Journal of Plant Growth Regulation (2023) 42:5796–5815
https://doi.org/10.1007/s00344-023-10966-5

Physiological and Molecular Effects of Calcium and Salicylic Acid on *Fusarium graminearum*-Infected Wheat Seedlings

Sherien Sobhy[1] · Essam-Eldeen M. Abo-Kassem[2] · Khalil M. Saad-Allah[2] · Elsayed E. Hafez[1]

Received: 4 August 2022 / Accepted: 24 February 2023 / Published online: 17 March 2023

Abstract

Fusarium graminearum is among the most virulent pathogens attacking wheat plants and significantly lowering its production globally. The present work aims to shed light on the interactions between two priming solutions, calcium (Ca) or salicylic acid (SA), and *F. graminearum* inoculation depending on the growth rate, physiological attributes, and molecular responses in wheat seedlings. In a pot experiment, *F. graminearum*-pre-inoculated or inoculum-free sandy soil was used to germinate wheat kernels after priming them for 12 h in distilled water, 5-mM $CaCl_2$, or 0.05-mM SA. The results demonstrated that *F. graminearum* inoculation decreased growth rate and chlorophyll content, but promoted carotenoids, stress markers (electrolytes leakage, lipid peroxidation, protein oxidation, hydrogen peroxide, and hydroxyl radical), antioxidant molecules (AsA, phenols, and flavonoids), osmolytes (GB, amino acids, and proline), and the antioxidant enzymes (CAT, GPX, SOD, PPO, and PAL). Additionally, the fungal infestation boosted the expression of CAT, GR, PR4, MT, and PCS genes. However, presoaking wheat kernels in Ca or SA solutions has contributed to mitigating the negative effects of fungal inoculation by restoring growth rate, chlorophyll content, and antioxidant capacity. It has also decreased the induced oxidative stress and downregulated the gene expression of *F. graminearum*-inoculated wheat seedlings. Consequently, by minimizing the negative repercussions of *F. graminearum* infestation, priming with Ca or SA could be used to appropriately stimulate growth and readjust the oxidative status of wheat seedlings.

Keywords Wheat seedlings · Calcium · Salicylic acid · *F. graminearum* · Grain priming · Molecular response

Introduction

Plants are subjected to biotic stress, which has an impact on and poses a threat to the agricultural sector. Fungal infections are a persistent global danger to crop production and food security. *Fusarium* species are one of the most common fungal infections in small grain cereals, causing extensive damage (Pereyra et al. 2004; Chouhan et al. 2022). *Fusarium* head blight (FHB), seedling blight, and *Fusarium* crown and foot brown rots (FCFR) are the most important diseases in wheat caused by *Fusarium graminearum* (AL Masri et al. 2017; Zheng et al. 2022). Climate change, reduced tillage, crop rotation practices, and/or the habit of throwing the straw in the field could therefore be contributing to the rise in FHB cases (Chen et al. 2019). *F. graminearum* was ranked as one of the top 10 fungal plant pathogens based on FHB severity and mycotoxicity (Dean et al. 2012). After infection and colonization of wheat heads, *F. graminearum* impairs overall wheat yield by interfering with kernel development and contaminating the surviving kernels with a mixture of mycotoxins, rendering them inappropriate for human and feed utilization (Mentges et al. 2020). The most prevalent *Fusarium* mycotoxins in cereal grains are deoxynivalenol, nivalenol aurofusarin, and fusarin C, with deoxynivalenol being the most important virulence factor for *F. graminearum* contamination (Chen et al. 2019).

By manipulating cytosolic calcium concentrations, as a key player in plant development and response, plants have developed efficient mechanisms for perceiving,

Handling Editor: Paloma Sanchez.

✉ Khalil M. Saad-Allah
khalil.saadallah@science.tanta.edu.eg

[1] Plant Protection and Bimolecular Diagnosis Department, Arid Lands Cultivation Research Institute (ALCRI), City of Scientific Research and Technological Applications (SRTA-City), New Borg El-Arab 21934, Egypt

[2] Botany Department, Faculty of Science, Tanta University, Tanta 31527, Egypt

translocating, and responding to a diverse array of biotic and abiotic stimuli (Reddy et al. 2011). Calcium (Ca^{2+}) is a versatile endogenous secondary messenger that triggers a variety of responses participating in growth, defense, and adaptation to biotic and abiotic stimuli in a diverse set of signaling pathways (Johnson et al. 2014; Kour et al. 2023). Moreover, Ca^{2+} is a tightly regulated ion within cellular compartments, and the spatial and temporal control of its concentration makes it a versatile signaling component in plants (Thor 2019). Calcium ions have also been shown to have a role in the crosslinking of pectic polysaccharides in the cell wall and middle lamella of plant cells, strengthening them and promoting tissue opposition to fungal enzyme activity (Hernández-Muñoz et al. 2006).

The involvement of phytohormones in reducing the detrimental consequences of biological and environmental stressors has been well documented. Salicylic acid (SA), one of the phytohormones, operates as a signaling and regulatory molecule in plant response to various stimuli by modulating metabolic and molecular processes (Khan et al. 2015; Liu et al. 2016). Consequently, seed germination, stomatal closure, ion uptake and transport, membrane permeability, photosynthesis, and plant development have all been demonstrated to be influenced by the exogenous application of SA (Ashraf et al. 2010; Aftab et al. 2011; Wang et al. 2013; Emamverdian et al. 2020). According to numerous studies, SA is a prominent phenyl propanoid compound that modulates plant tolerance to pathogenesis and other stress conditions. In plants, the systemic acquired resistance (SAR) phenomenon occurs when a pathogen encounters a plant organ, causing a local hypersensitivity reaction followed by signal transmission to surrounding plant organs, preventing additional infections (Heil and Bostock 2002; Bhar et al. 2018; Noman et al. 2020). These systemic responses are mostly based on hormonal cross-talk and small molecules interplay, which in turn induce a broad-spectrum resistance phenomenon (Shah et al. 2014). Salicylic acid-mediated signaling triggers the SAR (Grant and Lamb 2006; Klessig et al. 2018). Salicylic acid responses are correlated to the expression of pathogenesis-related proteins (PRPs), one of which is PRP1, a salicylic acid marker that is upregulated during SA response in many plants (Lemos et al. 2016).

Salicylic acid is an important plant defense hormone that helps plants defend themselves against a variety of diseases with biotrophic and hemibiotrophic lifestyles (De Vos et al. 2005). Elevated SA concentration at the site of pathogen attack can trigger H_2O_2 formation, resulting in a hypersensitive reaction and plant cell wall lignification at the pathogen infection site, which could promote disease resistance (Sorahinobar et al. 2016). Additionally, those authors found that SA treatment improved wheat resistance to *F. graminearum* infection via modulating the antioxidative pathway, thereby increasing H_2O_2 concentration and promoting pathogen-related genes. Furthermore, the significant correlation between H_2O_2 and SA levels affirms the interplay between these two signals in increasing plant defense, because they are intimately connected to the activity levels of PRPs (Herrera-Vásquez et al. 2015).

Wheat (*Triticum aestivum* L.) was categorized as the most fundamental agricultural commodity in 2014, with 730 million tons produced from over 220 million hectares (Arzani and Ashraf 2017). In many regions, it is utilized as the main staple food, such as bread, and it has been stated to be the most abundant source of dietary protein in human nutrition, accounting for the fifth of total dietary protein worldwide (Braun et al. 2010). Wheat grain is rich in phytochemicals, vitamins, antioxidants, and macro- and micronutrients, in addition to the major components of protein, carbohydrate, and lipids (Arzani 2019).

Previous studies have described how osmolyte molecules like glycinebetaine, proline, and free amino acids help the plant to cope with the invading microorganism through a variety of mechanisms, including adjusting cellular osmolality, ROS dissolution, maintaining membrane fluidity, and stabilizing cellular structures (Hayat et al. 2012; Sobhy et al. 2021). According to a comprehensive exploration of the literature, it is uncertain exactly how SA function to decrease *F. graminearum* pathogenicity to wheat and that there are no studies we are aware of that have determined the role of Ca in this pathogen's resistance. Hence, the key target of this study was to explore if priming with Ca or SA can indeed relieve the deleterious effects of *F. graminearum* inoculation on wheat seedlings and yields, with an emphasis on the growth rate, metabolic activity, antioxidant status, and molecular responsiveness at the seedling stage.

Materials and Methods

Wheat Kernels and Fungal Isolate

Wheat kernels (*Triticum aestivum* L., cv. Masr 1) were received from the Agricultural Research Center in Gemiza, Egypt and were chosen for conspicuous size and morphology homogeneity. The Mycological Center at Assiut University provided *Fusarium graminearum* Schwabe (isolate No. Fg8) for this investigation.

Inoculation of the Soil with *F. graminearum*

According to Sobhy et al. (2021), a sand-corn meal (SCM) medium was used to inoculate the soil with *F. graminearum* spores. Five mycelial discs were inserted in the SCM medium and incubated at 28 °C for 3 weeks after growing the fungal mycelia for 7 days on potato dextrose agar (PDA) medium. Before seeding wheat kernels, 5 g of SCM medium

were sprinkled in the prewashed sandy soil and left for two days.

Treatments and Setup for the Experiment

At the sowing time (November 2018), kernels of *Triticum aestivum* (cv. Masr 1) were externally disinfected for 5 min with 5% Clorox®, washed many times with tap water, and then rinsed in deionized water. According to the results of a preliminary study, the disinfected kernels were split into three groups: the 1st submerged in distilled water, the 2nd primed with 5-mM $CaCl_2$ solution, and the 3rd primed with 0.05-mM salicylic acid solution. After 12 h, the soaking solutions were removed and the kernels were washed with deionized water. Each kernel group was split into two subgroups, one of which was seeded in *F. graminearum* pre-inoculated sandy soil and the other in prewashed inoculum-free sandy soil. In a completely randomized design, the soil was enclosed in plastic pots (35 cm diameter × 27 cm depth) holding 8-kg soil and six treatments. In a completely randomized design with six treatments in total, the soil was confined in 35 × 27 cm plastic pots with 8-kg sand/pot. The pots were allowed to grow in controlled conditions (12:12 day/night, 25/15 °C, 2 and 70% relative humidity) and supplied with 70% of their field capacity distilled water whenever needed. Pots were trimmed into 10 uniform seedlings/pot upon seedling establishment, with each treatment replicated five times. Seedlings were collected after 21 days to be evaluated morphologically, biochemically, and molecularly. To emphasize reliability, the experiment was repeated, and the results were depicted as the average of two different experiments.

Plant Measurements

Growth Criteria

Wheat seedlings were harvested at 21 days old, separated into shoots and roots, and washed with tap water before being rinsed in deionized water. The length and water content of shoots and roots, as well as the leaf area, were determined using the harvested samples.

Estimation of Leaf Pigments

The photosynthetic pigments in the leaves of wheat seedlings were extracted in 80% cold acetone and assessed utilizing the technique of Metzner et al. (1965), with some modifications as described by Faryal et al. (2022).

Oxidative Stress Indices Estimation

Electrolyte leakage from the leaves of wheat seedlings was monitored according to the approach of Sairam et al. (1997), using a conductivity meters as adapted by Li et al. (2015). The level of malondialdehyde (MDA), as a product of peroxidative damage of unsaturated fatty acids in cell membranes, was evaluated in the leaves of wheat seedlings by following the procedures of Heath and Packer (1968), with slight modification as followed by (Golizadeh and Kumleh 2019). Protein oxidative damage in wheat leaves, as represented by the level of carbonyl content, was determined by the aid of 2,4-dinitrophenylhydrazine (Reznick and Packer 1994) following the modifications established by Dias et al. (2019).

Utilizing the method outlined by Velikova et al. (2000), the level of H_2O_2 in wheat seedlings was measured spectrophotometrically. The concentration of hydroxyl radicals ($OH^{\bullet}$) in the treated seedlings was determined using the method outlined by Halliwell et al. (1995), using 2-deoxyribose and thiobarbituric acid.

Antioxidant Molecules Determination

Based on a standard graph by AsA, the approach suggested by Oser (1979) and modified by Ragab and Saad-Allah (2020) was utilized to measure ascorbic acid (AsA) levels in wheat leaves. The content of phenolic compounds in wheat leaves was determined using Folin–Ciocalteu's reagent and a standard curve plotted using gallic acid according to the method of Jindal and Singh (1975) in accordance with the modifications proposed by (Ardestani and Yazdanparast 2007). $AlCl_3$, potassium acetate, and a standard flavonoid (quercetin) were used to determine the flavonoid concentration of wheat leaves as prescribed by Chang et al. (2002).

Osmoprotectant Molecules

The quaternary ammonium osmolyte glycine betaine (GB) was quantified in the aqueous extract of wheat leaves dry powder by the method outlined by Grieve and Grattan (1983) and modified by Escalante-Magaña et al. (2019) using KI-I_2 reagent and a standard graph developed by GB. The total free proline (TFPs) content of wheat leaves was determined using the acid ninhydrin reagent and a calibration curve created by proline (Bates et al. 1973). Using glycine as a standard and ninhydrin-citrate buffer-glycerol reagent, the total amino acids (TAAs) in the extract of wheat leaves were measured spectrophotometrically as proposed by Lee and Takahashi (1966).

Enzymatic Activities

Fresh wheat leaves were homogenized in liquid nitrogen and then extracted in phosphate buffer (50 mM, pH 7.0). Catalase (CAT) activity was measured by monitoring the initial rate of H_2O_2 breakdown at 240 nm and calculating the activity using an extinction coefficient (40 mM^{-1} cm^{-1}) (Kato and Shimizu 1987). The activity of guiacol peroxidase (GPX) was determined by measuring the brown-colored product tetraguaiacol produced by the reaction of guaiacol with H_2O_2 at 470 nm, and the activity was assessed using an extinction coefficient (26.6 mM^{-1} cm^{-1}) (Kato and Shimizu 1987). The increase in light absorption at 560 nm owing to the photochemical reduction of nitro blue tetrazolium (NBT) into formazan and the utilization of an extinction coefficient (21.1 mM^{-1} cm^{-1}) were used to evaluate superoxide dismutase (SOD) activity in wheat leaf extracts (Beyer and Fridovich 1987). The intensity of the generated color due to the oxidation of pyrogallol into purpurogallin was monitored at 420 nm, and polyphenol oxidase (PPO) activity was calculated using an extinction coefficient (26.4 mM^{-1} cm^{-1}) (Kumar and Khan 1982). In the presence of phenylalanine as a substrate, the absorbance of the resultant trans-cinnamic acid at 290 nm was used to detect phenylalanine ammonia-lyase (PAL) activity. PAL activity was calculated using the molar extinction coefficient of trans-cinnamic acid (10.24 mM^{-1} cm^{-1}) according to Wang et al. (2006). The activity of all measured enzymes was expressed as $\mu M.g^{-1}$ FM min.

Gene Expression Patterns Using qRT-PCR Analysis

The manufacturer's guidelines were followed to extract total RNA from wheat seedlings using the RNeasy Mini Kit (Qiagen). By reverse transcribing RNA in a total volume of 20 µL using a thermocycler (MJ Research, Inc., PTC-100™ Programmable thermal controller, USA), complementary DNA (cDNA) was generated using the reverse transcriptase (RT) enzyme. The reaction course involved an enzyme activation cycle for 60 min at 40 °C followed by a second enzyme deactivation cycle for 5 min at 95 °C.

The qRT-PCR was carried out in triplicate with a Rotor-Gene 6000 (QIAGEN, ABI System, USA) using SYBR Green PCR Master Mix (Fermentas, USA). A 25-µL mixture comprising primer pairs of the investigated genes: catalase (CAT), glutathione reductase (GR), pathogenesis-related 2 (PR2), pathogenesis-related 4 (PR4), metallothionein-like protein 1 (MT), or phytochelatin synthase (PCS), were utilized (Table 1) in each reaction, and data were retrieved during the extension stage. An initial dissociation phase at 95 °C for 10 min was followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s in the amplification process. To exclude the presence of non-specific amplicons, melting curves were acquired and as a reference, the β-actin gene was employed (Mo et al. 2012). The relative expression of the investigated genes was quantified and calculated using the $2^{-\Delta\Delta CT}$ approach developed by Livak and Schmittgen (2001).

Statistical Analysis

The findings of the current study were submitted to a two-way analysis of variance (ANOVA) to ascertain the relevance of both fungal inoculation and stimulant solutions (Ca and SA), along with their combined interactions on the measured variables (Supplementary materials Table S1). In addition, one-way ANOVA was used to separate means and determine the level of significance between the experimental treatments. All data analysis was carried out using Minitab 19.11 software under the

Table 1 The sequence of primers used in the current study

Primer name	Gene ID	Direction	Sequences 5–3
PR4 (Pathogenesis-related 4)	819632	Forward	GACCTGAATGCGGTCGTCAAGG
		Reverse	AGCATGTTTCTGGAATCAGGCTG
PR2 (Pathogenesis-related 2)	824893	Forward	TCACCAAACTATTGGATTTCAA
		Reverse	GACTCAATTTTTGACTTCTTAATCC
MT (Metallothionein-like protein 1)	543163	Forward	ACACCAAGGGCAGAGCATAG
		Reverse	CACTCGTGTGATGGTGTGAG
PCS (Phytochelatin synthase)	839354	Forward	CAGACCACCATCCACGACTT
		Reverse	ACAGCCTGTTCATTCCCTTT
GR (Glutathione reductase)	824631	Forward	CCTGATGCGGTATTTTCTCCTTA
		Reverse	GCACCATATGCGGTGTGAA
CAT1 (Catalase)	107799583	Forward	CCATCTGGCTCTCCTACTGG
		Reverse	AGAACTTGGACGACGGCCCTGA
ß-actin (Beta-actin)	10096	Forward	GTGGGCCGCTCTAGGCACCAA
		Reverse	CTCTTTGATGTCACGCACGATTTC

general linear model (GLM) using LSD as a post hoc test at 5% level. The data are a representation of results from two distinct trials, with each carried out in triplicate (i.e., $n = 6$). All data were reported as the average ± the standard deviation (SD).

Results

Plant Growth Parameters

Inoculation with *F. graminearum* or priming with Ca or SA, as well as their combined interactions, significantly ($P < 0.05$) affected the growth characteristics of wheat seedlings, as measured by shoot height, shoot water content,

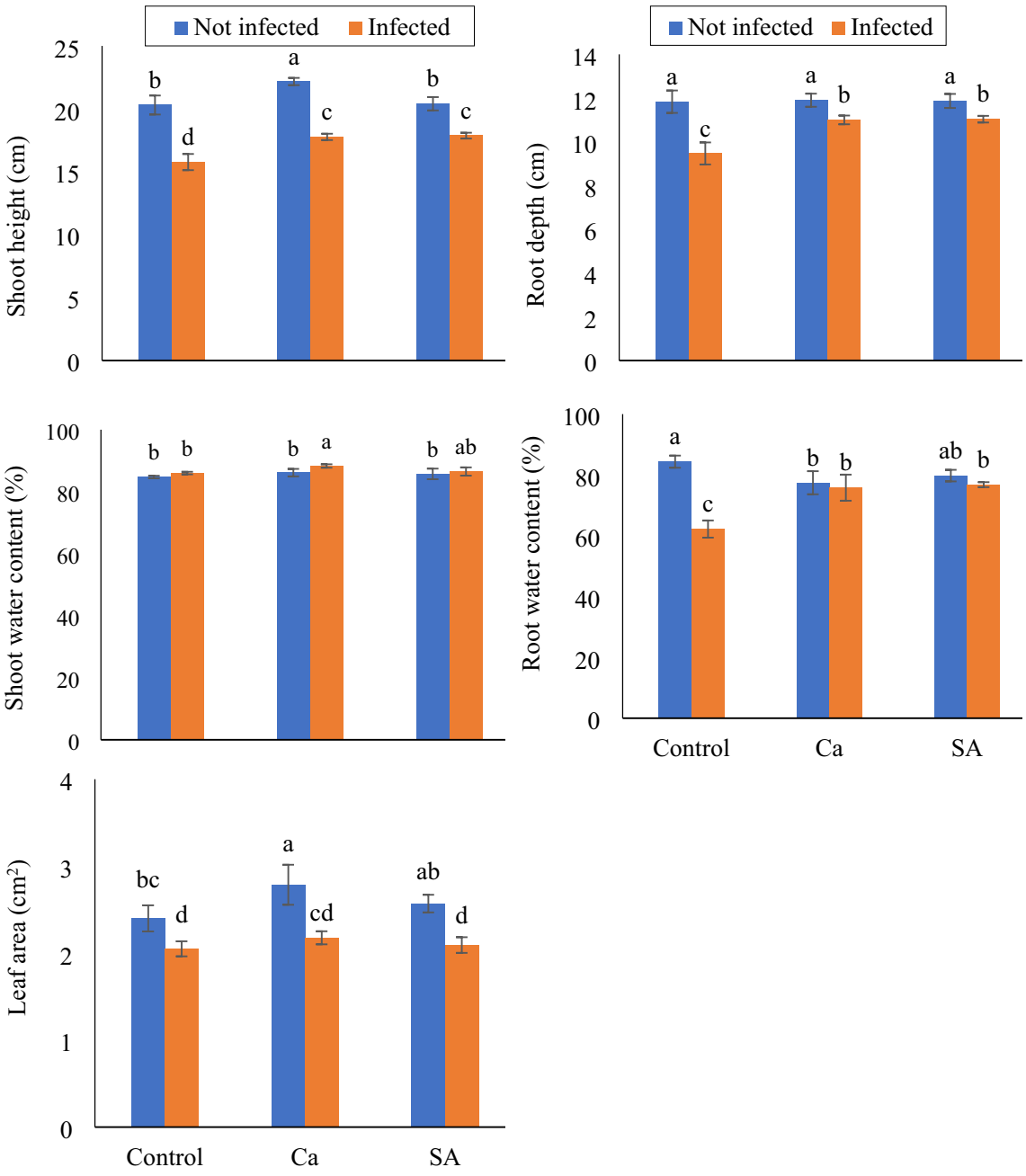

Fig. 1 Representative images of the impact of calcium or salicylic acid grain priming on the growth characteristics of wheat seedlings inoculated with *F. graminearum*

leaf area, root depth, and root water content (Fig. 1 and Fig. S1). When compared to the healthy-not infected control, *F. graminearum* inoculation evidently reduced shoot height, leaf area, root depth, and root relative water content, but had no obvious effect on the shoot water content of wheat seedlings. In comparison to the control treatment, seed priming with $CaCl_2$ increased shoot height and leaf area, did not affect shoot water content or root depth, and slightly decreased root water content of wheat seedlings. On the other hand, SA-treated wheat seedlings showed no change in shoot height, shoot water content or root depth, a mild drop in root water content, and a minor improvement in leaf area, in comparison to control seedlings. Even though the combination of fungal infection and priming treatments (Ca or SA) boosted shoot and root growth, the leaf area of SA-treated and *F. graminearum*-infected wheat seedlings showed more stress signals than infected seedlings. Overall, the effect of *F. graminearum* on wheat seedling growth was reduced in plants primed with Ca or SA solutions than in unprimed-infected control plants.

Photosynthetic Pigments Response

The statistical analysis of photosynthetic pigments response to *F. graminearum* inoculation, priming with Ca or SA, and their combined interactions revealed that individual treatments slightly affected Chl a and Chl b levels, but not carotenoids content in wheat seedling leaves. The combined treatments, on the other hand, did not affect Chl a, but had a significant effect on both Chl b and carotenoids (Fig. 2). Fungal inoculation decreased Chl a and b contents in a similar way, whereas Ca and Sa treatments separately boosted Chl a and slightly decreased Chl b, compared to the healthy-untreated control. When compared to plants individually inoculated with *F. graminearum*, combined treatments of Ca or SA with fungal inoculation resulted in a significant improvement in Chl a and b contents. In the case of carotenoids, fungal invasion resulted in a significant increase when compared to the control; however, priming with Ca or SA did not influence carotenoids content, whether used alone or in combination with fungal treatment.

Changes in Stress Markers

The stress markers in wheat seedlings, as measured by electrolytes leakage (EL), lipid peroxidation (as measured by MDA), protein oxidation (as measured by C=O), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\cdot}$) contents, were significantly affected by the single experimental treatments (fungal inoculation or priming either with Ca or SA). The combined treatments, on the other hand, showed a significant effect on El, MDA, and C=O levels, but nonsignificantly affected H_2O_2 or $OH^{\cdot}$ levels

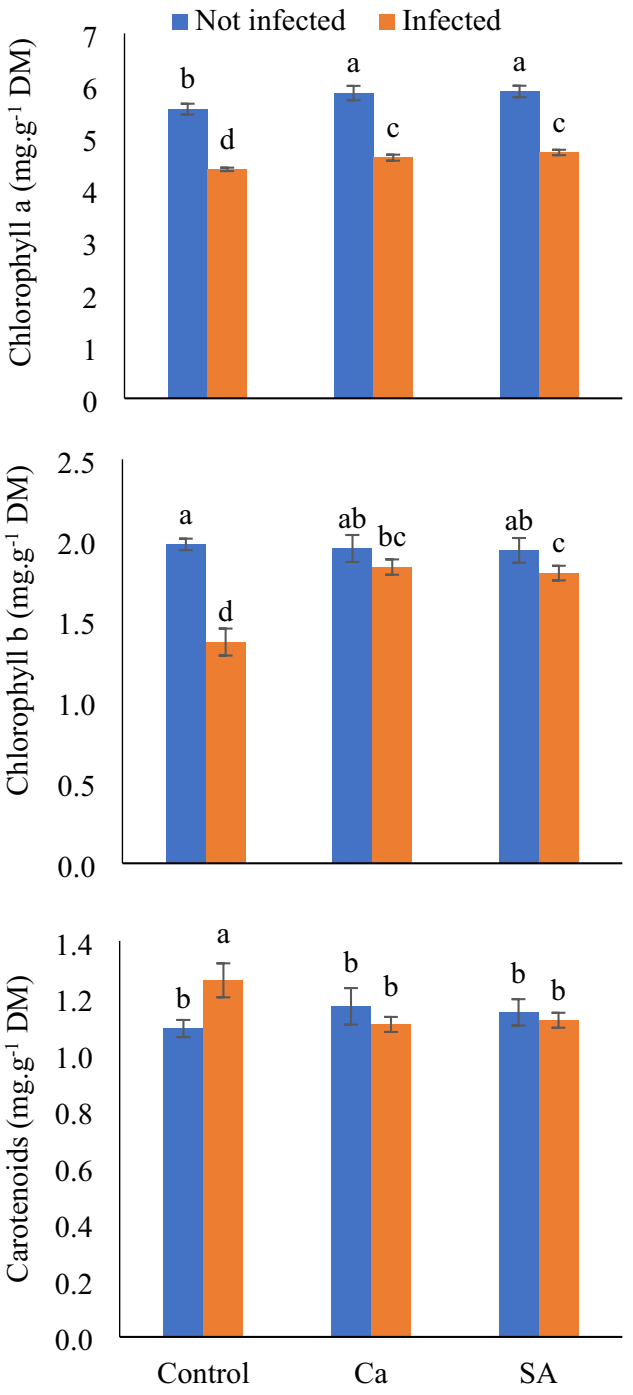

Fig. 2 Effect of calcium or salicylic acid grain priming on the photosynthetic pigments of wheat seedlings inoculated with *F. graminearum*. Different letters indicate statistically significant differences ($P \leq 0.05$)

(Fig. 3). When EL, MDA, C=O, H_2O_2, and $OH^{\cdot}$ contents were compared to the healthy-unstressed control values, *F. graminearum* inoculation resulted in 88.45, 184.63, 88.44, 33.11, and 51.83% increases, respectively. However, there was no discernible difference in the levels of

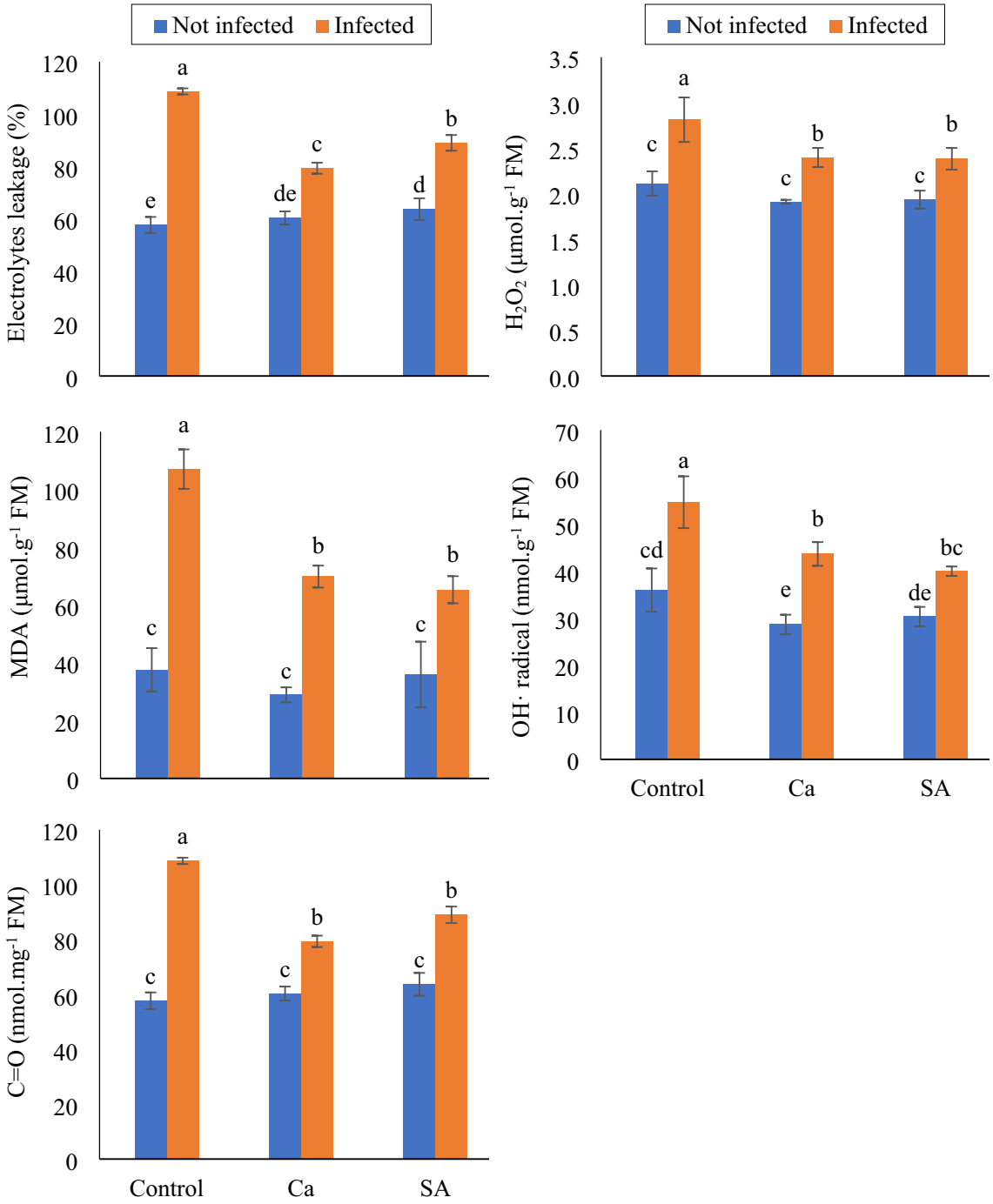

Fig. 3 Effect of calcium or salicylic acid grain priming on the stress markers of wheat seedlings inoculated with *F. graminearum*. Different letters indicate statistically significant differences ($P \leq 0.05$)

stress markers after the single administration of Ca or SA, except for $OH^{\cdot}$ content, which was somewhat reduced by both treatments. Nonetheless, when the priming solutions were combined with *F. graminearum*-inoculated treatment, the level of these stress markers declined in comparison to the stressed treatment, although they were still greater than the typical control values.

Alternation in Non-enzymatic Antioxidants

The non-enzymatic antioxidants, ascorbic acid (AsA), phenolic compounds, and flavonoids in the leaves of wheat seedlings, were substantially affected by the treatments used in this study (Fig. 4). The results of the two-way ANOVA analysis revealed that the contents of non-enzymatic antioxidants

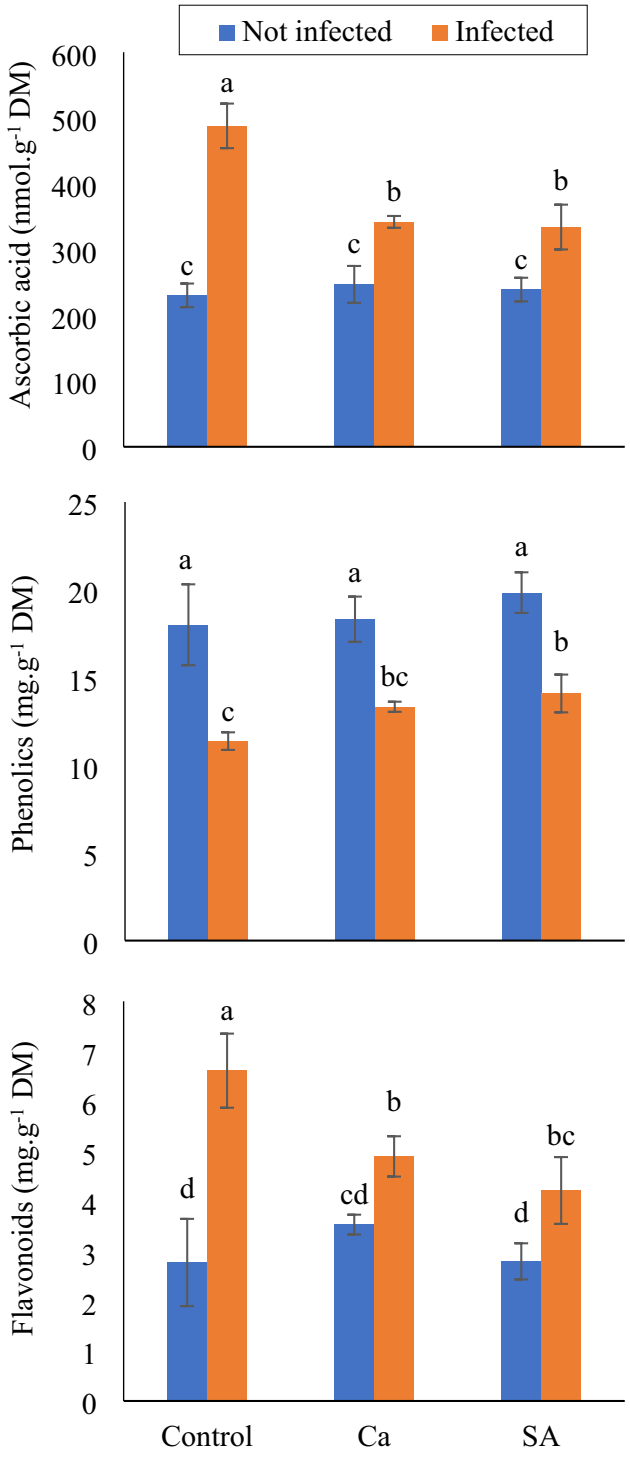

Fig. 4 Effect of calcium or salicylic acid grain priming on the non-enzymatic antioxidants of wheat seedlings inoculated with *F. graminearum*. Different letters indicate statistically significant differences ($P \leq 0.05$)

were significantly affected by fungal and priming treatments, either separately or together, except for the interaction of *F. graminearum* with priming solutions treatment, which nonsignificantly affected phenolic content (Table S1). When compared to control levels, AsA and flavonoids were significantly increased in response to *F. graminearum* infection, whereas phenolic compounds were decreased. Single priming treatments with Ca or SA, on the other hand, showed no change in the concentration of these compounds, except for a minor increment in flavonoids due to $CaCl_2$ priming. In comparison to the infected treatment, combined treatments resulted in decreased accumulation of AsA and flavonoids and increased accumulation of phenolic content in wheat seedlings.

Osmotic Homeostasis Molecules

Glycinebetaine (GB), free proline (FP), and free amino acids (FAAs) as osmotica were significantly affected ($P < 0.05$) in wheat seedlings by the experimental treatments as indicated by the statistical analysis (Fig. 5). Fungal inoculation treatment resulted in 87.10, 87.07, and 227.70% increases in GB, FP, and FAAs, respectively, when compared to the healthy control. Priming solutions sole treatments, on the other hand, exhibited no change in the level of these osmolytes in wheat seedlings. The interaction of priming and inoculation treatments contributed to a significant reduction in the amount of these molecules as contrasted to the unprimed-stressed control, while their level was still comparatively higher than the normal control treatment.

Variations in Antioxidant Enzymes

The effects of *F. graminearum* inoculation, seed priming with Ca or SA, and the combination of these treatments on catalase (CAT), guaiacol peroxidase (GPX), superoxide dismutase (SOD), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) activities in wheat seedlings are depicted in Fig. 6. Statistically, all the investigated enzymes were significantly ($P < 0.5$) affected by the experimental treatments. As apparent from the results, *F. graminearum* inoculation resulted in a significant increment in CAT, GPX, SOD, PPO, as well as PAL activities with percentages of 114.3, 93.9, 250.0, 253.0, and 172.7%, respectively, compared to the non-infected control. The individual priming treatments caused no any discernible change in the activity of the investigated enzymes, except for a slight increment in GPX activity attained following priming treatments, as compared to the control activities. Despite this, when seed priming and fungal inoculation treatments were combined, all of the aforementioned enzymes exhibited a significant decrease in activity compared to the infected treatment.

qRT-PCR Expression of the Investigated Genes

The growth, biochemical responsiveness, and yield of wheat infested with *F. graminearum* are largely dependent on the

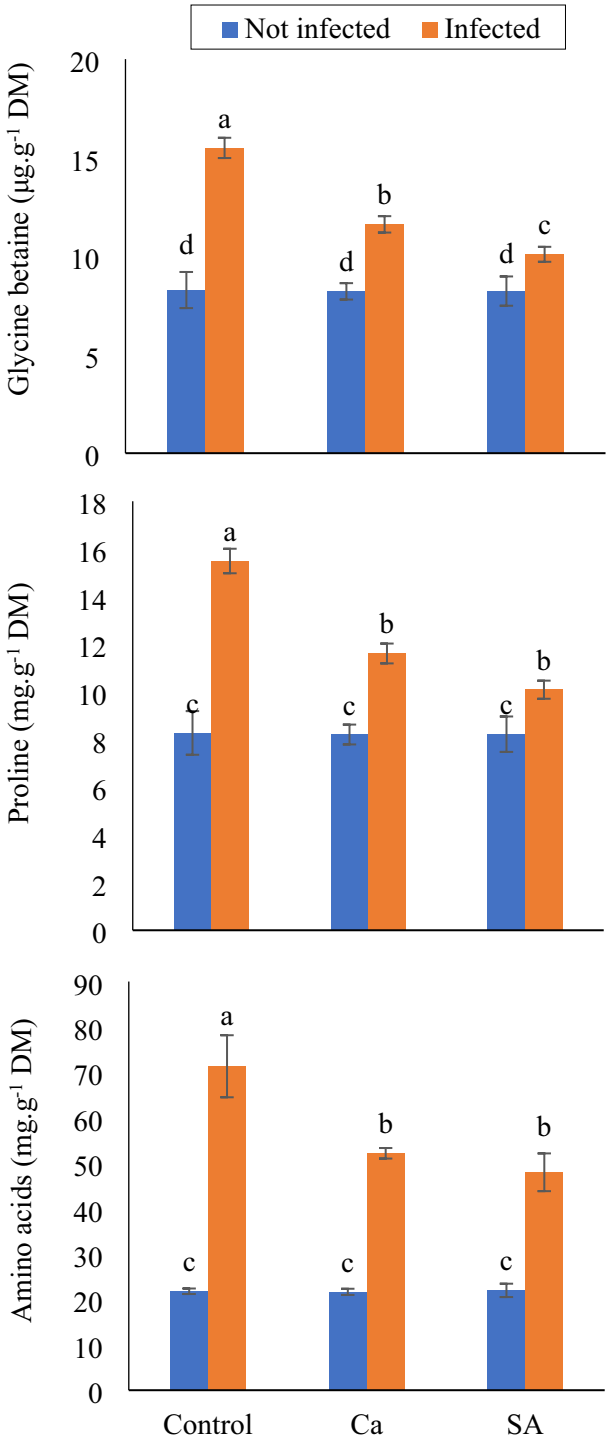

Fig. 5 Effect of calcium or salicylic acid grain priming on the osmoregulatory molecules of wheat seedlings inoculated with *F. graminearum*. Different letters indicate statistically significant differences ($P \leq 0.05$)

expression of specific genes that enable it to withstand such stressful conditions. In this study, we looked at how seed priming with Ca or SA affected the expression of antioxidant enzymes like catalase (CAT) and glutathione reductase (GR), pathogenesis-related proteins like PR2 and PR4, and metal-chelating proteins like metallothionein (MT), and phytochelatin synthase (PCS) in response to *F. graminearum* invasion in wheat seedlings (Fig. 7). Regarding *F. graminearum* inoculation, the data revealed highly significant increases in CAT, GR, PR4, MT, and PCS genes expression (379.1, 209.9, 2.6, 1.5 and 54.5 folds, respectively), and a non-significant decline (2%) in PR2 gene expression compared to the un-inoculated control. In the case of single priming treatments, Ca presoaking resulted in substantial increases in CAT (97.1 folds), GR (880.6 folds), PR4 (1.2 folds), and PCS (49.5 folds); however, it markedly decreased MT (98.0%) expression, with no effect on PR2 gene expression. Indeed, SA pretreatment showed increases of 126.3 folds in CAT, 11.6 folds in GR, 1.2 folds in MT, and 27.0 folds in PCS expression, but marginally (2.0%) lowered PR2 expression, with a complete lack of PR4 gene expression, compared to the unprimed-healthy control.

Regarding combined treatments of *F. graminearum* inoculation with Ca or SA, the expression of some genes was upregulated and others were downregulated. In this regard, the combined interaction of *F. graminearum* inoculation with Ca and SA reduced the expression of CAT by 74.39 and 98.61%, GR by 50.99 and 71.13%, and MT by 25.85 and 3.68%, respectively. Ca and SA when combined with *F. graminearum* inoculation, on the other hand, both boosted PCS expression by 10.61 and 16.22%, respectively. However, both priming solutions had differing effects on PR gene expression: Ca priming increased PR2 expression by 10.21%, while SA priming reduced it by 8.27%. Concerning the PR4 gene, Ca priming resulted in a 74.23% decrease in its expression, whereas SA entirely turned it off. Interestingly, the experimental treatments significantly ($P < 0.001$) affected the expression of the CAT, GR, and PR4 genes, whereas PR2 was nonsignificantly ($P > 0.05$) affected. Meanwhile, the single treatments possessed a significant effect on the expression of MT and PCS genes, but the combination treatments were non-significant (Table 2).

Discussion

As a pandemic cause of head blight and root rot diseases, *Fusarium graminearum* has a devastating effect on wheat growth and yield. The findings of our investigation demonstrated a notable reduction in wheat growth characteristics as affected by *F. graminearum* inoculation. These results were previously reported by several studies (Aucique-pérez et al. 2017; Baghbani et al. 2019; Sobhy et al. 2021). They ascribed the slowing of wheat growth caused by *F. graminearum* inoculation to biomolecules breakdown, disruptions in stomatal conductance, chlorophyll deterioration,

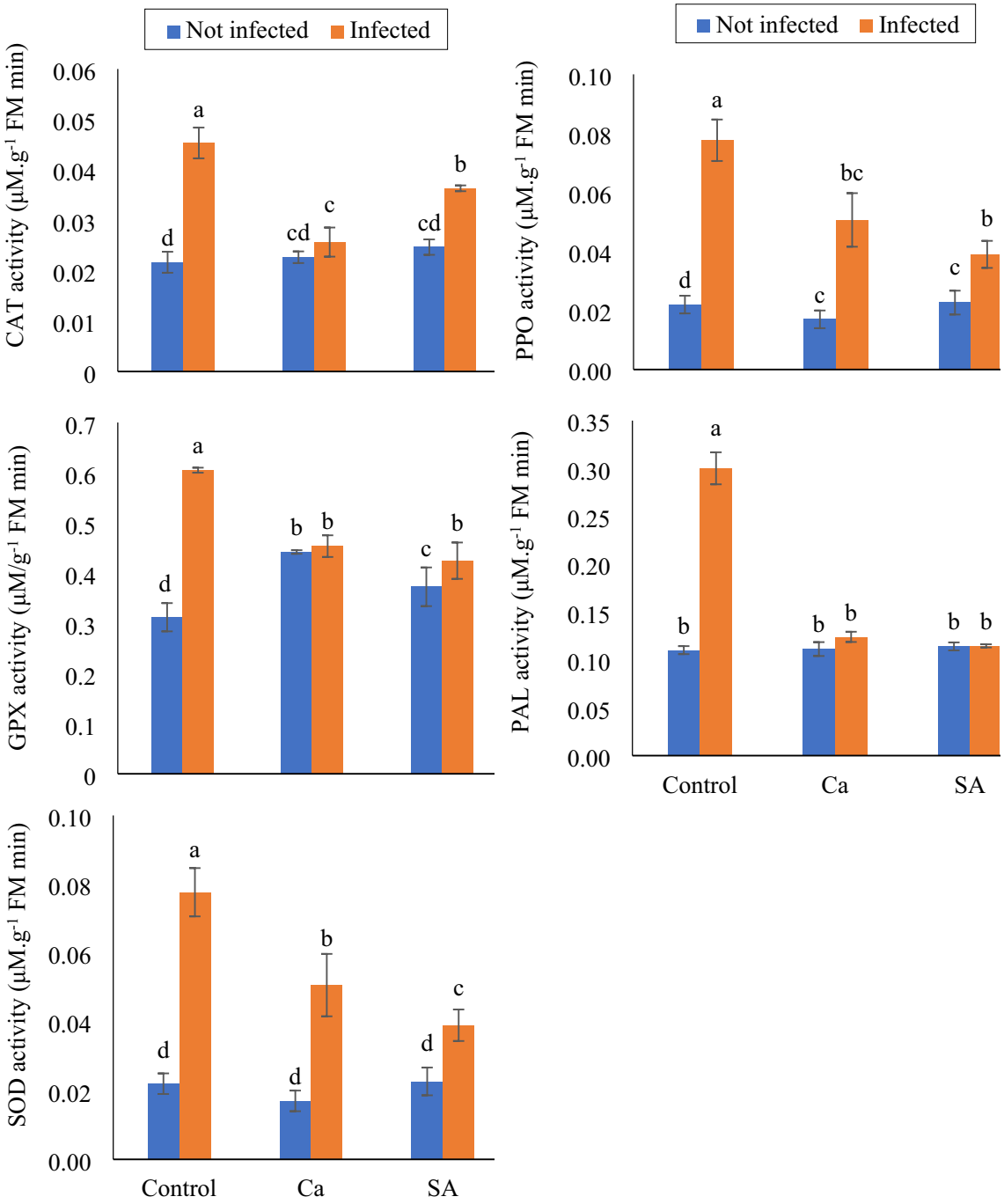

Fig. 6 Effect of calcium or salicylic acid grain priming on the antioxidant enzymes activity of wheat seedlings inoculated with *F. graminearum*. Different letters indicate statistically significant differences ($P \leq 0.05$)

and impairment of hydraulic homeostasis. Additionally, the study findings revealed that seed priming with either 0.05-mM salicylic acid (SA) or 5-mM $CaCl_2$ marginally boosted the growth rate of *F. graminearum*-infected wheat seedlings. It has been demonstrated that SA causes plants to develop systemic resistance to biotic and abiotic stressors (Shakirova et al. 2012). A substantial state of knowledge has been amassed up to this point and has suggested that SA is involved in the control of defense responses in several plants, including wheat, to phytopathogens and abiotic stressors (Shakirova et al. 2012; Verma et al. 2016). In their investigation, Qi et al. (2012) demonstrated that SA markedly retarded the mycelial growth of *F. graminearum* and inhibited the germination of its conidia. In agreement

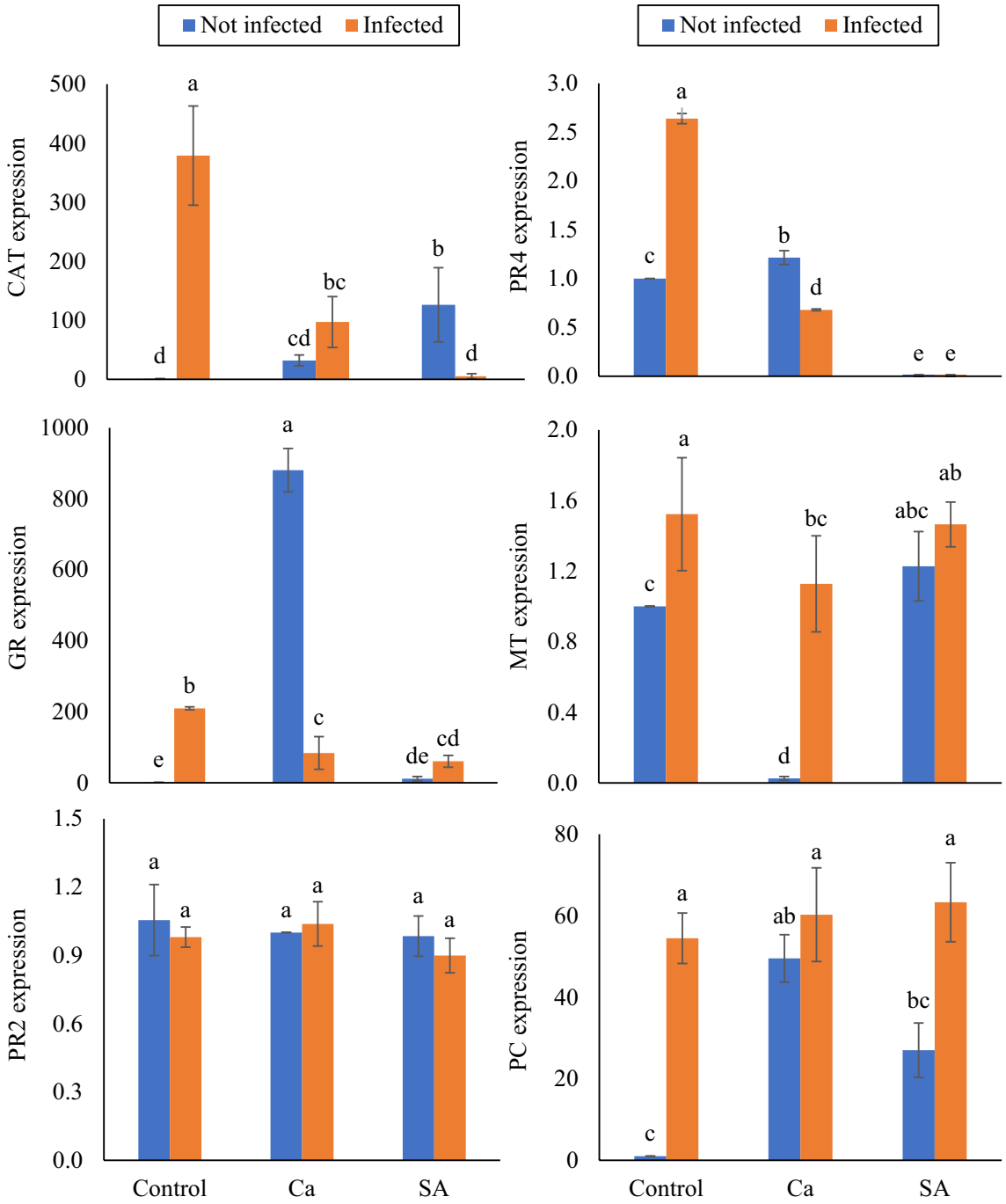

Fig. 7 Effect of calcium or salicylic acid grain priming on the expression of CAT, GR, PR2, PR4, MT, and PC genes of wheat seedlings inoculated with *F. graminearum*. Different letters indicate statistically significant differences ($P \leq 0.05$)

with our findings, Sorahinobar et al. (2021) concluded that wheat priming with SA promoted its defense responses for an effective and expeditious response to *F. graminearum* through modifying the antioxidative pathway for increasing H_2O_2 content, which contradicts our findings in this investigation because SA priming caused the drop in H_2O_2 concentration of wheat seedlings. Therefore, the elevated H_2O_2 production at the site of pathogen attack via SA contributes to both a hypersensitivity response and the solidification of plant cell walls, which in turn boost resistance against *F. graminearum* invasion (Sorahinobar et al. 2016).

In the same time, the results obtained herein showed an obvious improvement in the growth rate of wheat seedlings primed with $CaCl_2$ solution, as compared to *F. graminearum*

Table 2 The results of two-way ANOVA analyses of *F. graminearum* and priming solution (Ca or SA), as well as their interactions treatments on wheat-assessed parameters

Parameters	*F. graminearum*	Priming solution	*F. graminearum* × Priming solution
Shoot length	269.56***	23.13***	7.69**
Root depth	65.21***	9.93**	8.74**
Leaf area	57.81***	5.59*	1.42ns
WC Sh	7.79*	4.59*	0.63ns
WC R	43.77***	4.77*	24.22***
Chl a	778.71***	22.68***	0.365ns
Chl b	87.69***	20.53***	26.97***
Caro	1.50ns	1.60ns	11.69**
EC	572.00***	33.57***	54.22***
MDA	213.14***	21.81***	13.95***
C=O	634.88***	164.95***	147.74***
H_2O_2	70.97***	9.94**	1.48ns
$OH^{\cdot}$	83.66***	16.79***	2.74ns
Ascorbate	157.38***	14.91***	20.76***
Phenols	92.22***	4.74*	0.62ns
Flavonoids	62.87***	6.15*	8.59**
GB	217.50***	33.22***	31.75***
Proline	252.20***	46.83***	46.92***
Amino acids	494.02***	20.27***	20.57***
CAT	169.55***	31.43***	37.28***
GPX	93.19***	8.95**	50.86***
SOD	179.12***	20.08***	18.70***
PPO	132.93***	19.75***	52.49***
PAL	311.06***	239.70***	253.86***
CAT PCR	24.00***	14.43***	44.13***
GR PCR	141.27***	336.45***	425.99***
PR2	0.89ns	1.42ns	0.85ns
PR4	309.74***	2522.56***	976.96***
MT	44.93***	27.67***	7.55ns
PC	23.19***	5.22*	3.19ns

Numbers indicates *F* values at 0.05 level

***$P<0.001$; **$P<0.01$; *$P<0.05$; *ns* non-significant ($P>0.05$)

single treatment. These results are in line with Zielińska and Michniewicz (2001) findings on *Fusarium culmorum*, who postulated that the impact of Ca on the hormone balance in the plant may be the initial stage in the suppression of *F. culmorum* damage to wheat seedlings. Consequently, it is proposed that some plant hormones, including ethylene and ABA, participate in the interactions with *F. graminearum* (Wang et al. 2018), by regulating leaf chlorosis, senescence, and apoptosis, and ethylene helps to promote the establishment of disease symptoms (der Ent and Pieterse 2018). ABA was reported to have a positive or negative consequence on susceptibility depending on the timing and invading manner of the pathogen (Wang et al. 2018). To restrict pathogen penetration at the pre-invasive stage, ABA regulates stomata closure (Melotto et al. 2008). At the late post-invasive stage, however, ABA antagonistically inhibits SA- or JA-dependent defensive resistance, resulting in vulnerability (Yasuda et al. 2008). In addition, Chardonnet et al. (1999) described the mechanism by which exogenous calcium application can slow the progression of a fungal infection by thickening the fungal cell walls, thereby slowing the fungal mycelial expansion across the host cell walls, as well as increasing the resistance of plant cell walls to the pathogen invasion. Sasaki and Nagayama (1997) proposed that Ca stimulates the formation of Ca cross-linkages in the host cell walls, thereby inhibiting the pathogen cell wall-degrading enzymes, in addition to causing fungal cytoplasm to contract, resulting in the inactivation of fungal enzymes, as another potential mechanism by which calcium can resist fungal infection.

The observed decrease in chlorophyll content and the increase in carotenoids level brought on by *F. graminearum* inoculation in wheat seedlings is consistent with the findings of our earlier study (Sobhy et al. 2021). The decreased chlorophyll following fungal invasion could be ascribed to excessive water loss due to increased stomatal conductance (Baghbani et al. 2019). Another interpretation for the decrease in chlorophyll in wheat seedlings following *F. graminearum* infection could be the overproduction of ROS as a protective barrier against the fungal attack, which in turn accelerates chlorophyll breakdown. Carotenoids, as antioxidant molecules, are anticipated to increase in infected plants as a defensive mechanism. In light of this, increasing carotenoids content enhances *Fusarium* resistance by the infected plants (Boba et al. 2011).

Interestingly, Ca and SA priming positively affected the chlorophyll content of the infected wheat seedlings. As previously mentioned by Radwan et al. (2008), SA contributes to the improvement of chlorophyll content in diseased plants by inducing the biosynthesis of carotenoids, which is consistent with our findings. Additionally, SA treatment, according to Khodary (2004), could increase the chlorophyll concentration in diseased plants because it promotes the activity of rubisco enzyme. Moreover, SA promotes membrane permeability, which facilitates the utilization efficiency of minerals, particularly Mg and Fe, that are essential for the movement of photo-assimilates and the production of chlorophyll (Javaheri et al. 2012). Our findings of increased chlorophyll content following *F. graminearum* inoculation due to Ca priming supports the findings of Ahmad et al. (2015) that Ca helps stressed plants maintain high chlorophyll content. According to Guimaraes et al. (2011), Ca stabilizes the chloroplast and thylakoid membranes by interacting with their proteins and phospholipids. Additionally, Ca has a direct impact on chlorophyll since it is known that chloroplasts accumulate large amounts of calcium, which precipitates insolubly with phosphate and is used by the chloroplast to

produce ATP (Rocha and Vothknecht 2012). Additionally, Ca increases glucose oxidation, promoting the availability of ATP (Griffiths and Rutter 2009).

Stress markers are physiological indicators of the unfavorable environmental conditions, either biotic or abiotic, that the plant is encountering. Inoculating wheat with *F. graminearum* caused significant membrane deteriorations, including electrolyte leakage, lipid peroxidation, and protein oxidation, as well as H_2O_2 accumulation and the production of hydroxyl radicals. The increased levels of stress markers in wheat that had been invaded by *F. graminearum* were documented by other investigations (Sorahinobar et al. 2015; Spanic et al. 2020; Sobhy et al. 2021). Along with its function as a protective molecule against *Fusarium* invasion, H_2O_2 frequently acts as an elicitor in plant–pathogen interactions (Sorahinobar et al. 2017). H_2O_2 has been reported to be essential for the development of phytoalexins, cell wall thickening, and various plant genes involved in cellular defense against pathogen attack (Quan et al. 2008). Protein carbonylation, which is frequently employed as a measure of protein oxidation, is another consequence of oxidative stress causing damage to proteins as a response to the fungal attack (Shishatskaya et al. 2018). Additionally, it is proposed that the elevated electrolyte leakage observed in the infected wheat seedlings throughout this study is correlated to the membrane integrity loss that follows ROS development. Consequently, it has been suggested that the overproduction of hydroxyl radical after fungal invasion plays a significant part in the process of plant defense against fungal attack (Taheri and Kakooee 2017).

Regarding priming pre-treatments, the current findings showed that priming with either 5-mM $CaCl_2$ or 0.05-mM SA had contributed to a profound decrement in oxidative stress markers (EL, MDA, C=O, H_2O_2, and $OH^{\bullet}$ radical), as compared to wheat seedlings infected with *F. graminearum*. Earlier reports showed that Ca could improve the water status and seedling growth while reducing the damage to membranes by increasing the quantity of proline and glycine betaine (Nayyar 2003). Accumulating evidence also strongly suggests a cross-talk between Ca and H_2O_2 in the regulation of antioxidant enzymes activity (Hu et al. 2006). Moreover, Ca can regulate the activity of target proteins directly or via CaM, a ubiquitous calcium-binding protein, and the Ca/CaM complex regulates the activities of antioxidant enzymes along with several protein kinases and transcription factors (Rentel and Knight 2004). Ca functions as the central node in the overall signaling web and regulates a variety of mechanisms, including ion transport, gene regulation, cell motility, growth, proliferation, apoptosis, and stress tolerance. Supporting the alleviatory role of SA on *F. graminearum*-infected wheat seedlings, Szepesi (2005) reported that SA enhanced antioxidant enzyme activities of stressed tomato offering stress tolerance by decreasing oxidative stress. Moreover, Awate and Gaikwad (2014) asserted that SA treatment prompted the level of some secondary metabolites, such as coumarins, sterols, xanthoproteins, glycosides, and saponins. In addition to supporting plants recovery from biotic and abiotic stressors, these secondary metabolites may also diminish oxidative injury. According to Makandar et al. (2012), SA application promoted SA signaling, which improved the acquisition of innate immunity against *F. graminearum* in wheat.

Ascorbic acid (AsA) and flavonoids were demonstrated to increase in wheat seedlings as a result of *F. graminearum* inoculation. The same findings were reported earlier by Gauthier et al. (2015) and Sobhy et al. (2021). The increased accumulation of flavonoids has been attributed to the upregulation of flavonoids biosynthesizing genes in response to the infection with *F. graminearum* (Ravensdale et al. 2014). The most ubiquitous and efficient antioxidant, ASA, effectively reduces or prevents the damage caused by ROS to plants. It can also restore oxidized carotenoids or α-tocopherol, which effectively scavenge ROS, preserving membranes and reducing cellular injury through synergistic interactions with other antioxidants (Gill and Tuteja 2010). The antioxidant characteristics of flavonoids enable them to lower and remove ROS generated by both the pathogen and the plant during infection. Additionally, flavonoids are thought to contribute to the strengthening of plant structures and act as a mechanical barrier against fungal infestation (Treutter 2005, 2006). The integration of phenolic compounds into the cellulose fraction of the host plant cell walls to strengthen the plant's tolerance to pathogenic fungi may be responsible for the drop in their content following fungal invasion (Sobhy et al. 2021).

The assessed non-enzymatic antioxidants were unaffected by the single priming treatments with Ca or SA, but their interaction with *F. graminearum* reduced AsA and flavonoids while increased phenolics accumulation in comparison to seedlings that had been infected. The increased phenolic content following priming with Ca or SA can be explained by the ability of these solutions to inhibit the activity of the polyphenol oxidase enzyme. Both AsA and flavonoids have antioxidant characteristics that can counteract oxidative damage to cellular membranes and biomolecules, allowing plants to thrive in stressful situations. As the priming solutions could accommodate for their antioxidant role in plants' defense against the imposed fungal attack, the lowered accumulation of both compounds after priming with Ca or SA can be explained by the reduced demand for these molecules under such circumstances. Furthermore, the potential of these solutions to inhibit the activity of polyphenol oxidase can be used to elucidate why there is an increase in phenolic content after priming with Ca or SA (Singh et al. 2020).

The osmoregulatory molecules glycinebetaine (GB), free proline (FP), and free amino acids (FAAs) were stimulated by *F. graminearum* treatment in the present study. The accumulation of compatible solutes in plants has been documented to enhance their adaptability to stress conditions. Plants perform better under stress when compatible solutes are accumulated because they are valuable for the stability and maintenance of cellular biomolecules and enzymes (Mattana et al. 2005). As a component of the basic immune response, proline functions as a regulatory element of cell death to minimize the severity of disease progression in pathogen-infected cells. In addition, the elevated proline levels likely inhibit uncontrolled host cell death (Senthil-Kumar and Mysore 2012). In line with our findings, Lavanya and Amruthesh (2017) demonstrated that GB, as a versatile osmoprotectant, was effective in managing downy mildew infestation by eliciting host innate immunity.

When compared to *F. graminearum*-stressed seedlings, the proportion of osmoregulatory molecules evaluated in this study revealed a substantial decrease in their content as a result of Ca and SA priming. Ca priming promotes cells to develop stress tolerance by sustaining cellular membranes permeability and stability and by providing anti-stress responses (Hepler 2005). Additionally, Ca acts as a secondary messenger by sending a specific anti-stress signal that activates transcription factors associated with enzymatic antioxidants, preventing the accumulation of ROS and lipid peroxidation (Farooq et al. 2008). According to Hongna et al. (2021), SA treatment stimulated endogenous SA accumulation, which modified the distribution of endogenous phytohormones. Although there was no direct interaction between SA and ABA in response to abiotic stress, Alazem et al. (2019) detected the presence of crossta Sasaki lk between SA and ABA in plants under biotic stress. By acting as a molecular chaperone, GB was shown to scavenge ROS, upregulate stress-related genes, safeguard the photosynthetic apparatus, and sustain the structural integrity of proteins (Parveen et al. 2021). This implies that the used priming solutions (Ca and SA) potentially perform many roles like those of osmoregulatory molecules, negating the requirement for further accumulation of these osmolytes in response to *F. graminearum* infection.

Several antioxidant enzymes have been reported to become more active as a quick plant–pathogen interaction response to reduce the oxidative injury caused by the invading pathogen. As the first line of defense, superoxide dismutase (SOD) catalyzes the dismutation of superoxide radicals into O_2 and H_2O_2 preventing cell injury and tissue dysfunction. Catalase (CAT) and peroxidase (POD) simultaneously decompose the surplus H_2O_2 (Xu et al. 2013). Stress symptoms brought on by fungal invasion are caused by an imbalanced ROS-antioxidant system that oxidatively damages cellular membranes and macromolecules. Our findings showed that *F. graminearum* inoculation significantly increased the activity of all elucidated antioxidant enzymes, including CAT, guiacol peroxidase (GPX), SOD, polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL). Our results support the earlier findings of many authors (Sorahinobar et al. 2016; Spanic et al. 2017; Sobhy et al. 2021). The ability of a plant to withstand pathogen attack is based on the increased activity of antioxidant enzymes in response to the infection. SOD repairs the oxidative damage and fortifies the cell walls of the host (Zhang et al. 2013), and GPX and CAT eliminate the toxic ROS to avoid harming cells and organelles. In addition to generating lignin as a physical barrier against fungal invasion, PPO employs phenols as a substrate in ROS-consuming processes to produce fungitoxic quinones, which prevent fungal growth (Lattanzio et al. 2006). PAL plays a critical role in the plant defense system through its involvement in the biosynthesis of pivotal metabolites, like phenols, lignin, phytoalexins, and SA (Mandal et al. 2009). Because phenolic compounds are used as a substrate for PPO and PAL, the increased activity of these enzymes helps to explain why there was a decrease in phenolic compounds concentration after *F. graminearum* inoculation in the current study.

CAT, GPX, SOD, PPO, and PAL activities in wheat seedlings inoculated with *F. graminearum* were found to be declined to levels mostly close to that of the control by Ca and SA priming treatments. Because Ca sustains the integrity of the membrane and prevents the generation of free radicals, it may be responsible for the decrease in antioxidant enzymes activity (Tuberosa et al. 2007). This role of Ca may improve cell relief and adaptability. The involvement of Ca in signal transduction and gene expression under the oxidative stress brought on by fungi was also documented (McAinsh and Hetherington 1998; Trofimova et al. 1999). Nonetheless, the reduction in the antioxidant enzymes in *F. graminearum*-inoculated wheat seedlings following SA treatment may be attributed to that the activities of these enzymes are directly or indirectly regulated by SA. The decreased level of oxidative stress in seedlings treated with SA may potentially be attributed to its antioxidant potential in scavenging ROS (Popova et al. 2012). Makandar et al. (2012) demonstrated that SA, through activating responsive genes, promotes the basal resistance defense of wheat against *F. graminearum*. This decrease in antioxidant enzymes activity in the Ca- and SA-treated seedlings compared to the stressed ones raises the possibility that these molecules could mitigate the oxidative damage caused by *F. graminearum*.

Plant responses to biotic stress can be appropriately evaluated by monitoring alterations in gene expression. Here, we investigated the expression of wheat seedlings for some of the genes encoding for the antioxidant enzymes catalase (CAT) and glutathione reductase (GR), pathogenesis-related

(PR) proteins (PR2 and PR4), as well as metal-binding proteins, such as metallothionine (MT) and phytochelatin synthase (PCS). Except for the PR2 gene, *F. graminearum* treatment resulted in increased expression of the studied genes, as compared to the non-inoculated control. Overexpression of CAT enzyme has been shown to boost the efficiency of the infected seedlings to remove ROS and, as a result, to promote their resistance toward oxidative stress (Van Nguyen et al. 2012). To protect plant cells from oxidative damage, GPX uses GSH to eliminate H_2O_2 and lipid hydroperoxides (Quan et al. 2008). According to Asada (1994), lignin production, indole-3-acetic acid oxidation, and pathogen resistance are the three primary roles of GPX in plants. Similar to our findings, wheat plants treated with *Pyricularia oryzae* showed an increased GPX expression in their leaves (Debona et al. 2012). The increased GPX activity in response to the fungal assault was also demonstrated in *Plasmopara halstedii*-infected sunflower (Herbette et al. 2003). The stress-induced overexpression of the GPX gene may involve signaling components cross-talk, revealing the increasing complexity of the modulation of this gene (Agrawal et al. 2002).

In response to pathogen attack, plants immediately alter gene expression, resulting in the de novo synthesis of distinctive pathogenesis-related (PR) proteins (Sarowar et al. 2005). The current study revealed upregulation of pathogenesis-related protein 4 (PR4) in wheat seedlings as a consequence of *F. graminearum* infection. Correspondingly, Caruso et al. (1999) demonstrated that *F. culmorum* inoculation upregulated PR4 protein expression in wheat leaves. According to Zhou et al. (2021), PR4 (chitin-binding protein) is a key player in the activation of defense mechanisms against *Fusarium* infection. Because PR4 has enzymatic activity typical of ribonucleases, it has antifungal potential. The fact that PR4 proteins have antifungal activity toward a range of pathogenic fungi implies that they occupy main position in the defense mechanisms against pathogen attack (Singh et al. 2018).

The formation of metallothioneins (MTs) may be a relevant resistance mechanism in plants. These peptides with low molecular weight are abundant in cysteine residues (Cys) and frequently have a characteristic pattern of sulfur-containing amino acids. MTs were reported to be involved in ROS scavenging and metal homeostasis (Lukács et al. 2021). In wheat seedlings inoculated with *F. graminearum*, the acquired data showed that the expression of the MT gene was significantly upregulated. In line with our findings, Kim et al. (2001) documented that rice plants infected with *Magnaporthe grisea* blast fungus displayed increased gene expression of MTs. Similarly, the viral pathogen TMV substantially stimulated the *N. glutinosa* MTs gene expression (Choi et al. 1996). The role of MTs in modulating the availability of metal ions, which in turn influences the intracellular ROS generated in stressed plants, was identified by the authors as the reason for this upregulation in MTs gene expression. Phytochelatins (PCs) are small cysteine-rich peptides that are produced from glutathione as a precursor. By binding different heavy metals to sulfhydryl groups, PCs can sequester these metals in vacuoles and detoxify them (Chaudhary et al. 2018). Phytochelatin synthase (PCS) expression has been extensively documented under a variety of heavy metals stress situations, but to our knowledge, no prior studies have addressed the expression of this gene in a fungal invasion scenario. The overexpression of the PCS gene was reported to appraise the redox status of PR gene proteins, which indirectly interferes with the salicylic acid-induced genes encoding pathogen-related proteins, may be the mechanism underlying its effectiveness in pathogen defense (Dong 2004). To understand the precise mechanism and the transcriptional mechanisms controlling the expression of these genes, further detailed research on the potential involvement of phytochelatins in ROS homeostasis at the time of fungal attack is needed in future.

Conclusion

In this study, we have demonstrated that priming wheat kernels with either 5-mM $CaCl_2$ or 0.05-mM salicylic acid previous to *F. graminearum* inoculation significantly altered growth performance, leaf pigmentation, stress markers, oxidative status, osmoprotectant molecules, as well as the expression level some genes. Our findings exposed that priming solutions trigger metabolic and defensive processes, which may contribute to earlier and more efficient responses to the fungal assault. The decline in oxidative stress parameters (EL, MDA, C=O, H_2O_2, and $OH^{\cdot}$) together with the stimulated accumulation of non-enzymatic antioxidants (ascorbic acid, phenolics, and flavonoids) as well as osmoregulatory compounds (GB, proline, and amino acids), in addition to the enhanced antioxidant enzymes activity (CAT, GPX, SOD, PPO, and PAL), may be the key requisite for the survival of *F. graminearum*-infected wheat seedlings. Our findings imply that wheat-defensive mechanisms versus *F. graminearum* infection may involve MT and PCS upregulation. Additionally, the integrated implementation of the priming treatments and *F. graminearum* administration considerably minimized the pathogen effects by improving growth rate, boosting oxidative homeostasis, and modifying the expression levels of some stress-responsive genes in wheat-infected seedlings. Understanding the concise mechanisms by which Ca and SA priming could restrict *F. graminearum* toxicity in wheat plants requires more study on the signaling, molecular, and proteomic levels in the subsequent developmental phases. Additionally, in the investigation that follows, we should also take into account the

following query: Does exogenous Ca inhibit the pathogen itself?

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00344-023-10966-5.

Author Contributions SS involved in conceptualization, methodology, software, data curation, original draft preparation, software, and validation. E-EA-K involved in conceptualization, visualization, supervision, and reviewing of the manuscript. KS involved in conceptualization, visualization, methodology, investigation validation, software, original draft preparation, and reviewing and editing of the manuscript. EH involved in conceptualization, visualization, and investigation.

Funding Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB). This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Aftab T, Khan MMA et al (2011) Role of salicylic acid in promoting salt stress tolerance and enhanced artemisinin production in *Artemisia annua* L. J Plant Growth Regul 30:425–435. https://doi.org/10.1007/s00344-011-9205-0

Agrawal GK, Rakwal R, Jwa NS, Agrawal VP (2002) Effects of signaling molecules, protein phosphatase inhibitors and blast pathogen (*Magnaporthe grisea*) on the mRNA level of a rice (*Oryza sativa* L.) phospholipid hydroperoxide glutathione peroxidase (OsPHGPX) gene in seedling leaves. Gene 283:227–236. https://doi.org/10.1016/S0378-1119(01)00854-X

Ahmad P, Sarwat M, Bhat NA, Wani MR (2015) Alleviation of cadmium Toxicity in *Brassica juncea* L. (Czern. & Coss.) by calcium application involves various physiological and biochemical strategies. PLoS ONE 10:e0114571. https://doi.org/10.1371/journal.pone.0114571

AL Masri A, Hau B, Dehne H, Mahlein A (2017) Impact of primary infection site of *Fusarium* species on head blight development in wheat ears evaluated by IR-thermography. Eur J Plant Pathol 147:855–868. https://doi.org/10.1007/s10658-016-1051-2

Alazem M, Kim KH, Lin NS (2019) Effects of abscisic acid and salicylic acid on gene expression in the antiviral RNA silencing pathway in *Arabidopsis*. Int J Mol Sci 20:e2538. https://doi.org/10.3390/ijms20102538

Ardestani A, Yazdanparast R (2007) Antioxidant and free radical scavenging potential of *Achillea santolina* extracts. Food Chem 104:21–29. https://doi.org/10.1016/j.foodchem.2006.10.066

Arzani A (2019) Emmer (*Triticum turgidum* ssp. dicoccum) flour and bread. In: Preedy VR, Watson RR (eds) Flour and breads and their fortification in health and disease prevention, 2nd edn. Academic Press, pp 89–98

Arzani A, Ashraf M (2017) Cultivated ancient wheats (*Triticum* spp.): a potential source of health-beneficial food products. Compr Rev Food Sci Food Saf 16:477–488. https://doi.org/10.1111/1541-4337.12262

Asada K (1994) Production and action of active oxygen species in photosynthetic tissues. In: Foyer CH, Mullineaux PM (eds) Causes of photooxidative stress and amelioration of defense systems in plants. CRC Press, Boca Raton, pp 77–103

Ashraf M, Akram NA, Arteca RN, Foolad MR (2010) The physiological, biochemical and molecular roles of brassinosteroids and salicylic acid in plant processes and salt tolerance. CRC Crit Rev Plant Sci 29:162–190. https://doi.org/10.1080/07352689.2010.483580

Aucique-pérez CE, Eduardo P, Silva DM et al (2017) Photosynthesis impairments and excitation energy dissipation on wheat plants supplied with silicon and infected with *Pyricularia oryzae*. Plant Physiol Biochem 121:196–205. https://doi.org/10.1016/j.plaphy.2017.10.023

Awate PD, Gaikwad DK (2014) Influence of growth regulators on secondary metabolites of medicinally important oil yielding plant *Simarouba glauca* DC. under water stress conditions. J Stress Physiol Biochem 10:222–229

Baghbani F, Lotfi R, Moharramnejad S et al (2019) Impact of *Fusarium verticillioides* on chlorophyll fluorescence parameters of two maize lines. Eur J Plant Pathol 154:337–346. https://doi.org/10.1007/s10658-018-01659-x

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207. https://doi.org/10.1007/BF00018060

Beyer WF, Fridovich I (1987) Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal Biochem 161:559–566. https://doi.org/10.1016/0003-2697(87)90489-1

Bhar A, Chatterjee M, Gupta S, Das S (2018) Salicylic acid regulates systemic defense signaling in chickpea during *Fusarium oxysporum* f. sp. ciceri Race 1 infection. Plant Mol Biol Report 36:162–175. https://doi.org/10.1007/s11105-018-1067-1

Boba A, Kulma A, Kostyn K et al (2011) The influence of carotenoid biosynthesis modification on the *Fusarium culmorum* and *Fusarium oxysporum* resistance in flax. Physiol Mol Plant Pathol 76:39–47. https://doi.org/10.1016/j.pmpp.2011.06.002

Braun H-J, Atlin G, Payne T (2010) Multi-location testing as a tool to identify plant response to global climate change. In: Reynolds MP (ed) Climate change and crop production. CABI Wallingford, pp 115–138

Caruso C, Chilosi G, Caporale C et al (1999) Induction of pathogenesis-related proteins in germinating wheat seeds infected with *Fusarium culmorum*. Plant Sci 140:87–97. https://doi.org/10.1016/S0168-9452(98)00199-X

Chang CC, Yang MH, Wen HM, Chern JC (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 10:178–182

Chardonnet CO, Sams CE, Conway WS (1999) Calcium effect on the mycelial cell walls of *Botrytis cinerea*. Phytochemistry 52:967–973. https://doi.org/10.1016/S0031-9422(99)00315-5

Chaudhary K, Agarwal S, Khan S (2018) Role of phytochelatins (PCs), metallothioneins (MTs), and heavy metal ATPase (HMA) genes

in heavy metal tolerance. In: Prasad R (ed) Mycoremediation and environmental sustainability. Springer, Cham, pp 39–60
Chen Y, Kistler HC, Ma Z (2019) *Fusarium graminearum* trichothecene mycotoxins: biosynthesis, regulation, and management. Annu Rev Phytopathol 57:15–39. https://doi.org/10.1146/annurev-phyto-082718-100318
Choi D, Kim HM, Yun HK et al (1996) Molecular cloning of a metallothionein-like gene from Nicofiana glufinosa L. and its induction by wounding and tobacco mosaic virus infection. Plant Physiol 112:353–359
Chouhan D, Dutta A, Kumar A et al (2022) Application of nickel chitosan nanoconjugate as an antifungal agent for combating *Fusarium* rot of wheat. Sci Rep 12:e14518. https://doi.org/10.1038/s41598-022-18670-2
De Vos M, Van Oosten VR, Van Poecke RMP et al (2005) Signal signature and transcriptome changes of *Arabidopsis* during pathogen and insect attack. Mol Plant-Microbe Interact 18:923–937
Dean R, Van Kan JAL, Pretorius ZA et al (2012) The Top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol 13:414–430. https://doi.org/10.1111/j.1364-3703.2011.00783.x
Debona D, Rodrigues FÁ, Rios JA et al (2012) Biochemical changes in the leaves of wheat plants infected by Pyricularia oryzae. Phytopathology 102:1121–1129. https://doi.org/10.1094/PHYTO-06-12-0125-R
der Ent S, Pieterse CMJ (2018) Ethylene: multi-tasker in plant–attacker interactions. In: Annual plant reviews online. Wiley, pp 343–377
Dias MC, Mariz-Ponte N, Santos C (2019) Lead induces oxidative stress in *Pisum sativum* plants and changes the levels of phytohormones with antioxidant role. Plant Physiol Biochem 137:121–129. https://doi.org/10.1016/j.plaphy.2019.02.005
Dong X (2004) NPR1, all things considered. Curr Opin Plant Biol 7:547–552. https://doi.org/10.1016/j.pbi.2004.07.005
Emamverdian A, Ding Y, Mokhberdoran F (2020) The role of salicylic acid and gibberellin signaling in plant responses to abiotic stress with an emphasis on heavy metals. Plant Signal Behav 15:e1777372. https://doi.org/10.1080/15592324.2020.1777372
Escalante-Magaña C, Aguilar-Caamal LF, Echevarría-Machado I et al (2019) Contribution of glycine betaine and proline to water deficit tolerance in pepper plants. HortScience 54:1044–1054. https://doi.org/10.21273/HORTSCI13955-19
Farooq M, Basra SMA, Rehman H, Saleem BA (2008) Seed priming enhances the performance of late sown wheat (*Triticum aestivum* L.) by improving chilling tolerance. J Agron Crop Sci 194:55–60. https://doi.org/10.1111/j.1439-037X.2007.00287.x
Faryal S, Ullah R, Khan MN et al (2022) Thiourea-capped nanoapatites amplify osmotic stress tolerance in *Zea mays* L. by conserving photosynthetic pigments, osmolytes biosynthesis and antioxidant biosystems. Molecules 27:e5744. https://doi.org/10.3390/molecules27185744
Gauthier L, Atanasova-penichon V, Chéreau S, Richard-Forget F (2015) Metabolomics to decipher the chemical defense of cereals against *Fusarium graminearum* and deoxynivalenol accumulation. Int J Mol Sci 16:24839–24872. https://doi.org/10.3390/ijms161024839
Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930. https://doi.org/10.1016/j.plaphy.2010.08.016
Golizadeh F, Kumleh HH (2019) Physiological responses and expression changes of fatty acid metabolism-related genes in wheat (*Triticum aestivum*) under cold stress. Plant Mol Biol Rep 37:224–236. https://doi.org/10.1007/s11105-019-01150-9
Grant M, Lamb C (2006) Systemic immunity. Curr Opin Plant Biol 9:414–420. https://doi.org/10.1016/j.pbi.2006.05.013
Grieve CMM, Grattan SRR (1983) Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil 70:303–307. https://doi.org/10.1007/BF02374789
Griffiths EJ, Rutter GA (2009) Mitochondrial calcium as a key regulator of mitochondrial ATP production in mammalian cells. Biochim Biophys Acta (BBA) Bioenerg 1787:1324–1333. https://doi.org/10.1016/j.bbabio.2009.01.019
Guimaraes FVA, de Lacerda CF, Marques EC et al (2011) Calcium can moderate changes on membrane structure and lipid composition in cowpea plants under salt stress. Plant Growth Regul 65:55–63. https://doi.org/10.1007/s10725-011-9574-1
Halliwell B, Aeschbacht R, Loligert J, Aruoma OI (1995) The characterization of antioxidants. Food Chem Toxicol 33:601–617. https://doi.org/10.1016/0278-6915(95)00024-V
Hayat S, Hayat Q, Alyemeni MN et al (2012) Role of proline under changing environments: a review. Plant Signal Behav 7:1456–1466
Heath R, Packer L (1968) Photoperoxidation in isolated chloroplasts. Arch Biochem Biophys 125:189–198. https://doi.org/10.1016/0003-9861(68)90654-1
Heil M, Bostock RM (2002) Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. Ann Bot 89:503–512. https://doi.org/10.1093/aob/mcf076
Hepler PK (2005) Calcium: a central regulator of plant growth and development. Plant Cell 17:2142–2155. https://doi.org/10.1105/tpc.105.032508
Herbette S, Lenne C, Tourvieille De Labrouhe D et al (2003) Transcripts of sunflower antioxidant scavengers of the SOD and GPX families accumulate differentially in response to downy mildew infection, phytohormones, reactive oxygen species, nitric oxide, protein kinase and phosphatase inhibitors. Physiol Plant 119:418–428. https://doi.org/10.1034/j.1399-3054.2003.00186.x
Hernández-Muñoz P, Almenar E, Ocio MJ, Gavara R (2006) Effect of calcium dips and chitosan coatings on postharvest life of strawberries (*Fragaria x ananassa*). Postharvest Biol Technol 39:247–253. https://doi.org/10.1016/j.postharvbio.2005.11.006
Herrera-Vásquez A, Salinas P, Holuigue L (2015) Salicylic acid and reactive oxygen species interplay in the transcriptional control of defense genes expression. Front Plant Sci 6:e171. https://doi.org/10.3389/fpls.2015.00171
Hongna C, Leyuan T, Junmei S et al (2021) Exogenous salicylic acid signal reveals an osmotic regulatory role in priming the seed germination of *Leymus chinensis* under salt-alkali stress. Environ Exp Bot 188:104498. https://doi.org/10.1016/j.envexpbot.2021.104498
Hu X, Jiang M, Zhang J et al (2006) Calcium–calmodulin is required for abscisic acid-induced antioxidant defense and functions both upstream and downstream of H_2O_2 production in leaves of maize (*Zea mays*) plants. New Phytol 173:27–38. https://doi.org/10.1111/j.1469-8137.2006.01888.x
Javaheri M, Mashayekhi K, Dadkhah A et al (2012) Effects of salicylic acid on yield and quality characters of tomato fruit (*Lycopersicum esculentum* Mill.). Int J Agric Crop Sci 4:1184–1187
Jindal KK, Singh RN (1975) Phenolic content in male and female *Carica papaya*: a possible physiological marker for sex identification of vegetative seedlings. Physiol Plant 33:104–107. https://doi.org/10.1111/j.1399-3054.1975.tb03774.x
Johnson JM, Reichelt M, Vadassery J et al (2014) An *Arabidopsis* mutant impaired in intracellular calcium elevation is sensitive to biotic and abiotic stress. BMC Plant Biol. https://doi.org/10.1186/1471-2229-14-162
Kato M, Shimizu S (1987) Chlorophyll metabolism in higher plants. VII. Chlorophyll degradation in senescing tobacco leaves; phenolic-dependent peroxidative degradation. Can J Bot 65:729–735. https://doi.org/10.1139/b87-097

Khan MIR, Fatma M, Per TS et al (2015) Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. Front Plant Sci 6:1–17. https://doi.org/10.3389/fpls.2015.00462

Khodary SEA (2004) Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. Int J Agric Biol 6:5–8

Kim S, Ahn I, Lee Y (2001) Analysis of genes expressed during rice-*Magnaporthe grisea* interactions. Mol Plant-Microbe Interact 14:1340–1346. https://doi.org/10.1094/MPMI.2001.14.11.1340

Klessig DF, Choi HW, Dempsey DA (2018) Systemic acquired resistance and aalicylic acid: past, present, and future. Mol Plant-Microbe Interact 31:871–888. https://doi.org/10.1094/MPMI-03-18-0067-CR

Kour J, Khanna K, Singh AD et al (2023) Calcium's multifaceted functions: from nutrient to secondary messenger during stress. S Afr J Bot 152:247–263. https://doi.org/10.1016/j.sajb.2022.11.048

Kumar KB, Khan PA (1982) Peroxidase and polyphenol oxidase in excised ragi (*Eleusine corocana* cv PR 202) leaves during senescence. Indian J Exp Biol 20:412–416

Lattanzio V, Lattanzio VMT, Cardinali A (2006) Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. In: Imperato F (ed) Phytochemistry: advances in research. Trivandrum, Kerala, pp 23–67

Lavanya NS, Amruthesh NK (2017) Glycine betaine mediated disease resistance against *Sclerospora graminicola* in Pearl Millet. J Appl Biol Biotechnol 5:45–51. https://doi.org/10.7324/jabb.2017.50308

Lee YYP, Takahashi T (1966) An improved colorimetric determination of amino acids with the use of ninhydrin. Anal Biochem 14:71–77. https://doi.org/10.1016/0003-2697(66)90057-1

Lemos M, Xiao Y, Bjornson M et al (2016) The plastidial retrograde signal methyl erythritol cyclopyrophosphate is a regulator of salicylic acid and jasmonic acid crosstalk. J Exp Bot 67:1557–1566. https://doi.org/10.1093/jxb/erv550

Li X, Ahammed GJ, Zhang YQ et al (2015) Carbon dioxide enrichment alleviates heat stress by improving cellular redox homeostasis through an ABA-independent process in tomato plants. Plant Biol 17:81–89. https://doi.org/10.1111/plb.12211

Liu Z, Ding Y, Wang F et al (2016) Role of salicylic acid in resistance to cadmium stress in plants. Plant Cell Rep 35:719–731. https://doi.org/10.1007/s00299-015-1925-3

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262

Lukács M, Pálinkás DC, Szunyog G, Várnagy K (2021) Metal binding ability of small peptides containing cysteine residues. Chem Open 10:451–463. https://doi.org/10.1002/open.202000304

Makandar R, Nalam VJ, Lee H et al (2012) Salicylic acid regulates basal resistance to *Fusarium* head blight in wheat. Mol Plant-Microbe Interact 25:431–439. https://doi.org/10.1094/MPMI-09-11-0232

Mandal S, Mallick N, Mitra A (2009) Salicylic acid-induced resistance to *Fusarium oxysporum* f. sp. *lycopersici* in tomato. Plant Physiol Biochem 47:642–649. https://doi.org/10.1016/j.plaphy.2009.03.001

Mattana M, Biazzi E, Consonni R et al (2005) Overexpression of Osmyb4 enhances compatible solute accumulation and increases stress tolerance of *Arabidopsis thaliana*. Physiol Plant 125:212–223. https://doi.org/10.1111/j.1399-3054.2005.00551.x

McAinsh MR, Hetherington A (1998) Encoding specificity in Ca^{2+} signalling systems. Trends Plant Sci 3:32–36. https://doi.org/10.1016/S1360-1385(97)01150-3

Melotto M, Underwood W, He SY (2008) Role of stomata in plant innate immunity and foliar bacterial diseases. Annu Rev Phytopathol 46:101–122. https://doi.org/10.1146/annurev.phyto.121107.104959

Mentges M, Glasenapp A, Boenisch M et al (2020) Infection cushions of *Fusarium graminearum* are fungal arsenals for wheat infection. Mol Plant Pathol 21:1070–1087. https://doi.org/10.1111/mpp.12960

Metzner H, Rau H, Senger H et al (1965) Trace elements' uptake and antioxidant response to excess of manganese in in vitro cells of sensitive and tolerant wheat. Planta 65:186–194. https://doi.org/10.1007/bf00384998

Mo Y, Wan R, Zhang Q (2012) Application of reverse transcription-PCR and real-time PCR in nanotoxicity research. In: Reineke J (ed) Nanotoxicity: methods and protocols. Humana Press, Totowa, pp 99–112

Nayyar H (2003) Variation in osmoregulation in differentially drought-sensitive wheat genotypes involves calcium. Biol Plant 47:541–547. https://doi.org/10.1023/B:BIOP.0000041059.10703.11

Noman A, Aqeel M, Qari SH et al (2020) Plant hypersensitive response vs pathogen ingression: death of few gives life to others. Microb Pathog 145:e104224. https://doi.org/10.1016/j.micpath.2020.104224

Oser BL (1979) Hawk's physiological chemistry, 14th edn. McGraw-Hills, New York

Parveen A, Ashraf MA, Hussain I et al (2021) Promotion of growth and physiological characteristics in water-stressed *Triticum aestivum* in relation to foliar-application of salicylic acid. Water 13:e1316. https://doi.org/10.3390/w13091316

Pereyra SA, Dill-Macky R, Sims AL (2004) Survival and inoculum production of *Gibberella zeae* in wheat residue. Plant Dis 88:724–730. https://doi.org/10.1094/PDIS.2004.88.7.724

Popova LP, Maslenkova LT, Ivanova A (2012) Role of salicylic acid in alleviating heavy metal stress. In: Ahmad P, Prasad M (eds) Environmental adaptations and stress tolerance of plants in the era of climate change. Springer, New York, pp 447–466

Qi P-F, Johnston A, Balcerzak M et al (2012) Effect of salicylic acid on *Fusarium graminearum*, the major causal agent of fusarium head blight in wheat. Fungal Biol 116:413–426. https://doi.org/10.1016/j.funbio.2012.01.001

Quan LJ, Zhang B, Shi WW, Li HY (2008) Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. J Integr Plant Biol 50:2–18

Radwan DEM, Lu G, Fayez KA, Younis S (2008) Protective action of salicylic acid against bean yellow mosaic virus infection in *Vicia faba* leaves. J Plant Physiol 165:845–857. https://doi.org/10.1016/j.jplph.2007.07.012

Ragab G, Saad-Allah K (2020) Seed priming with greenly synthesized sulfur nanoparticles enhances antioxidative defense machinery and restricts oxidative injury under manganese stress in *Helianthus annuus* (L.) seedlings. J Plant Growth Regul. https://doi.org/10.1007/s00344-020-10240-y

Ravensdale M, Rocheleau H, Wang L et al (2014) Components of priming-induced resistance to Fusarium head blight in wheat revealed by two distinct mutants of *Fusarium graminearum*. Mol Plant Pathol 15:948–956. https://doi.org/10.1111/mpp.12145

Reddy ASN, Ali GS, Celesnik H, Day IS (2011) Coping with stresses: roles of calcium- and calcium/calmodulin-regulated gene expression. Plant Cell 23:2010–2032. https://doi.org/10.1105/tpc.111.084988

Rentel MC, Knight MR (2004) Oxidative stress-induced calcium signaling. Plant Physiol 135:1471–1479. https://doi.org/10.1104/pp.104.042663.1

Reznick AZ, Packer L (1994) Oxidative damage to proteins: spectrophotometric method for carbonyl assay. In: Wurtzel ET (ed) Methods in enzymology. Elsevier, pp 357–363

Rocha AG, Vothknecht UC (2012) The role of calcium in chloroplasts—an intriguing and unresolved puzzle. Protoplasma 249:957–966. https://doi.org/10.1007/s00709-011-0373-3

Sairam RK, Deshmukh PS, Shukla DS (1997) Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. J Agron Crop Sci 178:171–178. https://doi.org/10.1111/j.1439-037X.1997.tb00486.x

Sarowar S, Jin Y, Eui K et al (2005) Overexpression of a pepper basic pathogenesis-related protein 1 gene in tobacco plants enhances resistance to heavy metal and pathogen stresses. Plant Cell Rep 24:216–224. https://doi.org/10.1007/s00299-005-0928-x

Sasaki I, Nagayama H (1997) Induction of β-glucosidase in *Botrytis cinerea* by cell wall fractions of the host plant. Biosci Biotechnol Biochem 61:1073–1076

Senthil-Kumar M, Mysore KS (2012) Ornithine-delta-aminotransferase and proline dehydrogenase genes play a role in non-host disease resistance by regulating pyrroline-5-carboxylate metabolism-induced hypersensitive response. Plant Cell Environ 35:1329–1343. https://doi.org/10.1111/j.1365-3040.2012.02492.x

Shah J, Chaturvedi R, Chowdhury Z et al (2014) Signaling by small metabolites in systemic acquired resistance. Plant J 79:645–658. https://doi.org/10.1111/tpj.12464

Shakirova FM, Avalbaev AM, Bezrukova MV et al (2012) Hormonal intermediates in the protective action of exogenous phytohormones in wheat plants under salinity. In: Khan NA, Nazar R, Iqbal N, Anjum NA (eds) Phytohormones and abiotic stress tolerance in plants. Springer, Berlin, pp 185–228

Shishatskaya E, Menzyanova N, Zhila N et al (2018) Toxic effects of the fungicide tebuconazole on the root system of Fusarium-infected wheat plants. Plant Physiol Biochem 132:400–407. https://doi.org/10.1016/j.plaphy.2018.09.025

Singh A, Jain D, Tyagi C et al (2018) In silico prediction of active site and in vitro DNase and RNase activities of Helicoverpa-inducible pathogenesis related-4 protein from *Cicer arietinum*. Int J Biol Macromol 113:869–880. https://doi.org/10.1016/j.ijbiomac.2018.03.027

Singh A, Banerjee A, Roychoudhury A (2020) Seed priming with calcium compounds abrogate fluoride-induced oxidative stress by upregulating defence pathways in an indica rice variety. Protoplasma 257:767–782. https://doi.org/10.1007/s00709-019-01460-5

Sobhy SE, Abo-Kassem EEM, Sewelam NA et al (2021) Pre-soaking in weed extracts is a reasonable approach to mitigate *Fusarium graminearum* infection in wheat. J Plant Growth Regul. https://doi.org/10.1007/s00344-021-10442-y

Sorahinobar M, Niknam V, Ebrahimzadeh H, Soltanloo H (2015) Differential antioxidative responses of susceptible and resistant wheat cultivars against Fusarium head blight. Int J Farming Allied Sci 4:239–243

Sorahinobar M, Niknam V, Ebrahimzadeh H et al (2016) Central role of salicylic acid in resistance of wheat against *Fusarium graminearum*. J Plant Growth Regul 35:477–491. https://doi.org/10.1007/s00344-015-9554-1

Sorahinobar M, Soltanloo H, Niknam V, Ebrahimzadeh H (2017) Physiological and molecular responses of resistant and susceptible wheat cultivars to *Fusarium graminearum* mycotoxin extract. Can J Plant Pathol 39:444–453. https://doi.org/10.1080/07060661.2017.1379442

Sorahinobar M, Safaie N, Moradi B (2021) Salicylic acid seed priming enhanced resistance in wheat against *Fusarium graminearum* seedling blight. J Plant Biol. https://doi.org/10.1007/s12374-021-09329-y

Spanic V, Viljevac Vuletic M, Abicic I, Marcek T (2017) Early response of wheat antioxidant system with special reference to Fusarium head blight stress. Plant Physiol Biochem 115:34–43. https://doi.org/10.1016/j.plaphy.2017.03.010

Spanic V, Zdunic Z, Drezner G, Vuletic MV (2020) Differences in physiological traits at the initial stage of Fusarium head blight infection in wheat. Biol Plant 64:185–192. https://doi.org/10.32615/bp.2020.014

Szepesi Á (2005) Role of salicylic acid pre-treatment on the acclimation of tomato plants to salt-and osmotic stress. Acta Biol Szeged 49:123–125

Taheri P, Kakooee T (2017) Reactive oxygen species accumulation and homeostasis are involved in plant immunity to an opportunistic fungal pathogen. J Plant Physiol 216:152–163. https://doi.org/10.1016/j.jplph.2017.04.018

Thor K (2019) Calcium—nutrient and messenger. Front Plant Sci 10:1–7. https://doi.org/10.3389/fpls.2019.00440

Treutter D (2005) Significance of flavonoids in plant resistance and enhancement of their biosynthesis. Plant Biol 7:581–591. https://doi.org/10.1055/s-2005-873009

Treutter D (2006) Significance of flavonoids in plant resistance: a review. Environ Chem Lett 4:147–157. https://doi.org/10.1007/s10311-006-0068-8

Trofimova MS, Andreev IM, Kuznetsov VV (1999) Calcium is involved in regulation of the synthesis of HSPs in suspension-cultured sugar beet cells under hyperthermia. Physiol Plant 105:67–73. https://doi.org/10.1034/j.1399-3054.1999.105111.x

Tuberosa R, Giuliani S, Parry MAJ, Araus JL (2007) Improving water use efficiency in Mediterranean agriculture: what limits the adoption of new technologies? Ann Appl Biol 150:157–162. https://doi.org/10.1111/j.1744-7348.2007.00127.x

Van Nguyen T, Schöfer W, Bormann J (2012) The stress-activated protein kinase FgOS-2 is a key regulator in the life cycle of the cereal pathogen *Fusarium graminearum*. Mol Plant-Microbe Interact 25:1142–1156. https://doi.org/10.1094/MPMI-02-12-0047-R

Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Plant Sci 151:59–66. https://doi.org/10.1016/S0168-9452(99)00197-1

Verma P, Yadav AN, Khannam KS et al (2016) Molecular diversity and multifarious plant growth promoting attributes of Bacilli associated with wheat (*Triticum aestivum* L.) rhizosphere from six diverse agro-ecological zones of India. J Basic Microbiol 56:44–58

Wang JW, Zheng LP, Wu JY, Tan RX (2006) Involvement of nitric oxide in oxidative burst, phenylalanine ammonia-lyase activation and Taxol production induced by low-energy ultrasound in *Taxus yunnanensis* cell suspension cultures. Nitric Oxide 15:351–358. https://doi.org/10.1016/j.niox.2006.04.261

Wang Q, Liang X, Dong Y et al (2013) Effects of exogenous salicylic acid and nitric oxide on physiological characteristics of perennial ryegrass under cadmium stress. J Plant Growth Regul 32:721–731. https://doi.org/10.1007/s00344-013-9339-3

Wang L, Li Q, Liu Z et al (2018) Integrated transcriptome and hormone profiling highlight the role of multiple phytohormone pathways in wheat resistance against Fusarium head blight. PLoS ONE 13:1–24. https://doi.org/10.1371/journal.pone.0207036

Xu J, Duan X, Yang J et al (2013) Enhanced reactive oxygen species scavenging by overproduction of superoxide dismutase and catalase delays postharvest physiological deterioration of cassava storage roots. Plant Physiol 161:1517–1528. https://doi.org/10.1104/pp.112.212803

Yasuda M, Ishikawa A, Jikumaru Y et al (2008) Antagonistic interaction between systemic acquired resistance and the abscisic acid–mediated abiotic stress response in *Arabidopsis*. Plant Cell 20:1678–1692. https://doi.org/10.1105/tpc.107.054296

Zhang P, Zhou MP, Zhang X et al (2013) Change of defensive-related enzyme in wheat crown rot seedlings infected by *Fusarium graminearum*. Cereal Res Commun 41:431–439

Zheng N, Li G, Zhang K et al (2022) Analysis of Fhb1 gene and resistance to Fusarium head blight in 3,177 diverse wheat accessions. J Cereal Sci 104:103387. https://doi.org/10.1016/j.jcs.2021.103387

Zhou Z, Zhu Y, Tian Y et al (2021) MdPR4, a pathogenesis-related protein in apple, is involved in chitin recognition and resistance response to apple replant disease pathogens. J Plant Physiol 260:e153390. https://doi.org/10.1016/j.jplph.2021.153390

Zielińska M, Michniewicz M (2001) The effect of calcium on the production of ethylene and abscisic acid by fungus *Fusarium culmorum* and by wheat seedlings infected with that pathogen. Acta Physiol Plant 23:79–85. https://doi.org/10.1007/s11738-001-0026-9