expression of the apical bud in soybean (*Glycine max*). *Biochem. Biophys. Res. Commun.* **2013**, *437*, 544–549. [CrossRef]
12. Singh, H.; Singh, N.B.; Singh, A.; Hussain, I. Exogenous application of salicylic acid to alleviate glyphosate stress in So-lanum lycopersicum. *Int. J. Veg. Sci.* **2017**, *23*, 552–566. [CrossRef]
13. Khan, S.; Zhou, J.L.; Ren, L.; Mojiri, A. Effects of glyphosate on germination, photosynthesis and chloroplast morphology in tomato. *Chemosphere* **2020**, *258*, 127350. [CrossRef] [PubMed]
14. Sergiev, I.G.; Alexieva, V.S.; Ivanov, S.V.; Moskova, I.I.; Karanov, E.N. The phenylurea cytokinin 4PU-30 protects maize plants against glyphosate action. *Pestic. Biochem. Physiol.* **2006**, *85*, 139–146. [CrossRef]
15. Gomes, M.P.; Richardi, V.S.; Bicalho, E.M.; da Rocha, D.C.; da Silva, M.A.N.; Soffiatti, P.; Garcia, Q.S.; Sant'Anna-Santos, B.F. Effects of Ciprofloxacin and Roundup on seed germination and root development of maize. *Sci. Total Environ.* **2019**, *651*, 2671–2678. [CrossRef] [PubMed]
16. Zhao, P.; Lu, G.-H.; Yang, Y.-H. Salicylic Acid Signaling and its Role in Responses to Stresses in Plants. In *Mechanism of Plant Hormone Signaling under Stress*; Wiley: New York, NY, USA, 2017; pp. 413–441.
17. Khan, M.I.R.; Fatma, M.; Per, T.S.; Anjum, N.A.; Khan, N.A. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. *Front. Plant Sci.* **2015**, *6*, 462. [CrossRef]
18. Hayat, Q.; Hayat, S.; Irfan, M.; Ahmad, A. Effect of exogenous salicylic acid under changing environment: A review. *Environ. Exp. Bot.* **2010**, *68*, 14–25. [CrossRef]
19. Hasanuzzaman, M.; Nahar, K.; Bhuiyan, T.I.A.T.F.; Anee, T.I.; Inafuku, M.; Oku, H.; Fujita, M. Salicylic Acid: An All-Rounder in Regulating Abiotic Stress Responses in Plants. In *Phytohormones-Signaling Mechanisms and Crosstalk in Plant Development and Stress Responses*; IntechOpen: London, UK, 2017; pp. 31–75.
20. Wang, J.; Lv, M.; Islam, F.; Gill, R.A.; Yang, C.; Ali, B.; Yan, G.; Zhou, W. Salicylic acid mediates antioxidant defense system and ABA pathway related gene expression in Oryza sativa against quinclorac toxicity. *Ecotoxicol. Environ. Saf.* **2016**, *133*, 146–156. [CrossRef]
21. Radwan, D.E. Salicylic acid induced alleviation of oxidative stress caused by clethodim in maize (*Zea mays* L.) leaves. *Pestic. Biochem. Physiol.* **2012**, *102*, 182–188. [CrossRef]
22. Cui, J.; Zhang, R.; Wu, G.L.; Zhu, H.M.; Yang, H. Salicylic acid reduces napropamide toxicity by preventing its accumu-lation in rapeseed (*Brassica napus* L.). *Arch. Environ. Contam. Toxicol.* **2010**, *59*, 100–108. [CrossRef]
23. Liang, L.; Lu, Y.L.; Yang, H. Toxicology of isoproturon to the food crop wheat as affected by salicylic acid. *Environ. Sci. Pollut. Res.* **2012**, *19*, 2044–2054. [CrossRef]
24. Wang, C.; Zhang, Q. Exogenous salicylic acid alleviates the toxicity of chlorpyrifos in wheat plants (*Triticum aestivum*). *Ecotoxicol. Environ. Saf.* **2017**, *137*, 218–224. [CrossRef]
25. Liu, T.; Li, T.; Zhang, L.; Li, H.; Liu, S.; Yang, S.; An, Q.; Pan, C.; Zou, N. Exogenous salicylic acid alleviates the accumula-tion of pesticides and mitigates pesticide-induced oxidative stress in cucumber plants (*Cucumis sativus* L.). *Ecotoxicol. Environ. Saf.* **2021**, *208*, 111654. [CrossRef]

26. Shiferaw, B.; Smale, M.; Braun, H.-J.; Duveiller, E.; Reynolds, M.; Muricho, G. Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. *Food Secur.* **2013**, *5*, 291–317. [CrossRef]
27. Fayez, K.A.; Ali, E.F. Impact of Glyphosate Herbicide and Salicylic Acid on Seed Germination, Cell Structure and Physiological Activities of Faba Bean (*Vicia faba* L.) Plant. *Ann. Res. Rev. Biol.* **2017**, *17*, 1–15. [CrossRef]
28. Spormann, S.; Soares, C.; Fidalgo, F. Salicylic acid alleviates glyphosate-induced oxidative stress in *Hordeum vulgare* L. *J. Environ. Manag.* **2019**, *241*, 226–234. [CrossRef] [PubMed]
29. Akbulut, G.B.; Yigit, E.; Bayram, D. Investigation of the Effects of Salicylic Acid on Some Biochemical Parameters in Zea mays to Glyphosate Herbicide. *J. Environ. Anal. Toxicol.* **2015**, *5*, 271. [CrossRef]
30. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water-stress studies. *Plant Soil* **1973**, *39*, 205–207. [CrossRef]
31. Kramer, G.F.; Norman, H.A.; Krizek, D.T.; Mirecki, R.M. influence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids in cucumber. *Phytochemistry* **1991**, *30*, 2101–2108. [CrossRef]
32. Alexieva, V.; Sergiev, I.; Mapelli, S.; Karanov, E. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell Environ.* **2001**, *24*, 1337–1344. [CrossRef]
33. Swain, T.; Goldstein, L. *Methods in Polyphenol Chemistry*; Pridham, J.B., Ed.; Pergamon Press: Oxford, UK, 1964; pp. 131–146.
34. Ellman, G.L. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* **1959**, *82*, 70–77. [CrossRef]
35. Beauchamp, C.; Fridovich, I. Superoxide dismutase. Improved assay and an assay applicable to acrylamide gels. *Anal. Biochem.* **1971**, *44*, 276–287. [CrossRef]
36. Aebi, M. Catalase in vitro. *Methods Enzymol.* **1984**, *105*, 121–126.
37. Dias, M.A.; Costa, M.M. Effect of Low Salt Concentrations on Nitrate Reductase and Peroxidase of Sugar Beet Leaves. *J. Exp. Bot.* **1983**, *34*, 537–543. [CrossRef]
38. Smith, I.K.; Vierheller, T.L.; Thorne, C.A. Assay of glutathione reductase in crude tissue homogenates using 5,5′-dithiobis(2-nitrobenzoic acid). *Anal. Biochem.* **1988**, *175*, 408–413. [CrossRef]
39. Gronwald, J.W.; Fuerst, E.P.; Eberlein, C.V.; Egli, M.A. Effect of herbicide antidotes on glutathione content and gluta-thione S-transferase activity of sorghum shoots. *Pestic. Biochem. Physiol.* **1987**, *29*, 66–76. [CrossRef]
40. Varshney, S.; Khan, M.I.R.; Masood, A.; Per, T.S.; Rasheed, F.; A Khan, N. Contribution of Plant Growth Regulators in Mitigation of Herbicidal Stress. *J. Plant Biochem. Physiol.* **2015**, *3*, 160. [CrossRef]
41. Langaro, A.C.; Agostinetto, D.; Ruchel, Q.; Garcia, J.R.; Perboni, L.T. Oxidative stress caused by the use of preemergent herbicides in rice crops. *Rev. Ciência Agronômica* **2017**, *48*, 358–364. [CrossRef]
42. Savvides, A.; Ali, S.; Tester, M.; Fotopoulos, V. Chemical Priming of Plants Against Multiple Abiotic Stresses: Mission Possible? *Trends Plant Sci.* **2016**, *21*, 329–340. [CrossRef]
43. Soares, C.; Pereira, R.; Spormann, S.; Fidalgo, F. Is soil contamination by a glyphosate commercial formulation truly harmless to non-target plants?—Evaluation of oxidative damage and antioxidant responses in tomato. *Environ. Pollut.* **2019**, *247*, 256–265. [CrossRef]
44. Hayat, S.; Hayat, Q.; Alyemeni, M.N.; Wani, A.S.; Pichtel, J.; Ahmad, A. Role of proline under changing environments: A review. *Plant Signal Behav.* **2012**, *7*, 1456–1466. [CrossRef]
45. Hossain, M.A.; Li, Z.-G.; Hoque, T.S.; Burritt, D.J.; Fujita, M.; Munné-Bosch, S. Heat or cold priming-induced cross-tolerance to abiotic stresses in plants: Key regulators and possible mechanisms. *Protoplasma* **2018**, *255*, 399–412. [CrossRef] [PubMed]
46. Carvalho, F.E.L.; Silveira, J.A.G. H2O2-retrograde signaling as a pivotal mechanism to understand priming and cross stress tolerance in plants. In *Priming-Mediated Stress and Cross-Stress Tolerance in Crop Plants*; Hossain, M.A., Liu, F., Bur-ritt, D., Fujita, M., Huang, B., Eds.; Academic Press: Cambridge, MA, USA, 2020; pp. 57–78.