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Contribution of auxin to the resistance of a soft wheat variety (*Triticum aestivum* L.) to abiotic stress

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Abstract
The massive use of phytosanitary products to protect crops can lead to oxidative stress, cellular toxicity and disruption of the normal physiological functions of plants, which is detrimental to their growth and productivity. In order to limit these adverse impacts and improve plant growth, it is essential to use phytohormones. These plant hormones control essential processes such as mitosis and expansion and help to strengthen the resilience of plants against various stresses, including those caused by pesticides. Our study aims to examine the toxicity of a systemic herbicide (glyphosate) on the growth and development of a variety of soft wheat (*Triticum aestivum* L.), both in the presence and absence of auxin. The data obtained in this study indicate a decrease in the germination rate as well as a reduction in root and leaf growth. In addition, the induction of some enzymatic parameters such as catalase and superoxide dismutase, as well as non-enzymatic parameters such as malondialdehyde, was observed in soft wheat treated with glyphosate in the absence of auxin. However, in the presence of auxin, all the enzymatic and non-enzymatic parameters measured were lower than those obtained without auxin. In addition, increased root and leaf growth and a high germination rate were noted, demonstrating the importance of auxin in the development and preservation of the plant by mitigating the intensity of oxidative stress.

Keywords: *Auxin; glyphosat; soft wheat; oxidative stress; Triticum aestivum L.*

Introduction

In agriculture, herbicides are commonly used to control weeds and volunteer plants, particularly in cereal and pulse crops (Nedjah, 2015). Glyphosate is one of the main herbicides used to reduce the mass of weeds, by regulating fruit maturation processes, these compounds promote more uniform ripening and facilitate earlier harvesting, thereby improving fruit quality and production efficiency (Kumar *et al.,* 2022). Pesticides in soil can directly influence plant physiological processes, particularly hindering root and leaf development, they disrupt nutrient uptake and circulation, which impairs leaf and root growth (Awais *et al.,* 2017). Faced with this attack, plants have developed effective detection, communication and response mechanisms to manage these harmful stresses (Radhouane, 2011). According to He *et al.* (2018), various universal metabolites are associated with plant resistance to abiotic stresses, there are several universal metabolites associated with the response of plants to abiotic stresses, such as the cuticle as an external barrier, unsaturated fatty acids (UFAs) as membrane regulators and oxylipin precursors, reactive species scavengers, and chaperone molecules (which stabilize proteins and subcellular structures). Phytohormones represent one of the most important components of plant stress-response mechanisms. They function as signaling molecules that regulate cellular communication and coordinate physiological and molecular responses, even at very low concentrations. These hormones play a central role in enabling plants to perceive, integrate, and adapt to various abiotic stresses, thereby contributing to stress tolerance

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and survival (Kazen, 2023). Yadav *et al.* (2021) recently demonstrated that phytohormones, such as auxin, play a crucial role in plant resistance to stress by controlling cellular functions at the molecular level via the cell signaling mechanism. Among the various mechanisms that plants employ to cope with abiotic stress, phytohormones constitute a crucial signaling network. Acting as endogenous chemical messengers at low concentrations, they regulate plant growth, development, and stress-responsive pathways by coordinating complex physiological and molecular processes (Ali *et al.,* 2023). Auxin, a recently identified plant hormone, is also involved in multiple physiological actions, such as growth, rooting, seed germination, photosynthesis and protection against abiotic and biotic stresses (Maheshwari *et al.,* 2015). Auxin, regulates essential processes such as seed germination, root development, cell elongation, and overall plant growth. It also interacts with other phytohormones to coordinate plant responses to abiotic and biotic stresses, thereby enhancing adaptation and tolerance to adverse environmental conditions (Casal and Estevez, 2021; Wang et al., 2022). Our study falls within this specific framework, where we seek to define the impact of a plant hormone (auxin), on the growth and development of a variety of soft wheat exposed to glyphosate.

Materials and Methods

Presentation of the biological material

The biological material used in our study consists of soft wheat grains of the genus *Triticum aestivum* L, this is the variety HD 1220, the seeds of which were kindly supplied to us by field of grain supply, storage and distribution, Annaba, Algeria.

Chemical material

The chemical material used in this study is a glycine derivative, glyphosate, a non-selective herbicide with systemic action, used as a weedkiller in agriculture, particularly in maize and wheat crops. It acts by blocking the synthesis chain of amino acid precursors essential for plant function (Mazoyer, 2015).

Biochemical material

The biochemical material used in this study is indole-3-acetic acid (IAA). Most synthetic auxins are used as herbicides in horticulture and agriculture. They are mainly used as cutting and growth hormones (Zhao *et al.,* 2010).

Test conduct and treatment

Selected soft wheat (*Triticum durum* Desf.) seeds are carefully disinfected by exposing them to a 5% sodium hypochlorite solution for three minutes, followed by a thorough rinsing with distilled water. Before use, soft wheat seeds are carefully and precisely selected. They must be in perfect condition and show no visible irregularities (neither breakage nor obvious signs of disease). The seeds are planted in plastic cells filled with sterilized potting soil (10 g), usually with three seeds per cell. The seeds are planted in cavities about 1 cm deep, created with a pen. The phytohormone is applied by soaking soft wheat seeds in hormone solutions prepared at different concentrations for 6 hours. Subsequently, the seeds are washed and rehydrated at 25°C on sterile filter paper, using a ventilated oven, until they regain their original humidity. The glyphosate treatment was applied as soon as the seeds were sown, all dosages were carried out on the roots after one month of treatment, in the presence or absence of auxin (Table1).

Table 1. *The different concentrations of glyphosate and auxin used in this study.*

Product	T	C1	C2	C3
Glyphosate (µM)	0	100	250	500
Auxin (µM)	0	100	250	500

Parameters studied

Average root length. Root elongation parameters are determined in petri dishes and carried out on 5-day germinations, in the presence or absence of auxin. Average root length is monitored by marking with Chinese ounce (Mammadov, 1999).

Germination rate. The percentage of germinated seeds in relation to seeds sown at the beginning for a given time (each fortnight of the treatment) was calculated as a function of internal factors (embryonic dormancy and integumentary inhibitions) and external germination factors (temperature, humidity and light, etc.) (Kabil *et al.,* 2016).

Average length of the first leaf. After 1 month in culture, the length of the first leaf of each germinated wheat seed is measured using a tape measure. The average length was calculated for each pot and for each treatment condition (Mammadov, 1999).

Malondialdehyde (MDA) measurement. The evolution of malondialdehyde (MDA) concentration, de-

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termined according to the method of Alia et al. (1995), allowed the estimation of lipid peroxidation. Fresh roots were ground in 5% trichloroacetic acid (TCA) using a proportion of 10 ml for each gram of plant, then centrifuged for 15 minutes at a force of 12,000 g. An equivalent amount of 0.5% thiobarbituric acid (TBA) prepared in 20% trichloroacetic acid (TCA) was incorporated into the superimposed liquid phase. The mixture was incubated at a temperature of 100 °C for half an hour. The reaction was then stopped by immersing the tubes in an ice bath. The absorbance of the supernatant was measured following centrifugation at 10,000 g for five minutes at a wavelength of 532 nm.

***Monitoring of Catalase Activity* (CAT).** The catalase assay (CAT) is performed following the technique of Cakmak and Horst (1991). Absorbance is recorded as a decrease over three minutes at a wavelength of 240 nm and a molar extinction coefficient ε = 39400 $M^{-1}.cm^{-1}$.L.To obtain a total volume of 3 ml, the composition of the reaction mixture is as follows: 100 µL of crude enzyme extract, 50 µL of hydrogen peroxide (HO) at a concentration of 0.3%, and finally 2850 µL of phosphate buffer (50 mM, pH = 7.2). The instrument is calibrated without the need for the enzyme extract. The addition of hydrogen peroxide initiates the reaction. Catalase activity is measured in nmol/min/mg of protein.

***Monitoring of Superoxide Dismutase activity* (SOD).** Superoxide dismutase activity assessment was performed based on the technique of Rao *et al.* (2000). The NBT reaction mixture includes 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin, and 0.1 mM EDTA, the reaction mixture consisted of 3 mL of NBT reagent solution and 0.1 mL of enzyme extract. Reaction activation was performed by adding 2 µM riboflavin and exposing the tubes to 15 W fluorescent lamps for 15 minutes. A complete reaction mixture without enzyme extract was used as a control sample. The photochemical efficiency of NBT was quantified at a specific wavelength of 560 nm (Rao *et al.,* 2014).

Statistical analysis

The results presented are based on the mean and standard deviation. The analysis was performed using the one-way Analysis of Variance (ANOVA) statistical test, taking into account the time periods and concentrations implemented, using Minitab data analysis software (version 16.0).

Results

Impact of glyphosate on average root length according to the presence and absence of auxin

From the data obtained, we note a notable and significant reduction ($p \leq 0.001$) in the average length of roots subjected to increasing concentrations of glyphosate in the absence of auxin, compared to control roots. This decrease goes from 8 cm in control roots to 5 cm in roots treated with the highest concentration (C3). However, when auxin is applied, the average root length increases significantly to reach a maximum in roots treated with the highest dose (12.5 cm), an increase of (≈2) times compared to the control root (Fig. 1).

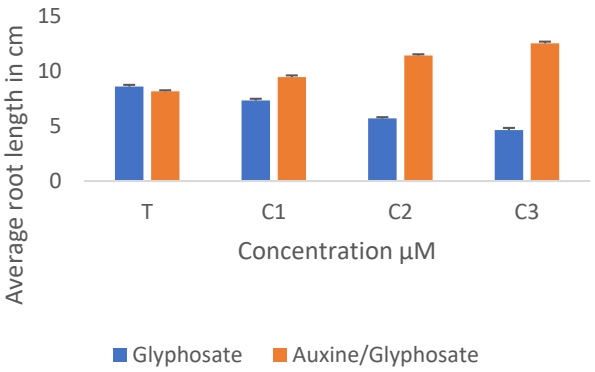

Figure1. *Variation in root length under glyphosate treatment with or without auxin.*

Effect of glyphosate on average leaf length in the presence and absence of auxin.

The data collected reveals a significant reduction ($p \leq 0.001$) in the average leaf length in plants treated with different concentrations of glyphosate in the absence of auxin. In fact, treatment of the seeds with glyphosate reduced the leaf growth of the seedlings according to the different concentrations C1, C2 and

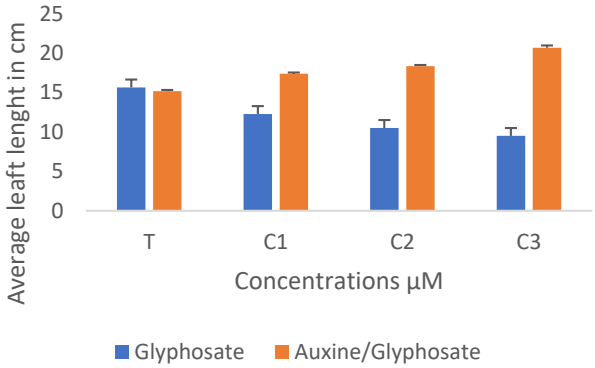

Figure 2. *Effect of glyphosate on the average length of soft wheat leaves in the presence and absence of auxin.*

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C3 to reach values of 12, 10 and 9 cm respectively, compared with the control value (15 cm). However, in the presence of auxin, the average leaf length increased significantly and proportionally to the dose ($p \leq 0.001$). This growth increased from 15 cm for the control leaves to a maximum of 20 cm for those treated with the highest concentration (Fig. 2).

Effect of glyphosate on germination percentage in the presence and absence of glyphosate.

According to the results obtained, the exposure of plants to different concentrations of glyphosate significantly inhibited ($P < 0.001$) the germination of seeds compared to the controls and in the absence of auxin, the germination rate goes from 73% in the control batch to 35% in the batch treated with the highest dose of xenobiotic, a decrease of 50%. However, in the presence of phytohormone (auxin) we recorded a dose-dependent increase in the percentage of germination, this increase goes from 73% in the control batch to 91% in the batch treated with the highest dose of auxin (Fig. 3).

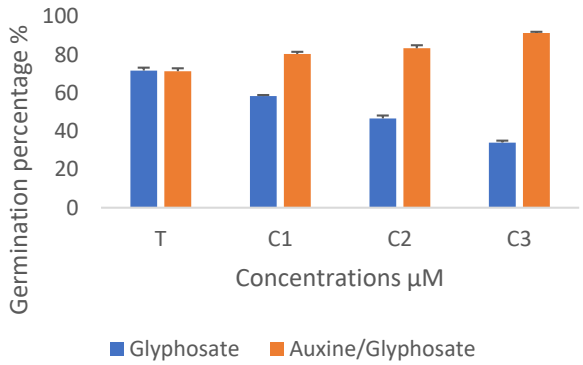

Figure 3. *Impact of glyphosate on the germination rate of soft wheat seeds according to the presence or absence of glyphosate.*

Effect of glyphosate on the level of malondialdehyde (MDA) in soft wheat roots in the presence and absence of auxin

A significant ($p \leq 0.001$) and dose-dependent increase in this rate was observed in roots exposed to increasing glyphosate concentrations, in the absence of phytohormone. This rate rose to (0.0651µmole/mg prot) for roots treated with C3, approximately six times higher than the control value (0.0090µmole/mg prot). However, the increase in MDA rate observed in the presence of auxin was less significant compared to that noted without auxin. This increase increased from 0.0033 µmole/mg prot in control roots to 0.0073 µmole/mg. prot in control roots to 0.0073 µmole/mg prot in roots subjected to treatment with the maximum dose (Fig. 4).

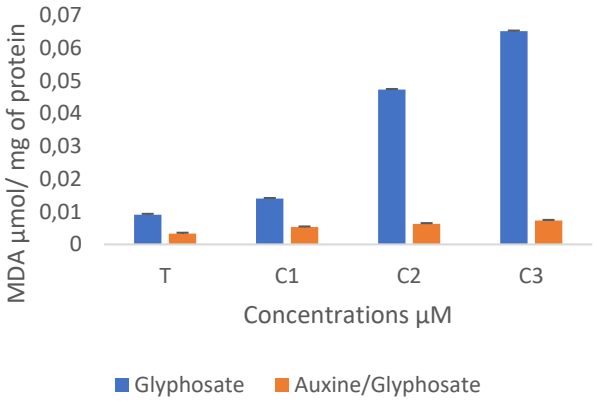

Figure 4. *Variation of MDA levels in soft wheat roots depending on the existence and absence of auxin.*

Monitoring Catalase Activity (CAT) in soft wheat roots in the presence and absence of auxin

Catalase activity reveals a significant stimulation ($p \leq 0.001$) of this in soft wheat roots exposed to various concentrations of glyphosate compared to control roots, and this in the absence of auxin. A peak is noted (0.000073 nmole/min/mg prot) at C3, almost 10 times the control values (0.0000034 nmole/min/mg prot), Similarly, CAT activity measured in glyphosate-treated soft wheat roots exposed to auxin revealed a considerable increase ($P \leq 0.001$) compared to control roots. The maximum value (0.00051 nmol/mn/mg Prot) was noted in roots exposed to C3. However, this increase remains significantly lower than that observed without the presence of auxin (Fig. 5).

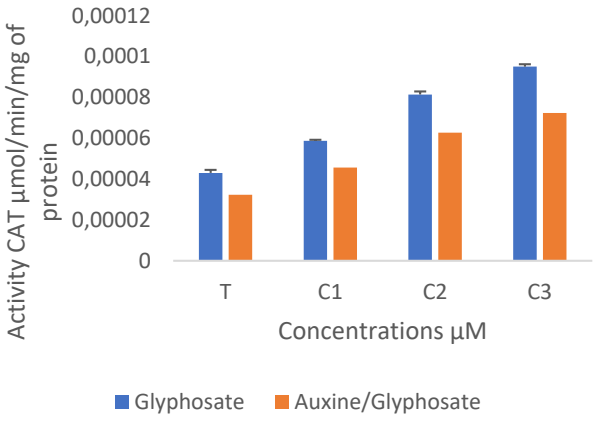

Figure 5. *Measurement of catalase activity in common wheat roots in the presence and absence of auxin.*

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Monitoring superoxide dismutase SOD activity in soft wheat roots in the presence and absence of auxin

The observation of superoxide dismutase activity indicates a marked increase ($p \leq 0.001$) in this activity in soft wheat roots exposed to glyphosate in the absence of auxin compared to the control group. This action is manifested at (0.00035 nmol/min/mg Prot) in untreated roots and reaches its peak (0.00093 nmol/min/mg Prot) in those subjected to the maximum concentration (C3). At the same time, a notable stimulation of SOD activity is noted in roots exposed to glyphosate and auxin. This activity reaches its peak (0.00058 nmol/min/mg Prot) in the case of roots subjected to the highest dose. However, this increase remains less marked compared to that observed without the presence of auxin (Fig. 6).

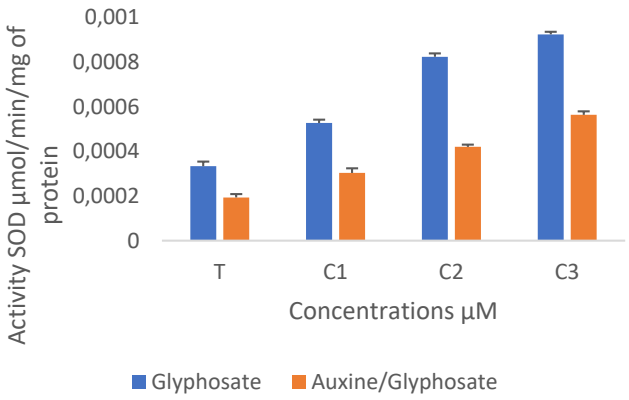

Figure 6. *Measurement of superoxide dismutase activity in common wheat roots in the presence and absence of auxin.*

Discussion

Pesticides, particularly herbicides, are phytotoxic and could induce dysfunction of major biological processes in plants, including stunted growth (Lysenko *et al.,* 2020). The results of this study indicated that *Triticum aestivum* L. root growth was considerably inhibited by the various glyphosate treatments. in this study, we observed a significant reduction in the average root length following exposure to different glyphosate concentrations. This inhibitory effect is likely associated with glyphosate-, leading to impaired amino acid biosynthesis, and reduced cell division and elongation in root tissues (Martinelli *et al.,* 2022; Wang *et al.,* 2022). The accumulation of glyphosate and its residues in the soil can impair root water uptake by disrupting root hydraulic conductivity, inducing oxidative stress, and altering membrane integrity, these physiological disturbances reduce cell expansion and elongation, ultimately limiting root growth (Boursiac *et al.,* 2022). Our results are in agreement with the work of (Meni *et al.,* 2015) who recorded a decrease in average root length in cayenne pepper (*Capsicum frutescens*) treated with different concentrations of sodium chloride.The application of exogenous auxin analogues, can modulate root development by regulating auxin-responsive pathways involved in cell division, elongation, and differentiation. Auxin signaling influences the expression of key genes associated with root, thereby promoting root architecture adjustment and improving root growth under stress conditions (Brumos *et al.,* 2020). Similarly, the study conducted by (Schoenaers *et al.,* 2018) shows that treatment with different concentrations of auxin stimulates root growth, this is due to a stimulation of cell division, since auxin activates genes responsible for cell division in the meristematic zone of the roots, thus promoting the creation of new cells (Balzan *et al.,* 2014), which is perfectly consistent with our observations. The results of this study indicated that the germination of *Triticum aestivum* seeds was significantly inhibited by the different glyphosate treatments. The percentage of germination was significantly reduced in seeds receiving increased concentrations of glyphosate. Similar data has been obtained previously in wheat plants (*Triticum aestivum* L.) exposed to six different metals (lead, nickel, iron, cadmium, aluminum, copper) (Pokorska-Niewiada *et al.,* 2018). The results obtained by Ali and Nas, (2018) reported that the interaction of heavy metals with important enzymes, mainly protease and amylase, can inhibit germination, such as Nickel (Ni), which affects the germination of seed plants by blocking the activities of the enzymes amylase, protease and ribonuclease (Sethy and Ghosh, 2013). Recent studies have shown that heavy metal stress can negatively affect seed germination by inducing osmotic imbalance, reducing water uptake, and disrupting metabolic processes essential for embryo activation. The decline in osmotic potential under metal toxicity conditions limits seed hydration, delays germination, and impairs early seedling establishment through oxidative stress and cellular damage (Kaur *et al.,* 2021; Rizwan *et al.,* 2022). However, we observed an increase in the germination rate in soft wheat plants exposed to various concentrations of glyphosate in the presence of auxin. Auxin, which is essential for breaking seed dormancy and initiating the germination process, stimulates cell expansion and embryonic development within the seed, thus facilitating seedling emergence

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(Heller *et al.,* 2014). Our findings support the research of Lymperopoulos *et al.* (2018) who reported that seed priming with indole acetic acid (1 ppm) stimulated the growth of *Bouteloua gracilis* and wheatgrass (*Agropyron elongatum*) seedlings. Our results are consistent with those of Youssef *et al.* (2025), who recorded an increase in the development of wheat crops treated with licorice extract. Similarly, previous studies have reported improved growth performance in wheat following exogenous auxin application. Foliar spraying with indole-3-acetic acid (IAA) significantly enhanced growth and physiological traits in different wheat genotypes under both normal and stress conditions (Khan *et al.,* 2023). At the same time, the length of the first leaf of soft wheat subjected to different concentrations of glyphosate was calculated. In the treated batches, glyphosate application inhibited the elongation of soft wheat leaves. Leaf elongation in the control plants remained higher than in the treated samples. our findings are in full agreement with those of Diaconu *et al.* (2020), who studied the impact of chromium and cadmium on the growth of *Lepidium sativum*. They stated that exposure to Cr and Cd in soil can affect the uptake and distribution of essential metals in plants, through interactive processes involving different transporters. In addition, Khan *et al.* (2015) stated that the negative impact of high doses of heavy metals on plant growth can be attributed to an alteration in chromosome structure. Also, Outa *et al.* (2020) pointed out that during long-term exposure, the stimulation of leaf elongation is most likely due to an anticipated accumulation of higher concentrations in macrophytes. In contrast, soft wheat crops treated with glyphosate in the presence of auxin show significant leaf growth development, our results are in agreement with Ren *et al.* (2011) who highlighted an increase in the average length of tomato (*Solanum lycopersicum*) leaves this is mainly due to the softening of the cell wall, allowing cells to elongate, which contributes to leaf expansion. Also, the results of Wu *et al.,* (2018) show that the growth development of tomato (*Solanum lycopersicum*) leaves is due to the interaction with ARF (Auxin Response Factors) proteins as auxin acts by binding to receptors (TIR1/AFB), which releases ARFs to activate specific growth-related genes. Abiotic stressors produce reactive oxygen species (ROS), which leads to oxidative stress in the majority of plants. Malondialdehyde (MDA) is a major end product of membrane lipid peroxidation and is widely recognized as a reliable biochemical marker of oxidative damage in plants subjected to abiotic stress. Increased MDA accumulation reflects the severity of membrane injury caused by excessive reactive oxygen species (ROS) under stress conditions (Hasanuzzaman *et al.,* 2020). In addition, some authors (Alayat *et al.,* 2015) report that Malondialdehyde levels increase with cadmium concentration, salinity and degree of cold. Recently, Laib *et al.* (2020) found that MDA levels increased in chickpea (*Cicer arietinum*) under cadmium stress. This work is in agreement with our results, which show an increase in this level as a function of glyphosate concentrations in common wheat. This increase is attributed to the oxidative burst generated by the accumulation of ROS, thus causing lipid peroxidation. On the other hand, Singh *et al* (2020) indicate that the application of different concentrations of copper and mercury caused a significant increase in MDA levels in the duckweed *Spirodela polyrhiza*. On the other hand, we observed an increase in CAT activity after treatment with various concentrations of glyphosate. The increase in CAT activity is explained by cell damage caused by exposure to contaminants (Mesnoua *et al.,* 2016). Our findings are consistent with those of Ferfar *et al.* (2016), who observed an increase in this activity in two types of wheat (Simeto and Cirta) exposed to two sulfonylurea herbicides, on the leaves and roots of wheat (*Triticum aestivum* L). Similarly, our results are in agreement with those of Hussein and Orabi (2025) who recorded an increase in catalase activity in grain sorghum plants sprayed with alpha-tocopherol and thiamine. The results obtained in our study show that glyphosate induced superoxide dismutase (SOD) activity in wheat roots. Our results corroborate those of Rao and Shekhawat (2014) who demonstrated an induction of SOD activity in *Brassica juncea* exposed to ZnO nanoparticles. Other work elucidates the role of antioxidant enzymes and shows that SOD activity is stimulated in *Oryza sativa* plants exposed to nano-ZnO (Sheteiwy *et al.,* 2015). Similar to the research of Junmei *et al.* (2024), an increase in SOD activity in *Sorghum bicolor* roots exposed to copper and zinc was observed. It is possible that the increase in SOD in *Cucumis sativus* tissues is due to the ability of the tested NPs, specifically CuO and ZnO, to penetrate the cell membrane and create aggregates, either among themselves or with other metals already present in the cells (Kim *et al.,* 2012). Phytohormones, such as abscisic acid (ABA) and auxin, regulate plant responses to abiotic stresses by coordinating signaling pathways, growth, and development (Waadt *et al.,* 2022). Their interaction with reactive oxygen species (ROS) contributes to improved stress tolerance by modulating

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oxidative status and activating enzymatic and non-enzymatic antioxidant defense systems (Al-Zahrani *et al.,* 2022). According to the findings of this research, a decrease in stress intensity was observed in plants treated in the presence of auxins. Auxins mitigate the deleterious effect of oxidative stress caused by the applied herbicide, as they function as secondary mediators to stimulate antioxidants and have the ability to capture reactive oxygen species in affected plants (Shoaib *et al.,* 2022). Our findings are consistent with several investigations, showing that application of exogenous abscisic acid at 30 µM to young wheat plants stimulated the activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX), increased the level of malondialdehyde (MDA) and enhanced resistance to pesticide stress (Bousba *et al.,* 2020). In Chinese thuja (*Platycladus orientalis*) seedlings, exogenous auxin (0.5, 1, 10, 100, and 200 µM) significantly reduced oxidative stress by regulating ROS metabolism, enhancing SOD, POD, and CAT activities (by 69%, 91%, and 94%, respectively), as well as MDA (Yao *et al.,* 2020). According to Najafi-Kakavand *et al.* (2019), two groups of *Alyssum inflatum* exposed to nickel (100, 200, and 400 µM) and treated with jasmonic acid (5 and 10 µM) decreased oxidative stress, increasing their resistance to nickel toxicity. According to Ghaffari *et al.* (2020), sugar beet (*Beta vulgaris* L.) plants exposed to water stress and treated with jasmonic acid (10 µM) showed a significant increase in the activities of various antioxidant enzymes such as APX (290%), CAT (80%), and SOD (94%), as well as improved stress tolerance.

Conclusion

Since plants are in constant contact with the soil, they can be exposed to high levels of pollutants, causing an oxidation reaction that initiates a series of reactions harmful to biological macromolecules. The data collected demonstrated that high levels of glyphosate lead to a stimulation of the enzymatic activities examined (Catalase and Superoxide dismutase) related to the antioxidant defense mechanism, associated with an increase in MDA levels. However, the application of auxin leads to a decrease in all the measured parameters compared to those obtained without its intervention. This underlines the role of auxin in plant defense, increasing their ability to cope with environmental stress, including that caused by pesticides. It therefore represents a biological solution to traditional chemical pesticides.

Conflicts of Interes:
The authors declare no conflicts of interest.

List of abbreviations
C: Control
C1: Low concentration
C2: average concentration
C3: high concentration
MDA: Malondialdehyde
SOD: Superoxide dismutase
TBA Thiobarbituric acid
NBT: Nitro Blue Tetrazolium
TCA: Trichloroacetic acid
DCPIP 2,6-dichlorophenolindophenol
CAT: Catalase
APX: Ascorbate Peroxidase
EDTA: Ethylenediaminetetraacetic acid

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