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Priming of wheat plant with weed extracts, calcium and salicylic acid for contribution to alleviating the oxidative stress imposed by *Fusarium graminearum* and lead toxicity

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Abstract

Biotic and abiotic stress factors drastically limit plant growth and productivity through changing the physiological, biochemical, and cellular processes. In this study, 100 mM of lead (Pb) was used as an abiotic stress source, while *Fusarium graminearum* represented a biotic one on wheat plant. Compared to the control, Pb treatment and *F. graminearum* inoculation led to remarkable reductions in the wheat seedlings leaf area that reached 21 %, and 12.5 %, respectively. Moreover, the current results showed an enhanced activity of Phenylalanine ammonia lyase (PAL) that reached 173 % in the stressed wheat grains and seedlings, decreased mineral contents in N^{-3}, P^{+3}, K^{+}, and Ca^{+2} in the shoot of wheat seedlings by 25, 42, 23 and 44 %; respectively, substantial increase (79 %) in the total soluble carbohydrates (TSC) and a highly significant reduction (28 %) in the total soluble proteins (TSP), compared with the non-treated control plants. On the other hand, wheat seed priming with weed extracts [i.e., *Portulaca oleracea* L. (purslane) and *Beta vulgaris* L. (chard)], or chemical solutions; mainly Calcium (Ca^{+2}) and Salicylic acid (SA) applied exogenously, resulted in a noteworthy increase in the leaf area, compared with the stress treatments. Furthermore, seed priming ameliorated the toxic effects induced by Pb and *F. graminearum* treatments on the photosynthetic pigments; where it significantly increased the pigments content, while the chlorophyll (Chl) a/b ratio was reduced. Furthermore, priming treatments significantly increased the mineral contents (*i.e.*, N, P, K, and Ca) and counteracted the imposed effects of stress treatments on TSC and TSP. The differential display polymerase chain reaction (DD-PCR) technique was performed to identify the variations in gene expression between the different treatments of wheat plants at three intervals of 7, 14, and 21 d old. This study aimed to investigate the use of plant extracts as potentially effective and environmentally safe green bio-control agents to control the infection of wheat plant by *F. graminearum*, ameliorate the biotic and abiotic

stresses, and compete with the currently used deleterious chemical fungicides in the wheat farms.

Keywords: Wheat, Weed extracts, *Fusarium graminearum*, Salicylic acid, Lead toxicity, Gene expression

1. Introduction

Being sessile, plants are challenging a range of biotic and abiotic stresses. Heavy metal toxicity is the furthermost common abiotic stresses that contaminate the soil, air, and water bodies universally (Ahmad *et al*., 2015; Das *et al*., 2021). Moreover, lead (Pb) toxicity leads to a variety of biochemical and physiological dysfunctions; comprising growth rate, water status, seed germination and nitrate assimilation (Lamhamdi *et al*., 2013). In addition, Pb badly affects carbon dioxide assimilation and the plant photosynthetic rate (Huang and Cunningham, 1996). At the metabolic level; Pb interacts with the proteins present in the cell cytoplasm, and a higher concentration of Pb may decrease the protein pool (Garcia *et al*., 2006).

In addition to abiotic stress, plants experience biotic stress as well. The fungal pathogens, which are a main threat to crop production and food security, are an unceasing threat worldwide (Sobhy *et al*., 2021). *Fusarium* species is considered as one of the chief groups of fungal pathogens that commonly cause severe damage to the cereals (Pereyra *et al*., 2004). In this regard, *Fusarium* head blight (FHB), seedling blight, and *Fusarium* crown and foot brown rots (FCFR), are the most damaging diseases in wheat that are incited by *Fusarium graminearum* (AL Masri *et al*., 2017).

The use of natural methods (*i.e*., herbal extracts) other than the chemical methods seems as an eco-friendly choice to advance the performance of plants under terminal stresses (Benito *et al*., 2023). Interestingly, to control plant diseases as well as abiotic stresses, natural products of some plants have been widely used (Momin and Nair, 2001; Farooq *et al*., 2009).

Portulaca oleracea L. that is known globally as purslane; is a glabrous fleshy herb with edible juicy stems and leaves, slightly acidic, and has a taste as spinach. For hundreds of years, purslane has been used in food as salads and in medicine as a medicinal plant. Additionally, purslane is a main source of glutathione; vitamin A and C, B-complex, and β-carotene among other phenolic compounds. This herb also contains several minerals, including iron; calcium, phosphorus, potassium and magnesium (Uddin *et al*., 2014).

Swiss chard (Beta vulgaris L. subsp. *cicla*) is a widely spread herb all over the world, and is used as a green vegetable (Liu *et al*., 2013). Chard is ranked among the ten main powerful vegetables, and its antioxidant capacity is attributed to its high phenolic contents (Kähkönen *et al*., 1999). Besides, it is a chief source of flavonoid glycosides derivative of apigenin; known as vitexin (Gennari *et al*., 2011), vitamin C, and essential minerals such as Na, Ca, and Mg (Pokluda and Kuben, 2002). Furthermore, chard is also a great source of fatty acids; mainly linoleic acid followed by oleic acid (Ninfali and Angelino, 2013).

Plants have developed some operative mechanisms to recognize, translocate, and respond to a wide range of biotic and abiotic signals; through controlling the cytosolic calcium level (Reddy *et al*., 2011; Sobhy *et al*., 2023), which plays a main role in plant growth, development, and response to the various environmental stresses (Ahmad *et al*., 2015). Calcium (Ca^{+2}) is a versatile intracellular secondary messenger that operates in various signaling pathways, which starts a wide range of responses involved in growth, defense, and tolerance to biotic and abiotic stresses. The endogenous and exogenous signals stimulate elevation of the cytoplasmic Ca, which is

accountable for the suitable downstream responses (Johnson *et al*., 2014).

The role of plant phytohormones in ameliorating the opposing effects of the abiotic and\ or biotic stresses on plants has been widely described (Uçarli, 2021). Among the plant hormones; salicylic acid (SA), which functions as a signaling and regulatory molecule during plant responses to the various environmental stresses; via the SA-mediated control of the metabolic and molecular processes (Khan *et al*., 2015; Liu *et al*., 2016). Furthermore, exogenous application of SA stimulates a range of diverse processes in plants, including seed germination; stomatal closure, mineral uptake and transport, membrane permeability, photosynthesis, and whole plant growth (Aftab *et al*., 2011).

Wheat (*Triticum aestivum* L.) is used as a main principal food in several countries worldwide (Mălinas *et al*., 2022). In addition to being a chief source of proteins, carbohydrates, and lipids; wheat grain is a main component of other health- related ingredients, including phytochemicals; vitamins, antioxidants, macro- and micro-nutrients (Arzani, 2019), and is also a valued source of vitamin E (Falk and Munné-Bosch, 2010).

Therefore, the objectives of the current study were to estimate the toxic effects of Pb stress and *F. graminearum* invasion on wheat plant at the seedling and yield stages, and to evaluate the effects of wheat grain priming using either chemical stimulants (*i.e*., Calcium and Salicylic acid) or natural weed extracts (*i.e*., purslane and chard) in alleviating the deleterious effects of both stressors on the wheat growth rate; photosynthetic indices, antioxidant status, and gene expression response of the wheat seedlings.

2. Materials and methods

2.1. Plant material

Grains of wheat (*Triticum aestivum* L.) cv. (Masr 1) were obtained from the Agricultural Research Center (ARC), Egypt, and were selected carefully for a uniform size and shape.

2.2. Preparation of weed extracts

Purslane and chard weeds were gathered from several local agricultural fields near Tanta city, Egypt, washed several times with dist. water, and finally with deionized H_2O. The leaves were separated; air dried in open air, and a known leaf weight of each weed was extracted in dist. water (1:4 w/v) to get 25 % weed extracts, according to preliminary experiments (Sobhy *et al*., 2021). Parallel to weed extract application, aliquots of each extract were vacuum-dried under reduced pressure using a rotary evaporator (FOUR E'S, USA) till full dryness. The obtained crystalline phytochemicals were re-dissolved in aqueous methanol (20: 80 v/v), and preserved at 4°C till further use in the following quantitative estimation procedures.

2.3. Phytochemical screening analysis of the weeds extracts

A number of important compounds and elements were detected in each weed extract, including flavonoids; phenolics, and ascorbate, which were evaluated according to the methods conducted by Jindali and Singh, (1975); Oser, (1979); Chang *et al*., (2002). Saponin was assessed quantitatively by the method designated by Hiai *et al*., (1975), while tannins content was determined referring to Broadhurst and Jones, (1978). The nitrogen (N) content was estimated according to the previous methods conducted by Tetlow and Wilson, (1964), whereas phosphorous (P) was determined spectrophotometerically using the molybdenum blue method reported by Allen *et al*., (1974). Endogenous calcium (Ca) and potassium (K) were quantified according to Allen *et al*., (1974). Moreover, proline; glycine, betaine, and total amino acids were assessed according to the previous methods reported by Lee and Takahashi, (1966); Bates *et al*., (1973); Grieve and Grattan, (1983). The antioxidant capacities of both weed extracts were evaluated using the 2,2 Diphenyl-1-picrylhydrazyl (DPPH) and

Phosphomolybdate (PMA) assays, as described previously by Brand-Williams *et al*., (1995); Bondet *et al*., (1997); Jayaprakasha *et al*., (2006).

2.4. Preparation of *F. graminearum* inoculum

Pathogenic *F. graminearum* isolate was provided by the Mycological Center, Faculty of Science, Assiut University, Egypt. Soil inoculation with the fungal isolate (*F. graminearum*) was carried out according to Porter and Merriman, (1983). *F. graminearum* was grown on potato dextrose agar (PDA) medium at 28°C for 7 d until the sporulation had completed. For soil treatment, sand-corn meal medium was used with some modifications. The sand-corn meal medium composed of 200 g of sand, 100 g of corn meal, and 100 ml of deionized H_2O in a 500 ml Erlenmeyer flask, and then autoclaved for 20 min. After cooling, five agar discs cut from the 7 d growing *F. graminearum* culture using a sterile cork borer were inoculated aseptically into the pre-sterilized sand-corn meal and incubated for 3 weeks at 28°C. Nearly five grams of the medium were added two days before sowing of the primed wheat grains to the soil.

2.5. Seed treatments and wheat growth conditions in the greenhouse

Based on preliminary experiments conducted by Sobhy *et al*., (2019); Sobhy *et al*., (2021), a pre-soaking period of wheat grains for 12 h individually in 25 % (w/v) of both weeds extracts, 5 mM $CaCl_2$, and 0.05 mM SA, were selected for the consequent experiments. During the season of plant growth (November, 2017), wheat grains (cv. Masr 1) were disinfected using commercial Clorox (1:1 v/v) for 5 min., washed with de-ionized H_2O many times, and then immersed in sterile dist. water. The disinfected grains were separated into five main groups; the first group was soaked in dist. water for 12 h and was considered as control. The second and third groups of grains were soaked in 25 % aqueous purslane extract and in 25% aqueous chard extract; respectively, as natural weed extracts. The fourth group was soaked in 5 mM $CaCl_2$ solution, while the fifth group was primed in 0.05 mM salicylic acid solution. After 12 h of priming, the soaking solutions were drained out and the grains were washed again with sterile dist. water. In the greenhouse, the grains of each group were individually sown in plastic pots (20 cm in diameter × 20 cm depth) containing sandy soil (3 pots for each treatment), which were pre-washed with 50 % HCl and then washed 3 times with dist. water, and then were further divided into three sub-groups as follows:

Sub-group A: This sub-group was irrigated with dist. water and considered as control for the previously mentioned soaking solutions.

Subgroup B: The sown grains were watered with 100 mM $Pb(CH_3COO)_2$ (lead acetate) solution (after 6 d from sowing), and were considered as abiotic stress treatment.

Sub-group C: The grains were sown in soil pre-inoculated with *F. graminearum*, as described previously.

For all experiments, the pots were watered with tap water and left to grow under controlled conditions (12:12 day/night, 25/15°C± 2, and nearly 70 % relative humidity) in the greenhouse for 60 d till the sampling time. All treatments were represented in triplicates, and the experimental design was completely randomized. Finally, analyses of the growth parameters of wheat plant were carried out.

2.6. Plant analyses

2.6.1. Plant growth parameters

Treated wheat seedlings were harvested after 21 d from sowing and the leaf area was estimated. The other harvested seedlings were also separated into roots and shoots. Shoots were weighed as fresh weight and dry weight after incubation at 60°C till a constant weight. The water content of the collected shoots was then calculated according to Goering and Van Soest, (1970) as follows:

Shoot water content = [(fresh weight - dry weight) / fresh weight] × 100

2.6.2. Estimation of the photosynthetic chlorophyll indices

The harvested fresh leaf samples were used to determine the chlorophyll contents (a and b) and the carotenoids contents according to Metzner *et al*., (1965). The obtained results were used to estimate the values of chl a\b and chl a+b\carotenoids, in addition to the total pigments.

2.6.3. Antioxidants capacity

2.6.3.1. DPPH* radical scavenging activity

Slightly modified previous methodologies that were conducted by Brand-Williams *et al*., (1995); Bondet *et al*., (1997) were followed to estimate the antioxidant capacity. The dried shoots (1 g) of plant tissue were extracted in 10 ml (95 %) ethanol. An aliquot of 0.1 ml of shoot sample extract was added to 3.9 ml of a DPPH (2, 2 Diphenyl -1- picrylhydrazyl) solution (0.03 g\ l in methanol). The mixture was shaken vigorously and then left in darkness at room temperature for 1 h. The decrease in absorbance was checked at 517 nm using a spectrophotometer (Bio-Rad model 3550, Germany) after 1 h of starting the reaction against a blank. The percentage (%) of scavenged DPPH was determined using the following equation:

$$\text{DPPH}^{*}\text{scavenging (\%)} = \frac{Ao-As}{Ao}\ x\ 100$$

Where; Ao: is the absorbance of the blank, while As: is the absorbance of the sample measured at 517 nm

2.6.3.2. Phosphomolybdate (PMA) assay

The total antioxidant capacity was determined using the phosphomolybdate assay that was reported by Jayaprakasha *et al*., (2006). An aliquot of 0.1 ml of the plant shoot ethanolic extract (1g of dry tissue\10 ml) was combined with 3 ml of phosphomolybdate mixture; composed of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The samples were placed in a boiling water bath at 95 °C for 90 min., and then cooled to room temperature. Finally, the sample absorbance was evaluated at 695 nm against the blank using a spectrophotometer (Bio-Rad model 3550, Germany). The total antioxidant capacity was expressed as mg equivalents\ g.

2.6.3.3. Phenylalanine ammonia lyase (PAL) activity

Phenylalanine ammonia lyase (PAL) enzyme activity was quantified at 290 nm following the previous method conducted by Zucker, (1965); Rosler *et al*., (1997). About 1 ml of the assay mixture reaction comprised 15 mM phenylalanine, 30 mM sodium borate buffer (pH 8.8), and 200 µl of plant extract. The PAL enzyme activity was expressed in mM/g FM\ min.; where FM refers to the frequency modulation.

2.7. Determination of the mineral contents

2.7.1. Estimation of the endogenous calcium and potassium contents

The mixed acid-digestion assay was used for endogenous Ca and K element contents determination according to Allen *et al*., (1974). About 1.5 g of oven dried plant samples (*i.e*., root, shoot, and grains) was mixed with 2 ml of 30 % perchloric acid, and 4 ml of conc. H_2SO_4. The mixture was heated gently until sulphuric acid fumes disappeared and the complete mixture had turned into a clear solution without charring. The solution was then diluted to a constant volume (50 ml) with dist. water. This extract was used to determine of the mineral contents (i.e. potassium and calcium) using an atomic absorption flame emission spectrophotometer (Model Perkin Elmer 2380, Canada).

2.7.2. Estimation of nitrogen content

The nitrogen content of treated wheat plants was assessed according to the method adopted by Tetlow and Wilson, (1964). The dried plant tissue was digested in nitric acid and hydrogen peroxide, and then the digested samples were titrated against 0.6 N NaOH using a few drops of phenolphthalein as an indicator until a pink color appeared. Thereafter, 1 ml of phenol-

sodium nitroprusside reagent was added and mixed, followed by adding 1 ml of sodium hydroxide-sodium hypochlorite reagent. The mixture was incubated at 37º C for 15 min., diluted to 10 ml, and the absorbance was measured at 630 nm using a spectrophotometer (Bio-Rad model 3550, Germany) against a blank. Nitrogen concentration in the samples was expressed as mg\g DM; where DM refers to dry mass of the plant tissue.

2.7.3. Phosphorous estimation

Phosphorous (P) content was determined spectrophotometerically by the molybdenum blue method of Allen *et al*., (1974). About 1 ml of the digested samples was titrated against 8 N NaOH solution using a phenolphthalein indicator until the pink color appeared, followed by the addition of 1 ml of ammonium molybdate mixture (composed of 25 g ammonium-molybdate dissolved in 400 ml of dist. water + 280 ml of conc. H_2SO_4). Thereafter, 1 ml of stannous chloride reagent (0.5 g stannous chloride that was dissolved in 250 ml 2 % HCl) was added, diluted to 10 ml, and then left for 30 min. The absorbance was measured against a blank at 700 nm. The content of phosphorus was determined as mg\ g DM using a calibration curve of standard phosphorus (*i.e*., KH_2PO_4) solutions.

2.8. Quantitative assessment of total soluble proteins and carbohydrates

2.8.1. Preparation of the treated wheat grains extract

The grain extract used for estimation of total soluble proteins and carbohydrates extraction was prepared following the method designated by Naguib *et al*., (1968). Approximately 0.1 g of the treated wheat grain fine powder was added to 5 ml of borate buffer (composed of 28.63 g boric acid + 29.8 g potassium chloride + 3.5 g sodium hydroxide in 1 l of dist. water, at pH 8.0), kept standing at 4°C for 24 h, and then centrifuged for 15 min. at 3000 rpm. The supernatant obtained after filtration using a Whatman filter paper (1) was completed to a known volume and used for estimation of the total soluble proteins and the total soluble carbohydrates.

2.8.2. Determination of the total soluble proteins

The total soluble proteins content of the treated wheat grains was determined as described previously by Bradford, (1976). Exactly 0.1 ml of borate buffer extract was well mixed with 3 ml Commasie Brillient Blue (CBB) reagent (100 mg of CBB was dissolved in 50 ml of 95 % ethanol + 100 ml of 85 % phosphoric acid, and then completed to 1 l with dist. water). After 2 min., the absorbance was measured at 595 nm. The concentration of total soluble proteins was estimated at mg/ g DM.

2.8.3. Determination of total soluble carbohydrates

The phenol sulfuric acid method had been used to evaluate the total soluble carbohydrates content of the treated wheat grains following the method reported by Dubois *et al*., (1956). About 0.1 ml of borate buffer was pipetted into a test tube containing 1ml of 5 % phenol and 5 ml of conc. H_2SO_4, and placed in a water bath at 30 ºC for 20 min. The absorption of the resulting color was measured at 490 nm.

2.9. Quantifying the changes in gene regulation in the treated wheat seedlings

2.9.1. Total RNA extraction and cDNA synthesis

Total RNA from the control and the treated wheat seedlings was extracted using the RNeasy Mini Kit (QIAGEN, Germany), according to the manufacturer's instructions. Complementary DNA (cDNA) was attained by reverse transcribing RNA, and the reaction components and conditions were conducted as previously described by Aseel *et al*., (2019a); Sobhy *et al*., (2021); Aseel *et al*., (2022).

2.9.2. Differential display polymerase chain reaction (dd-PCR)

The differential display polymerase chain reaction (dd-PCR) was carried out using four different primers listed in Table (1) for 45 cycles; where the mixture and

Table 1.The sequence of primers used in DD-PCR assay

No.	Primer	Gene name	Direction	Sequences 5 - 3
1	PR4	Endogluconase	Forward	GACCTGAATGCGGTCGTCAAGG
2	PR4		Reverse	AGCATGTTTCTGGAATCAGGCTG
3	PR3	Chitinase	Reverse	CGGCGCCACGGTCGGCGTCTGA
4	PR5	Thaumatine-like protein	Forward	ATGGGCTACTTGACATCTTCTT

conditions were conducted according to Rashad *et al*., (2018); Aseel *et al*., (2019b). The products of dd-PCR were separated using 2 % agarose gel electrophoresis with a DNA marker, and then photographed using a gel documentation system (Shaikhaldein *et al*., 2018; Aseel *et al*., 2019b).

2.10. Statistical analysis

The obtained results were statistically analyzed using One way analysis of variance (ANOVA), to determine the degree of significances of the obtained variations for the applied treatments. All statistical methods applied in this study were conducted according to Bishop, (1983), while the analysis was carried out by Costat under Windows software. The data were expressed as the means of three replicates ± standard deviation (SD).

3. Results

3.1. Chemical composition of the weed plant extracts

The results presented in Table (2) revealed that both weed extracts contained high levels of antioxidant compounds; with the prevalence of high ascorbate content (46.6 mg/g DM) in purslane and a high phenolic content (51 mg /g DM) in chard. Purslane was considered as a high source of saponins (39 mg/g DM), while tannins were present in a higher quantity in chard (25 mg/g DM). In addition, high levels of P and K were recorded in the purslane extract (6.5 and 32 mg/g DM, respectively), whereas the chard extract contained a higher values of N and Ca (22 and 38 mg/g DM, respectively). Regarding the osmoprotectants, glycine betaine (GB) was present in purslane with a remarkable amount of 20 mg/g DM; however, the total amino acids content was higher in chard (56 mg/g DM). Moreover, the total recorded antioxidant activities, including PMA (19 and 7 %), while the DPPH scavenging activities recorded 12 and 17 % scavenging of purslane and chard extracts, respectively.

3.2. Plant growth parameters

The data revealed that both Pb and *F. graminearum* treatments displayed a noteworthy reduction in the leaf area ($p< 0.05$), where the percentage of decrease was 21 % and 12.5 %l respectively, relative to the control treatment as shown in Fig. (1).

Moreover, seed priming in purslane and chard extracts or exogenous application of Ca and SA solutions showed a substantial increase in the leaf area relative to the stress treatments. SA was the most powerful treatment towards leaf area as it recorded an increment of 21 %, compared to Pb treatment. Nonetheless, the most remarkable effect of *F. graminearum* treatment was recorded through an increase in chard solution by 21 %, compared to the control treatment inoculated with *F. graminearum* only.

Regarding the shoot water content, results presented in Fig (1) showed that Pb and *F. graminearum* treatments revealed a highly significant decrease in the shoot water content, recording 47 % and 58 %; respectively, compared to the control.

Table 2.The phytochemical screening of purslane and chard weed extracts

Category	Tested parameters	Purslane extract	Chard extract	Measuring unit
Antioxidant	Flavonoids	48.0	47.0	
	Phenols	13.7	51.0	
	Ascorbic acid	46.6	20.8	
Secondary metabolites	Saponin	39.0	24.0	mg/g DM
	Tannins	12.5	25.0	
Minerals	Nitrogen	17.0	22.0	
	Phosphorus	6.5	4.8	
	Potassium	32.0	21.0	
	Calcium	30.4	38.0	
Osmo-protectants	Proline	6.3	4.1	
	Glycine betaine (GB)	20.0	7.0	
	Total amino acids	34.0	56.0	
Antioxidant activity	Total antioxidant activity (PMA)	19.0	7.0	
	DPPH scavenging activity	12.0	17.0	%

Where; DM is the dry mass of the used weeds, % is the percentage of scavenging activity

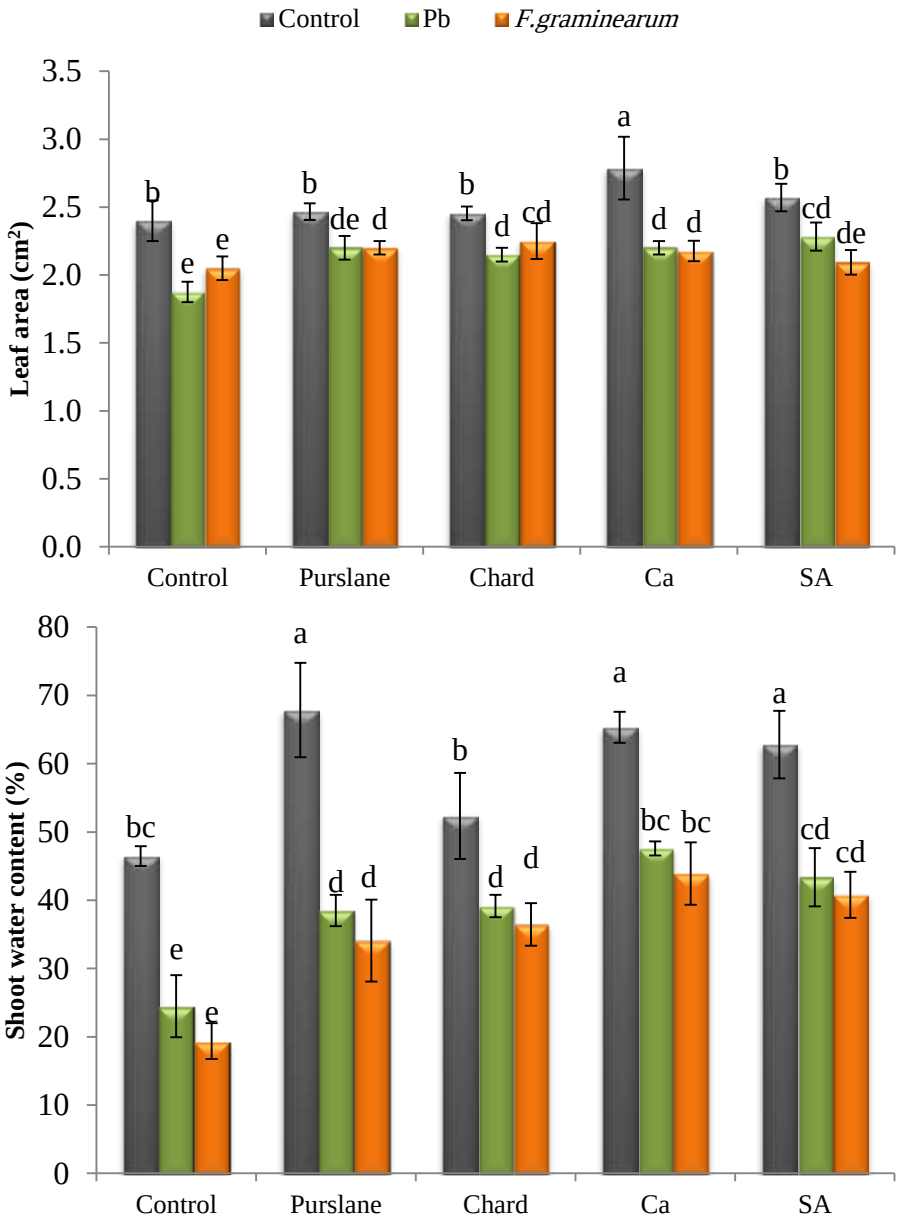

Fig.1: Effect of grain priming in weeds extract (25 % purslane and chard) and growth promoting chemicals (5 mM Ca and 0.05 mM SA) on leaf area (A) and shoot water content (B) of wheat seedlings under 100 mM Pb-stress and *F. graminearum* inoculation. Where; Presented data are means of three replicates ± SD (Standard deviation). Different superscript letters reveal statistically significant variations ($p< 0.05$)

Meanwhile, the priming treatments had enhanced the shoot water content of the wheat seedlings, where Ca was the most powerful priming treatment in the case of both stresses (*i.e*., Pb and *F. graminearum*); recording an increase in the shoot water content by 94 % and 126 %, respectively.

3.3. Estimation of the photosynthetic chlorophyll indices

The effects of seed pre-soaking in weed extracts (*i.e*., purslane and chard) and\or in growth promoting chemicals (i.e., Ca and SA) on the photosynthetic parameters of Pb and *F. graminearum* -stressed wheat seedlings were studied. The data revealed that Pb treatment caused a substantial reduction in the total photosynthetic pigments as well as Chl (a+b)/carotenoids ratio; where the percentages of decrease were 21 % and 38 %; respectively, relative to the control treatment. On the other hand; as shown in Table (3), the Chl a/b ratio expressed a noteworthy improvement with Pb stress treatment, which was represented by a 16 % increase, compared with the control.

Regarding *F. graminearum* treatment, the results revealed that such treatment caused a major decline in the total photosynthetic pigments as well as Chl (a+ b)/carotenoids ratio, recording percentage of 18 % and 34%; respectively, relative to the non-infected control treatment. While the Chl a/b ratio showed a substantial increase of 15 %.

The wheat seed priming treatments using purslane and chard extracts and\or Ca and SA solutions treatments, improved the observed Pb and *F. graminearum*-induced reductions in the photosynthetic parameters. When compared with the two stress treatments, seed priming in purslane extract resulted in the most noticeable increases in the total pigments as well as the Chl (a+b)/ carotenoids ratio. Interestingly, the best values recorded for Chl a/b ratio belong to the combined stress treatments and purslane extract, where their values were 2.6 % in Pb and 2.5 % in *F. graminearum* treatments, which were close to their control counterparts.

3.4. Antioxidant potential

The results presented in Fig. (2) showed that Pb stress led to a noticeable increase in PMA and DPPH activities of the plant extract recording 139 % and 58.5 %; respectively, and a highly significant increase in PAL activity (182 %). Meanwhile, *F. graminearum* infection increased the percentage of PMA and DPPH to reach 95 % and 66 %; respectively, while the PAL activity was increased to reach 173 %, relative to the control treatment.

All presoaking treatments decreased PMA, DPPH, and PAL activities in the treated wheat seedlings to values close to those of the controls. The most operative treatment in decreasing PMA, DPPH, PAL activities was for Ca solution, recording reductions by 42, 25, and 58 %; respectively, compared with Pb stress treatment.

On the other hand, soaking treatments offset the deleterious effects imposed by *F. graminearum* inoculation, where Ca solution achieved the best ameliorative effect as it decreased the PAL activity by 41 %, compared to the inoculated treatment. The purslane extract was the best soaking treatment regarding DPPH content, with a decreased percentage that reached 30 %, compared with the *F. graminearum* treatment.

The natural plant extracts (i.e., purslane and chard) side by side with the chemical solutions (Ca and SA) produced all the same ameliorative impacts on PAL activity recording an amelioration percentage of 60 %, compared with *F. graminearum* treated wheat seedlings.

3.5. Wheat shoots, roots, and grains mineral contents

The results presented in Table (4) demonstrate the effects of wheat seeds presoaking in weed extracts

Table 3. Changes in photosynthetic parameters (i.e., total pigments, Chl a/b ratio and Chl (a+b)/carotenoids) of wheat seedlings primed in weed extracts (*i.e*., 25 % purslane and chard), or signaling molecules (5 mM Ca and 0.05 mM SA), under 100 mM Pb stress or *F. graminearum* inoculation

Treatments	Total pigments	Chl a/b	Chl (a+b)/ carotenoids
	(mg/g DM)	%	
Control	8.6± 0.13^{c}	2.8± 0.08^{cd}	6.9± 0.15^{a}
Pb	6.8± 0.01^{e}	3.3± 0.13^{a}	4.3± 0.09^{c}
F. graminearum	7.0± 0.02^{e}	3.2± 0.21^{a}	4.6± 0.28^{c}
Purslane	8.6± 0.10^{c}	2.8± 0.16^{ef}	6.9± 0.26^{a}
Purslane+Pb	7.7± 0.06^{d}	2.6± 0.09^{f}	5.9± 0.24^{b}
Purslane+*F. graminearum*	7.7± 0.10^{d}	2.5± 0.05^{f}	5.9± 0.07^{b}
Chard	8.8± 0.15^{b}	2.8± 0.09^{bcd}	6.9± 0.12^{a}
Chard+Pb	7.7± 0.19^{d}	2.7± 0.18^{ef}	5.6± 0.09^{b}
Chard+*F. graminearum*	7.6± 0.09^{d}	2.6± 0.16^{ef}	5.8± 0.09^{b}
Ca	9.0± 0.15^{a}	3.0± 0.11^{bc}	6.7± 0.12^{a}
Ca+Pb	7.7± 0.13^{d}	2.6± 0.03^{ef}	5.9± 0.07^{b}
Ca+*F. graminearum*	7.6± 0.04^{d}	2.5± 0.09^{f}	5.8± 0.03^{b}
SA	9.0± 0.44^{a}	3.0± 0.56^{b}	6.8± 0.39^{a}
SA+Pb	7.6± 0.34^{d}	2.6± 0.23^{def}	5.8± 0.18^{b}
SA+*F. graminearum*	7.6± 0.14^{d}	2.6± 0.38^{def}	5.8± 0.14^{b}

Where; Presented data are means of three replicates ± SD (Standard deviation). Different superscript letters reveal statistically significant variations ($p< 0.05$), DM: Dry mass

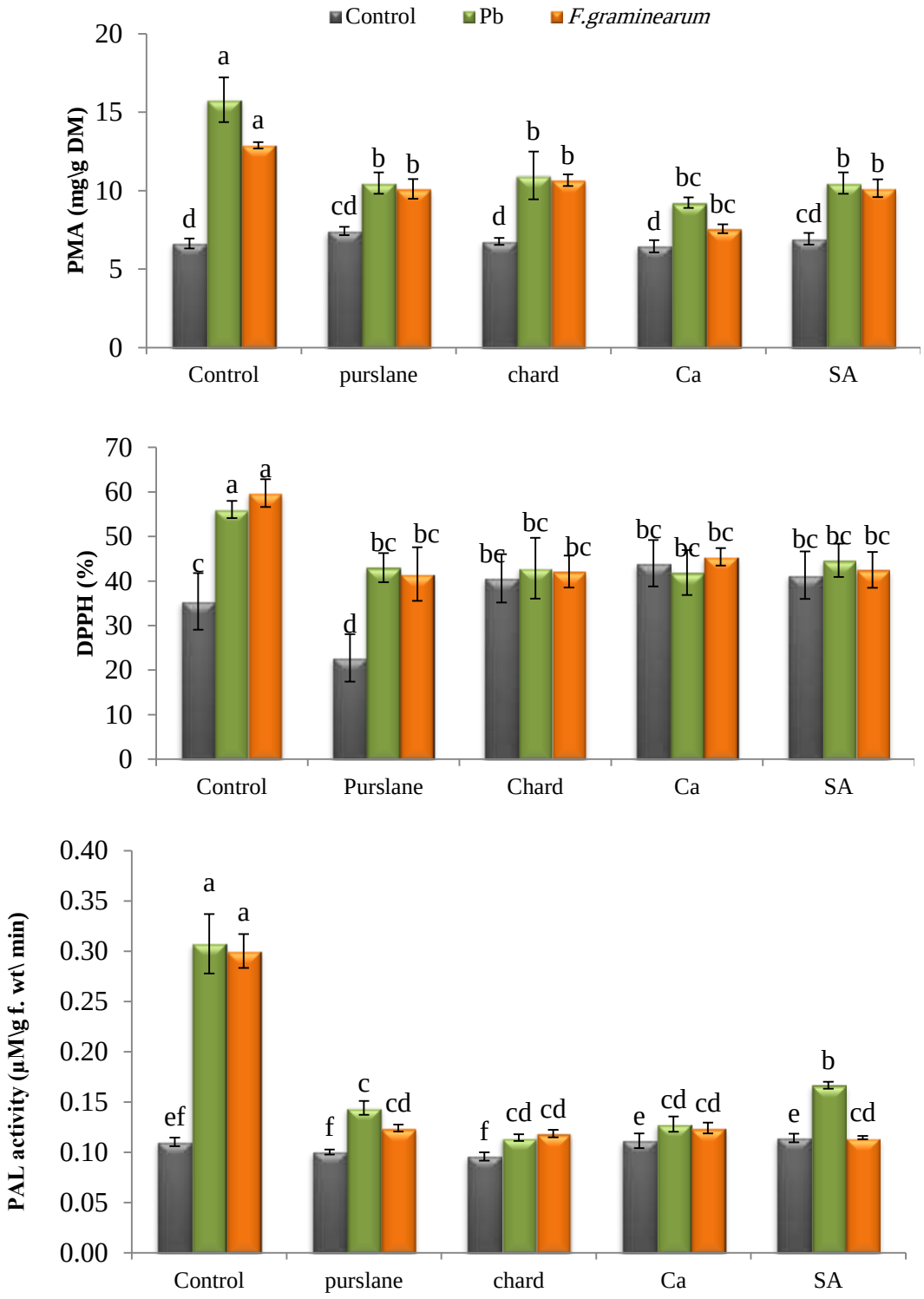

Fig. 2: Changes in antioxidant activities of phosphomolybdate assay (PMA), diphenylpicryl hydrazine (DPPH), and phenylalanine ammonia lyase (PAL) of wheat seedlings primed in weed extracts (25 % purslane and chard), or signaling molecules (5 mM Ca and 0.05 mM SA) under 100 mM Pb stress or *F. graminearum* inoculation. Where; Presented data are means of three replicates ± SD (Standard deviation). Different superscript letters reveal statistically significant variations (p< 0.05), DM: Dry mass

Table 4. Effect of grain priming in weeds extract (i.e., 25 % purslane and chard) and growth promoting chemicals (*i.e*., 5 mM Ca and 0.05 mM SA) on N, P, K, and Ca contents of wheat seedlings shoot and root; following 100 mM Pb stress and *F. graminearum* inoculation

Treatments	K^+	Ca^{2+}	N^{3+}	P^{3+}
	Shoot (mg/g DM)			
Control	30.8± 0.3^{b}	15.0±1.1^{cd}	13.3±1.2^{a}	5.5±0.9^{a}
Pb	20.8± 1.0^{f}	10.0± 1.8^{e}	9.6± 0.5^{d}	3.2± 0.8^{e}
F. graminearum	17.1± 0.0^{f}	11.2± 0.8^{e}	11.0± 0.6^{e}	3.7± 0.3^{e}
Purslane	31.7± 0.6^{b}	17.8± 1.5^{a}	13.2± 0.3^{a}	5.8± 0.3^{a}
Purslane+Pb	28.5± 0.5^{c}	13.9± 1.2^{d}	12.8± 0.3^{ab}	4.4± 0.3^{cd}
Purslane+*F. graminearum*	24.1± 0.3^{e}	14.3± 0.9^{cd}	11.6± 0.2^{de}	4.0± 0.2^{e}
Chard	33.8± 0.8^{a}	17.2± 1.1^{ab}	13.2± 0.3^{a}	5.5± 0.7^{a}
Chard+Pb	25.2± 0.2^{e}	13.9± 0.6^{cd}	12.7± 0.2^{be}	4.1± 0.1^{d}
Chard+*F. graminearum*	22.4± 0.5^{e}	12.7± 0.3^{e}	12.1± 0.2^{cd}	3.7± 0.3^{e}
Ca	28.3± 0.3^{c}	17.4± 0.7^{ab}	13.6± 0.5^{a}	4.8± 0.3^{bc}
Ca+Pb	24.4± 0.2^{e}	14.5± 0.5^{cd}	12.7± 0.2^{bc}	4.2± 0.3^{d}
Ca+*F. graminearum*	25.1± 0.3^{e}	13.1± 0.2^{cd}	12.4± 0.6^{cd}	3.7± 0.2^{e}
SA	28.2± 0.3^{c}	15.8± 0.9^{bc}	13.6± 0.6^{a}	5.4± 0.5^{a}
SA+Pb	26.5± 0.5^{d}	13.8± 0.6^{d}	12.3± 0.2^{c}	5.1± 0.1^{ab}
SA+*F. graminearum*	24.0± 0.1^{e}	12.4± 0.5^{e}	12.8± 0.2^{bc}	4.5± 0.2^{cd}
	Root (mg/g DM)			
Control	22.5± 0.5^{b}	4.1± 0.1^{c}	14.4± 1.0^{a}	5.2± 0.9^{a}
Pb	17.4± 0.4^{e}	2.3± 0.2^{g}	10.8± 0.3^{e}	3.0± 0.1^{c}
F. graminearum	18.0± 1.0^{e}	2.9± 0.2^{g}	9.8± 0.4^{d}	2.7± 0.3^{b}
Purslane	23.7± 0.8^{a}	4.5± 0.2^{b}	14.1± 0.5^{a}	5.6± 0.4^{a}
Purslane+Pb	18.3± 0.3^{e}	3.2± 0.0^{f}	11.7± 0.6^{d}	3.9± 0.3^{b}
Purslane+*F. graminearum*	18.9± 0.3^{d}	3.4± 0.2^{ef}	10.4± 0.8^{d}	3.2± 0.3^{b}
Chard	22.1± 0.3^{b}	5.2± 0.2^{a}	13.8± 0.4^{a}	5.0± 0.4^{a}
Chard+Pb	19.4± 0.5^{d}	3.5± 0.1^{e}	12.3± 0.6^{cd}	2.9± 0.1^{c}
Chard+*F. graminearum*	20.3± 0.2^{c}	3.7± 0.1^{d}	11.9± 0.3^{bc}	2.8± 0.3^{b}

Ca	24.3± 0.0^{a}	4.3± 0.3^{b}	13.8± 0.4^{a}	5.5± 0.6^{a}
Ca+Pb	20.5± 0.3^{c}	3.9± 0.2^{cd}	12.1± 0.2^{cd}	4.0± 0.2^{b}
Ca+*F. graminearum*	20.2± 0.1^{c}	3.5± 0.2^{de}	11.5± 0.1^{c}	3.4± 0.2^{b}
SA	22.4± 0.4^{b}	4.0± 0.1^{c}	14.1± 0.5^{a}	5.2± 0.3^{a}
SA+Pb	19.6± 0.6^{d}	3.7± 0.1^{de}	12.9± 0.4^{bc}	3.0± 0.1^{c}
SA+*F. graminearum*	20.3± 0.1^{c}	3.1± 0.1^{f}	12.6± 0.5^{b}	3.3± 0.5^{b}

Where; *Presented data are means of three replicates ± SD (Standard deviation). Different superscript letters reveal statistically significant variations ($p< 0.05$), DM: Dry mass

(purslane and chard) and\or the growth promoting chemicals (i.e., Ca and SA) on the shoot mineral contents (N, P, K, and Ca) of Pb and *F. graminearum* - stressed wheat seedlings. It was obvious from the obtained results that compared with the control plants contents, application of Pb caused a marked decrease in N, P, K, and Ca ions contents recording 9.6, 3.2, 20.8, and 10 mg/g DM; respectively, in the shoot, and 10.8, 3, 17.4, and 2.3 mg/g DM; respectively, in the root of treated wheat seedlings. All presoaking treatments counteracted the Pb effects on the shoot and root mineral contents by increasing the N, P, K, and Ca ion contents. With regard to purslane, chard, Ca, and SA treatments of the shoot, the N content increased to 12.8, 12.7, 12.7, and 13.8 mg/g DM compared to Pb treatment (9.6 mg/g DM), while the P content increased to 4.4, 4.1, 4.2, and 5.1 mg/g DMM, compared to 3.2 mg/g DM in Pb treatment. The increase in K was 28.5, 25.2, 24.4 and 26.5 mg/g DM when compared to 20.8 mg/g DM in the Pb treatment. Finally, the increase in Ca content was 13.9, 13.9, 14.5 and 13.8 mg/g DM, compared with of Pb treatment (10 mg/g DM). In the root tissue, with regard to the treatments with purslane, chard, Ca, and SA, the N content had an increase by 11.7, 12.3, 12.1 and 12.9 mg/g DM compared to 10.8 mg/g DM in Pb treatment, while the content of P recorded an increase by 3.9, 2.9, 4.0 and 3.0 mg/g DM, compared to Pb treatment (3.0 mg/g DM). The recorded increase in K content was 18.3, 19.4, 20.5 and 19.6, compared to 17.4 mg/g DM of Pb treatment, while the Ca content had an increase by 3.2, 3.5, 3.9 and 3.7 mg/ g DM, compared to of Pb treatment (.2.3 mg/g DM). Purslane extract was the most effective treatment in increasing N content (33 %) in wheat shoot, while SA extract was the most effective in increasing N content of the root (19 %), compared to Pb treatment. Moreover, SA solution was the most effective priming solution treatment with regard to P content in the shoot (59 %), while Ca was the best treatment in the root (33 %), compared to Pb-stressed treatment (3.2 and 3.0 mg/g DM in the shoot and root, respectively), where $p< 0.05$.

In addition to the increase in N and P contents, soaking treatments altered the concentrations of K and Ca in the shoot and root of wheat seedlings. In the shoot, priming with purslane and exogenous application of Ca were the most effective treatments with regard to K and Ca contents, recording 28.5 mg/g DM and 14.5 mg/g DM, respectively. In the root, exogenous Ca application represented the best ameliorative treatment on both of K and Ca contents (20.5 and 3.9 mg/g DM, respectively), compared to the stress treatment. *F. graminearum* inoculation resulted in a noteworthy decline in N, P, K and Ca ions endogenous contents of the shoots and roots of wheat seedlings; recording 17, 33, 44 and 25 % for the shoots; respectively, and 32, 48, 20 and 29 % for the roots; respectively, compared with the control contents of these ions ($p< 0.05$). The soaking treatments significantly reduced the deleterious effects imposed by *F. graminearum* inoculation and increased the

mineral contents, compared to the non-inoculated treatment. The content of N in both of the shoots and roots of wheat seedlings was effectively increased by priming in SA, recording 13.6 and 14.1 mg/g DM in shoot and root; respectively, compared with the *F. graminearum* treatment (11.0 and 9.8 mg/g DM in shoot and root, respectively). Moreover, SA and exogenous Ca increased the P content of wheat shoots and roots (recording 5.4, 5.5 mg/g DM, respectively), compared with the *F. graminearum* treatment (3.7 and 2.7 mg/g DM, respectively). In regard to K content, pre-soaking in Ca solution was more effective in increasing the shoot content of K (47 %), while priming in SA and chard extract resulted in the same effect on K content in the root and achieved the best value compared to inoculated treatment (13 %) ($p< 0.05$). Additionally, Ca content reached its greatest value with weed extracts pre-soaking, purslane in shoot (28 %) and chard in root (27 %), when compared to the pathogen inoculated wheat seedlings ($p< 0.05$). In regard to the grains mineral contents, the results presented in Table (5) revealed that 100 mM Pb treatment led to a reduction in the N, P, K and Ca contents in the wheat grains; recording decrease of 33, 47, 39, and 37 %; respectively, relative to the control. On the other side, the Pb-induced reductions in the mineral contents of wheat grains were recovered upon seed priming treatments with purslane and chard extracts, and\ or Ca and SA solutions. The most pronounced increase in K grain content was attributed to seed priming in chard extract, while the highest recorded values for P, N, and Ca belonged to the exogenous treatment with Ca. The data showed that *F. graminearum* treatment of wheat grains resulted in a noteworthy decline in N, P, K, and Ca ions contents; where the percentages of decrease were 38, 53, 36, and 39 %; respectively, compared to the non-infected control ($p< 0.05$). Nonetheless, soaking treatments resulted in an obvious increase in N, P, K, and Ca grains contents upon *F. graminearum* inoculation. SA solutions increased the N content (24 %) of wheat grains significantly. Additionally, Ca and SA solutions were the most effective priming treatments regarding P and K contents of wheat grains recording an increase by 35 % and 15 %; respectively, while chard extract had the best ameliorative effect with respect to the Ca content of wheat grains with an increase of 75 %, compared to *F. graminearum* inoculation treatments ($p< 0.05$).

3.6. Differential display PCR

Data analysis of dd-PCR using endogluconase (PR-4) forward primer had recorded 21 up-regulated genes in 7-d old seedlings; where about 21 fragments of up-regulated genes that ranged from 200 to 550 bp had been observed for the following treatments: Pb; *F. graminearum*, purslane, purslane+Pb, purslane+*F. graminearum*, chard, chard+Pb, chard+*F. graminearum*, Ca, Ca+Pb, and Ca+*F. graminearum*. Moreover, in 14 d-old samples; 34 turn-on and turn-off gene expressions were observed from 220 to 1300 bp. A 28 turn-on genes were recorded in the following treatments: Pb; *F. graminearum*, chard, chard+Pb, chard+*F. graminearum*, Ca, Ca+Pb, Ca+*F. graminearum*, SA, SA+Pb, and SA+*F. graminearum*, and 6 turn-off gene expressions in purslane, Ca+Pb and SA+Pb treatments that ranged from 220 to 350 bp. Furthermore, 21-d-old samples revealed 35 up regulated genes with range from 150 to 550 bp in Pb; *F. graminearum*, purslane, purslane+Pb, purslane+*F. graminearum*, chard, chard+Pb, chard+*F. graminearum*, Ca, Ca+Pb, Ca+*F. graminearum*, SA and SA+Pb treatments (Fig. 3A).

The results of dd-PCR using endogluconase (PR-4) reverse primer revealed 8 up-regulated genes in 7-d old seedlings, four fragments of up-regulated genes, one of which is approximately at 220 bp in purslane treatment and three high-density genes in chard+*F. graminearum* treatment. On the other side, 4 bands were detected as down-regulated genes with a molecular weight of 290 bp for (Ca+*F. graminearum*; SA, SA+Pb, and SA+*F. graminearum*). Additionally, in 14-d-old samples, 16 turn-on and turn-off genes were observed, with 8 up-regulated genes in the following treatments: chard; chard+Pb, Ca, Ca+Pb, Ca+*F. graminearum*, SA, SA+Pb, and SA+*F. graminearum*), with a size of about 700 bp.

Table 5. Effect of grain priming in weeds extract (i.e., 25% purslane and chard) and growth promoting chemicals (*i.e*., 5 mM Ca and 0.05 mM SA) on N, P, K, and Ca contents of wheat grains; following 100 mM Pb stress and *F. graminearum* inoculation

Treatments	Yielded Grains (mg/g DM)			
	K^{+}	Ca^{2+}	N^{3+}	P^{3+}
Control	6.3±0.3^{c}	1.7±0.1^{bc}	14.6±2.0^{a}	3.8±0.4^{ab}
Pb	4.3±0.3^{e}	0.9±0.1^{e}	8.9±0.4^{e}	2.4±0.4^{f}
F. graminearum	3.9±0.2^{e}	0.8±0.1^{e}	9.4±0.9^{f}	2.3±0.3^{d}
Purslane	6.9±0.4^{ab}	1.5±0.4^{cd}	13.8±1.4^{ab}	4.2±0.9^{a}
Purslane+Pb	4.9±0.3^{d}	1.2±0.0^{de}	10.9±0.4^{d}	3.1±0.1^{de}
Purslane+*F. graminearum*	4.3±0.1^{de}	1.3±0.1^{de}	10.2±0.3^{ef}	2.9±0.1^{cd}
Chard	7.4±0.5^{a}	2.1±0.1^{a}	12.9±1.2^{bc}	3.9±0.7^{ab}
Chard+Pb	5.2±0.2^{d}	1.1±0.2^{de}	10.6±0.4^{d}	2.7±0.2^{ef}
Chard+*F. graminearum*	4.5±0.3^{d}	1.4±0.2^{cd}	9.7±0.5^{ef}	2.6±0.2^{cd}
Ca	6.5±0.0^{bc}	2.4±0.2^{a}	14.0±0.4^{ab}	3.9±0.4^{ab}
Ca+Pb	5.0±0.4^{d}	1.6±0.4^{c}	11.9±0.2^{cd}	3.3±0.2^{bc}
Ca+*F. graminearum*	4.4±0.1^{d}	1.2±0.4^{de}	11.2±0.3^{de}	3.1±0.3^{bc}
SA	6.1±0.2^{c}	2.0±0.0^{ab}	13.1±0.8^{ab}	4.1±0.4^{a}
SA+Pb	4.7±0.0^{de}	1.4±0.3^{cd}	11.8±0.3^{cd}	3.1±0.2^{cd}
SA+*F. graminearum*	4.5±0.1^{d}	1.1±0.4^{de}	11.7±0.3^{cd}	3.1±0.2^{bc}

Where; *Presented data are means of three replicates ± SD (Standard deviation). Different superscript letters reveal statistically significant variations ($p< 0.05$), DM: Dry mass

Meanwhile, 8 turn-off genes in chard+*F. graminearum*; Ca, Ca+Pb, and Ca+*F. graminearum* that ranged approximately from 180 to 200 bp. In addition, 21-d-old samples investigated 13 up and down-regulated genes, containing 10 up-regulated genes in *F. graminearum*; purslane, purslane+*F. graminearum*, chard, chard+Pb, Ca, Ca+Pb, Ca+*F. graminearum*, SA and SA+*F. graminearum*. However, 3 down- regulated genes were observed in Pb treatment at nearly 220, 300 and 330 bp, as shown in Fig. (3B).

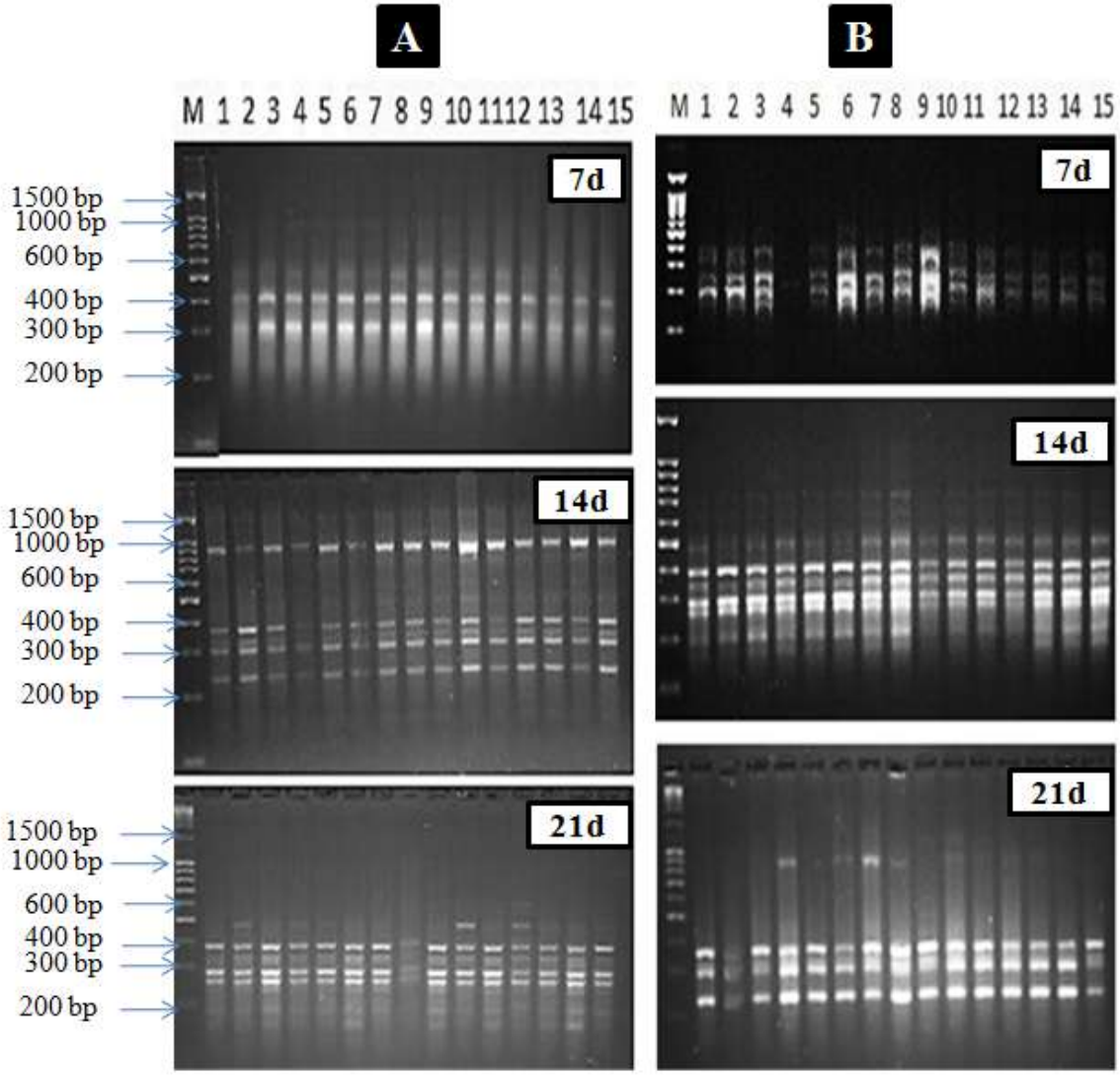

Fig. 3: Gene expression of differential display (DD-PCR) using different arbitrary defense primers; (A) Endogluconase (PR-4F), (B): Endogluconase (PR-4R). M: 1.5 Kbp DNA marker; 1: untreated (control); 2: Pb; 3: *F. graminearum*; 4: Purslane; 5: Purslane+Pb; 6: Purslane+*F. graminearum*; 7: Chard; 8: Chard+Pb, 9: Chard+*F. graminearum*; 10: Ca; 11: Ca+Pb; 12: Chard+*F. graminearum*; 13: SA; 14: SA+Pb; and 15: SA+*F. graminearum*

The results of Chitinase primer (PR-3) were displayed by dd-PCR (Fig. 4A) at 7, 14, and 21-d old wheat samples. The current data recorded 87 up and down-regulated genes in 7-d old samples. About 58 fragments of up-regulated genes that ranged from 150 to 1000 bp were detected for the following samples: Pb; *F. graminearum*, purslane, purslane+Pb, purslane+ *F. graminearum*, chard+Pb, chard+*F. graminearum*, Ca, Ca+Pb, Ca+*F. graminearum*, SA, SA+Pb, and SA+*F. graminearum*. Moreover, 29 bands were observed as down-regulated genes with molecular weights ranging from 190 to 250 bp for Pb; chard, chard+*F. graminearum*, Ca, Ca+Pb, Ca+*F. graminearum*, and SA+*F. graminearum*. Furthermore, in 14-d-old samples, 19 turn-on and turn-off genes were observed, with 7 turn-on genes in chard; chard+Pb, chard+*F. graminearum*, and Ca+Pb treatments, ranging approximately from 850 to 1000 bp. There were 12 down-regulated genes in the following treatments: purslane; purslane+*F. graminearum*, chard, and Ca+*F. graminearum*, with sizes of 220 and 330bp. Furthermore, in 21-d-old samples, investigated 25 up-regulated genes in the treatments of Pb; *F. graminearum*, purslane, purslane+Pb, purslane+*F. graminearum*, chard, chard+Pb, chard+*F. graminearum*, Ca, Ca+Pb, Ca+*F. graminearum*, SA, SA+Pb, and SA+*F. graminearum*, were detected with a range from 150 to 950 bp.

Thaumatine-like protein (PR-5) was used in the dd-PCR. Data revealed the existence of 10 up and down-regulated genes in 7-d old seedlings, and 2 fragments of up-regulated genes in Pb treatment at 380 to 580 bp. On the contrary, 6 bands were observed in down-regulate genes with a molecular weight ranged from 110 to 400 bp for Pb; *F. graminearum*, purslane+ *F. graminearum*, and SA. Besides, in 14-d-old samples, 3 turn-on and turn-off genes were observed, but only one band at 410 bp showed up-regulation in purslane+*F. graminearum* treatment. A set of 2 turn-off genes in purslane and purslane+Pb treatments was detected with a size of 450 bp. Additionally, in 21-d-old samples; 7 down-regulated genes in purslane+Pb, chard+*F. graminearum*, SA+Pb, and SA+*F. graminearum* treatments were recorded; with a size that ranged from 420 to 500bp (Fig. 4 B).

3.7. Total soluble carbohydrates and total soluble proteins

In the yielded wheat grains, the Pb treatment led to a highly significant increase in the total soluble carbohydrates (TSCs) and an extreme reduction in total soluble proteins (TSPs), where the percentages of change were 79 % and 28 %; respectively, relative to the control treatment (Fig. 5). On the other hand, the Pb-induced changes in the TSCs and TSPs were recovered upon seed priming treatments in purslane and chard extracts and\ or Ca and SA solutions. The most evident ameliorative effect was attributed to seed priming in Ca solution, where the TSCs and TSPs values were significantly close to their control counterparts, recording a decrease by 26 %, and an increase by 29 %; respectively, compared with the Pb treatment. Furthermore, *F. graminearum* treatment resulted in a noticeable increase in TSCs and a highly significant decrease in TSPs. The percentage of these changes reached 28 % in both parameters relative to the un-infected control (Fig. 5). On the contrary, soaking treatments in purslane, chard, Ca and SA counteracted the negative effects of *F. graminearum* inoculation, as they decreased the TSCs contents of wheat grains to 701, 687, 720 and 681 mg/g DM; respectively, close to that of the *F. graminearum* (544 mg/g DM), and increased the TSPs contents of wheat grains to 225, 227, 244 and 246 mg/g DM; respectively, which were close to that of the control (276 mg/g DM); where $p< 0.05$. Additionally, SA solution was the most effective priming solution for TSCs and TSPs levels; with values that were close to the un-inoculated control, recording a decrease by 25.5 % and an increase by 22 %; respectively, compared to *F. graminearum* treatment.

4. Discussion

A plants' water status is the function of three interdependent processes; mainly water uptake, water

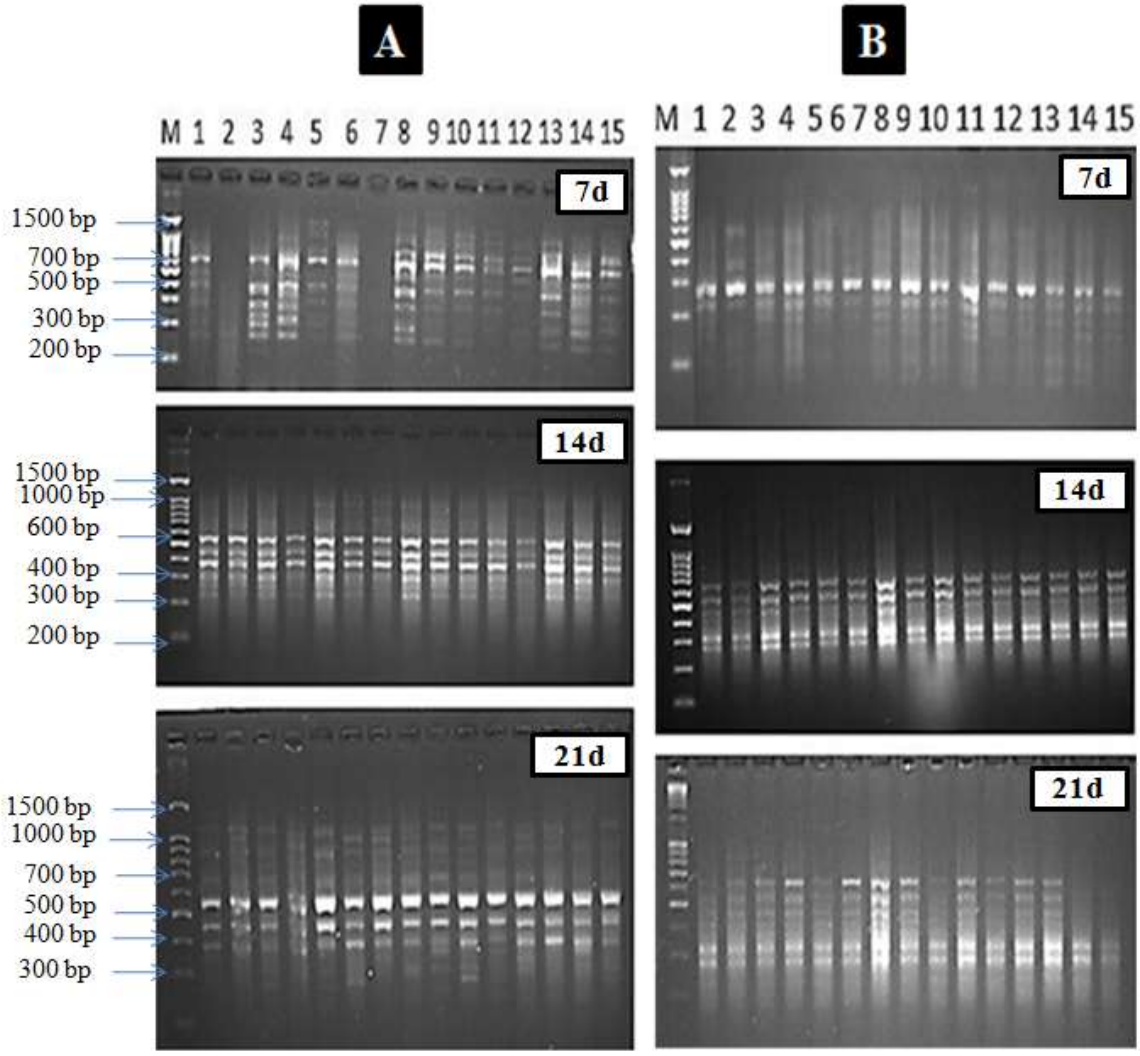

Fig. 4: Gene expression of dd-PCR using different arbitrary defense primers; (A): Chitinase (PR-3R), (B): Thaumatin like protein (PR-5F). M: 1.5 Kbp DNA marker; 1: untreated (control); 2: Pb; 3: *F. graminearum*; 4: Purslane; 5: Purslane+Pb; 6: Purslane+ *F. graminearum*; 7: Chard; 8: Chard+Pb, 9: Chard+*F. graminearum*; 10: Ca; 11: Ca+Pb; 12: Chard+*F. graminearum*; 13: SA; 14: SA+Pb; and 15: SA+*F. graminearum*

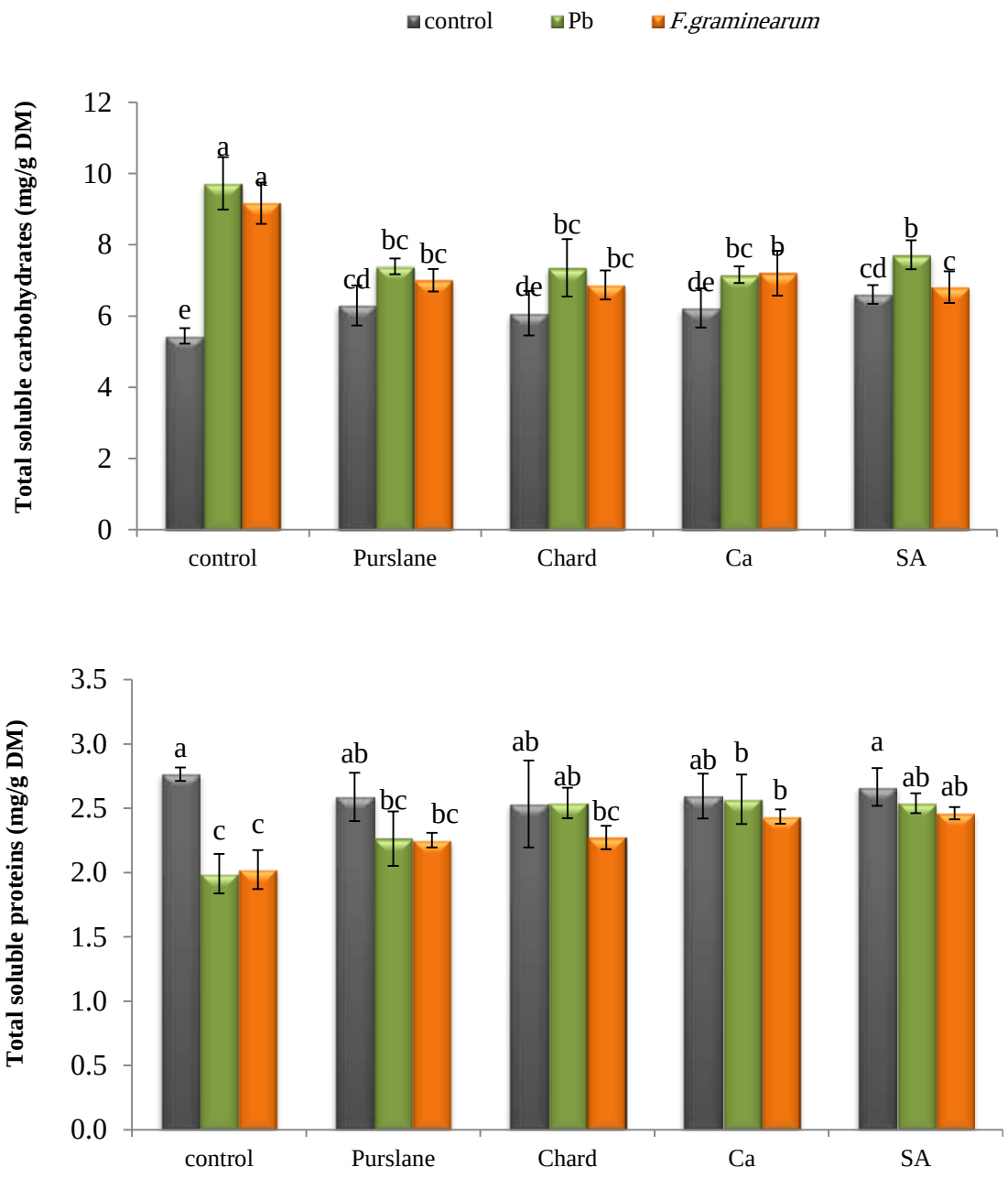

Fig. 5: Changes in total soluble carbohydrates (TSCs) and total soluble proteins (TSPs) of wheat grains primed in weed extracts (25 % purslane and chard), or signaling molecules (5 mM Ca and 0.05 mM SA) under 100 mM Pb stress or *F. graminearum* inoculation. Where; Presented data are means of three replicates ± SD (Standard deviation). Different superscript letters reveal statistically significant variations ($p< 0.05$)

transport, and water loss. The current results indicated that Pb treatment and *F. gra*minearum inoculation resulted in a substantial decrease in the shoot water contents of wheat seedlings. This result is in accordance with that of the previous study conducted by Kastori *et al*., (1992) on Pb-stressed sunflower, and Sayed, (1999) on Pb-stressed safflower plant (*Carthamus tinctorius* that belongs to the family *Asteraceae*). The reduction in water contents following the plants exposure to heavy metals could be accredited to the reduction in water availability in the soil as well as the reduction in soil water potential to values lower than that of the plant. Thus, plants grown in metal-contaminated soils often suffer from drought stress; mainly due to the poor physicochemical properties of the soil and shallow root systems (Gavrilescu, 2021). According to Weryszko-chmielewska and Chwil, (2010), a CO_2/O_2 imbalance in plants due to Pb-induced oxidative phosphorylation and respiratory disorders may also disrupt the plants water status.

Lamhamdi *et al*., (2013) reported that Pb application causes a decrease of most macronutrients; mainly K, Ca, P, Mg, and Na, and micronutrients, including Fe, Cu, and Zn in the plant tissues, due to the block of their absorption sites on the roots surface. Godbold and Kettner, (1991) documented that enormous reductions of ionic contents induce disturbance of the water contents.

On the contrary, the current purslane and chard priming treatments have resulted in a substantial increase in the shoot water contents, which may be accredited to the high mineral contents (i.e., N, P, K, and Ca) of these weeds. The roles of these minerals may be adjusting the osmotic pressure and retaining the internal water status.

In this regard, the positive impact of exogenously applied calcium (Ca) was manifested in the enhancement of plant water contents in the treated wheat seedlings, in accordance with Ortiz *et al*., (1994) study that was conducted on NaCl-stressed *Phaseolus vulgaris*; where Ca diminished the water loss and allowed more water contents upon growth restoration. Additionally, the presence of Ca ion competes with the heavy metals efficiently and prevent these cations from adhering to the plasma of the plant cells, and subsequently causes a decrease in their accumulation (Davis *et al*., 1983).

The enhancing role of salicylic acid (SA) treatment on the shoot water contents may be attributed to the fact that SA can induce glycinebetaine (GB) accumulation. The accumulation of GB in stressed plants regulates the cell osmotic balance; stabilizes the membrane integrity, and detoxifies the toxic ions (Ashraf and Foolad, 2007). Moreover, SA is involved in increasing the proline level, which has several functions, including osmotic pressure regulation; protection of membrane integrity, stabilization of enzymes and proteins, maintaining proper $NADP^+$/NADPH ratios, and scavenging of free radical metabolism under the abiotic stresses (Khan and Khan, 2013).

In parallel to the inhibitory effects of Pb on the plant growth parameters, there was a significant reduction in the total pigments and a remarkable increase in Chl a/b ratio. These results are matching with those of Yang *et al*., (2015), where the total pigment contents of *Robinia pseudoacacia* significantly decreased with Pb stress. The decrease in the pigment contents may be attributed to the fact that Pb is known to prevent chlorophyll synthesis either through diminished uptake of Mg and Fe ions by the plants (Burzynski, 1987), and\ or because it enhances the chlorophyllase enzyme (Drazkiewicz, 1994). The current result of the Chl a/b ratio was in harmony with the previous study conducted by Pätsikkä *et al*., (2002), who reported that the reduction of the grana structure of the chloroplast was consistent with the increased Chl a/b ratio. Moreover, this indicates that synthesis of the photosystem cores takes metabolic reference over synthesis of the light-harvesting complex II (LHC2 of PSII); where in these

photochemical reactions, Chl b is more prevalent than Chl a (Aravind and Prasad, 2004).

In the meantime, the present results indicated a remarkable reduction in the total pigments as a consequence of *F. graminearum* inoculation. This result is in accordance with Lorenzini *et al*., (1997), where *F. verticillioides* infection resulted in a significant reduction in the pigments concentration in the infected maize plants. Furthermore, Sanchez *et al*., (1983) reported that water loss upon fungal inoculation causes a decrease in the total pigments content.

On the contrary to stress action, the current treatments with weed extracts of purslane and chard caused a significant increase in the pigment contents, compared with Pb and *F. graminearum* treatments. This finding may be attributed to the high contents of flavonoids in these weeds. Flavonoids are known to reduce the production and quench the reactive oxygen species (ROS), through suppressing the singlet oxygen (*i.e*., inhibition of enzymes generating ROS like cyclooxygenase; lipoxygenase, mono-oxygenase, and xanthine oxidase); chelating the transition metal ions that catalyze ROS production, quenching the cascades of free-radical reactions in lipid peroxidation, and re-cycling of the other antioxidants (Arora *et al*., 2000). Meanwhile, chard is known to possess a main antioxidant capacity through its major betalains content (Kugler *et al*., 2004). Furthermore, chard is a great source of Mg, which is vital for chlorophyll biosynthesis as revealed previously by Pokluda and Kuben, (2002).

Moreover, the current results showed a significant increase in the total pigments content and a reduction in Chl a/b ratio in response to Ca priming treatment, compared with both stressor treatments. This result is in agreement with that of Sewelam *et al*., (2017), where Ca application increased Chl a and Chl b of drought-stressed wheat seedlings. The ameliorative actions of exogenous Ca might be attributed to Ca signaling, which is involved in the defense mechanisms induced by stress and in the maintenance of the K^+/Na^- ion selectivity (Chen and Murata, 2002).

Furthermore, the present study results indicated that priming of wheat grains in SA ameliorated the toxic effects induced by Pb and *F. graminearum* on the photosynthetic pigments, where it significantly increased the pigment contents, while the Chl a/b ratio was reduced. These results agree with Alamri *et al*., (2018), where SA application increased Chl a, Chl b and decreased Chla/b ratio in Pb-stressed wheat seedlings. This may be attributed to the roles of SA in reducing the damage caused by Pb on the synthesis of these pigments, in addition to improving the nutrient balance and the antioxidative system (Wang *et al*., 2011). In the same line, Shi *et al*., (2009) reported that SA has a positive role in protecting the photosynthetic pigments and apparatus under Cd stress conditions.

Assays for phosphomolybdate (PMA) and DPPH radical scavenging activities are the most commonly used methods for assessment of the antioxidant properties (Maisuthisakul *et al*., 2007). Results of the current work revealed that treating wheat seedlings with 100 mM Pb has resulted in a substantial increase in the PMA and DPPH activities of these seedlings. This finding is in accordance with Sarker *et al*., (2018) on *Amaranthus* leaves that have been subjected to salinity stress.

The antioxidant scavenging activities (*i.e*., PMA and DPPH) imposed by both stressors were reduced on priming the wheat grains in 25 % of purslane or chard extracts. The high contents of minerals such as K in these extracts may effectively lower the total antioxidant scavenging activity. According to Cakmak, (2005), plants that receive K nutrition can significantly reduce the radical's production through decreasing the activity of NADPH oxidases while maintaining the photosynthetic electron transport.

In the same context, the results displayed a significant decrease in PMA and DPPH activities of wheat seedlings with a priming exogenous treatment of 5 mM Ca. This finding may be accredited to the fact that Ca is a crucial regulator of growth and development in plants, so Ca helps in many biosynthetic processes and hence helps in cell relief; as manifested by Sobhy *et*

al., (2023) in their recent work on *F. graminearum* stressed wheat.

The present results highlighted the role of SA priming exogenous treatment in alleviating the oxidative stresses on wheat seedlings by decreasing the PMA and DPPH activities. These results validate the roles of SA on plants facing biotic stress; where SA serves as a signal molecule through up-regulating the H_2O_2 concentration, which can then act as a secondary messenger throughout the defense signaling route (Klessig and Malamy, 1994).

Phenylalanine ammonia-lyase (PAL) enzyme plays an essential role in the phenylpropanoid pathway and has been described to be affected by both of the biotic and abiotic stresses, including pathogen attack; wounding, cold, and UV light (Huang *et al*., 2010). The current results showed the enhanced activity of PAL as a magnitude of Pb stress and *Fusarium* infection compared with the non-treated control. These findings are matching with Ali et al. (2007) study on Cu-stressed Panax ginseng, Hashem *et al*., (2018) work on *F. graminearum* inoculated wheat, and on chickpea subjected to *F. oxysporum* inoculation (Rathod and Vakhariya, 2016). The increase in PAL activity upon fungal inoculation may be attributed to rapid production of phytoalexins, which are a part of the defense mechanism of plants and may also have a regulatory role in the biosynthesis of these secondary metabolites (Rathod and Vakhariya, 2016); in addition, they may also function as antioxidants due to their free-radical trapping properties.

Priming treatments in natural weed extracts (*i.e*., purslane and chard) had significant ameliorative effects that were represented by a decrease in PAL activity, when compared with stress treatments such as Pb or *F. graminearum*. These findings may be ascribed to the high contents of several antioxidant compounds such as flavonoids and phenolics in these extracts, which in-turn may decrease the oxidative stress imposed by both stressors. The phenolic compounds have chief roles in plants development; principally in lignin formation and biosynthesis of pigments. They also afford structural integrity and skeletal support to the plants (Bhattacharya and Rao, 2010).

Likewise, exogenous treatment with Ca significantly reduced the injurious impacts of Pb and *F. graminearum* on wheat seedlings through reducing the PAL activity. This finding is in agreement with Hossain *et al*., (2005); as Ca treatment reduced PAL activity of Al-stressed wheat. The reduction in PAL may be attributed to the fact that Ca can increase the ionic strength of the nutrient media (Noble and Sumner, 1988) and competes with Pb ions for the binding sites in either the symplast or apoplast of roots; thereby reducing the oxidative damage. In addition, enhanced Ca supply ameliorates the proton rhizo-toxicity in the growing root tips (Koyama *et al*., 2001). Accordingly, all these recognized Ca characters have counteracted the toxic effects imposed by Pb through increasing PAL activity, which is thus considered worthless.

Furthermore, compared to Pb and *F. graminearum* treatments, pre-treatment of wheat seedlings with SA enhanced their defense mechanisms through diminishing the activity of PAL enzyme. These effects may be attributed to the fact that SA may serve as a direct ROS scavenger; especially for the hydroxyl radicals and the iron-chelating compounds, which are produced via the Fenton reaction (Halliwell *et al*., 1995).

The healthy life cycle of a plant essentially depends on the balanced uptake of essential nutrients. The current study results displayed that the application of 100 mM Pb and *F. graminearum* treatment had resulted in an extensive decline in some minerals (*i.e*., N, P, K, and Ca) in the shoots and roots of wheat seedlings. These findings are matching with the previous results of Lamhamdi *et al*., (2013) on Pb-stressed wheat and spinach plants, and in *Fusarium* inoculated tomato plant (Bidellaoui *et al*., 2019).

The recorded current decreases in N, P, and Ca contents in the shoots of wheat seedlings exposed to Pb may be attributed to the increased competition

among the divalent cations on the same membrane channels, breakdown of the membrane functions, and possibly also as a product of additional ion leakage from the Pb-stressed plants (Lamhamdi *et al*., 2013). The reduced endogenous Ca level in the treated plants may adversely affect the cell division and elongation, and may be also responsible for the observed reduction in the leaf surface area (Cook, 1997). Pb affects the K^+-ATPase and -SH groups of the cell membrane proteins; causing an efflux of K^+ from the roots (Sharma and Dubey, 2005). Also, Sengar *et al*., (2008) suggested that the reduction in N concentration in Pb-stressed plants may be brought up by the reduced activity of nitrate reductase enzyme, which is responsible for the nitrate assimilation process.

Concerning weed extracts, the current results revealed that soaking of wheat grains in 25 % purslane and chard extracts caused an increase in some of the abovementioned mineral contents. This finding may be attributed to the increase in water contents of wheat seedlings upon these weed priming treatments, and hence more minerals become available to the plant cells. Moreover, this result may be attributed to attaining the cell membrane integrity, which decreases the leakage of such ions from the treated plant cells.

Priming of wheat grains in Ca solution significantly increased the minerals content (*i.e*., N, P, K, and Ca) of the wheat seedlings, compared with both stressors. The same results were attained by Khalil *et al*., (2017) on Pb- and Ni-stressed bean plants. The exogenous application of Ca increased the endogenous Ca contents of the stressed plant, in accordance with Tan *et al*., (2011), which appears to be related to the acquisition of tolerance (Larkindale and Knight, 2002). Ca is a secondary nutrient known to regulate the various cell functions; starting from nutrient uptake to changes in the cell status, and thus can be used to help the plants to cope with the biotic and abiotic stresses (Hirschi, 2004). Increasing the Ca levels in plant tissues can greatly decrease the pathogen's ability to invade the leaf (Conway *et al*., 1994).

Regarding the SA priming treatment, the obtained results indicated an increase in the contents of some measured minerals (i.e., N, P, K, and Ca). These outcomes are matching with previous results reported by Sheng *et al*., (2015), where SA dramatically promoted the accumulation of Ca in Mn-stressed plants. Furthermore, SA raised the N, P, K, and Ca contents in both the shoots and roots of salt-stressed-cucumber relative to the control (Yildirim *et al*., 2008). In a previous study reported by El-Tayeb *et al*., (2006), exogenous application of SA raised K, Ca, and P contents in the shoots and roots of barley seedlings relative to those of non-treated ones under salt stress. These findings may be attributed to that SA induces minerals transport from the nutrient solution to plants. It is well known that H^+-ATPase in cell plasma membranes plays a chief role in the transport of multiple ions; in addition, there are investigations indicating that SA can induce H^+-ATPase activity, which may account for the increased absorption of some minerals (*i.e*., K, Ca, Mg, and Fe) by SA under Cd toxicity (Palmgren and Harper, 1999).

The current ongoing study and elucidations of control mechanisms of the plant stress tolerance at the molecular level may enable the use of molecular mechanisms for evolving more tolerant plants, which are mainly centered on the pattern (up or down) of specific genes related to stress. The differential display reverse transcriptase polymerase chain reaction (DDRT-PCR) is a competent, profound and reproducible technology, which is more advantageous to gene expression analysis in several ways more than the other traditionally used methods (Singh and Ali, 2016). Moreover, the simplicity and wide applicability of this technique made it very novel. This dd-PCR technique has been introduced and developed to accelerate the identification of differentially expressed genes, in order to overcome the shortcomings of the earlier known methods, which were sensitive to error, unresponsive, and strenuous.

The dd-PCR profiles of wheat samples showed considerable variations in gene patterns in response to the Pb treatment and infection with *F. graminearum*,

and this finding is matching with that of Rashad *et al*., (2018) on *Rhizoctonia solani* infected maize. Moreover, the gene expression may have a crucial role in the regulation of plant status in presence of both stressors. Another dd-PCR study conducted by Liu and Baird, (2004) on sunflower for gene expression under un-treated, salt or drought stress plants revealed different up and down-regulated genes.

In regard to the priming treatments, the current results of dd-PCR showed up- and down-regulations of some genes in wheat seedlings as a response to the priming treatments in purslane or chard extracts, demonstrating the induction/suppression of some genes. This result was consistent with the previous study reported by Rashad *et al*., (2018), where these genes may be defence or antioxidant genes that increase the plant tolerance against Pb or *F. graminearum* stress.

The TSPs content in the plant cells is a vital indicator of their physiological state in response to an extensive variety of stressors (Singh and Tewari, 2003). The obtained results illustrated a highly significant decrease in the TSP of wheat grains subjected to 100 mM Pb stress. This finding is matching with that reported by Khalil *et al*., (2017), who revealed that application of Pb significantly reduced the production of the total proteins content in common bean. The toxic metals reduce the enzyme activity and the synthesis of protein (Lin and Kao, 2006), and/or cause an increase in its decomposition (Dietz *et al*., 1999). This toxicity may be attributed to binding of metals to the sulfhydryl groups in proteins; resulting in inhibition of activity, disruption of structure, or causes the displacement of an important element, leading thus to deficiency effects (Capuana, 2011).

Moreover, the results showed a significant decrease in the TSP contents of wheat grains inoculated with *F. graminearum* compared with the non-inoculated grains. This finding is in agreement with Akbari-Vafaiia *et al*., (2013) who stated that *F. culmorum* displayed a significant reduction in protein content of the wheat tissues as a result of pathogen infection. Similarly, Hassanein *et al*., (2016) reported a reduction in total protein of wheat shoots infected with *F. graminearum*. Both studies have attributed this reduction in TSP to some plant activities related to a hypersensitive response. Meanwhile, Arad and Richmond, (1976) study attributed the decrease in protein synthesis to the increase in RNase under stress.

Nonetheless, the obtained data regarding priming in SA treatment revealed a significant increase in TSP and this finding is in accordance with El-Tayeb *et al*., (2006), where the application of SA resulted in a higher accumulation of TSP in sunflower plants under Cu-toxicity. This attitude of SA may be accredited to its involvement in the expression of specific proteins or defense-related enzymes in order to cope with Pb stress (Popova *et al*., 2012). This was manifested in the increase of nitrogen fixation due to SA-improved activity of nitrate reductase (Yusuf and Fariduddin, 2012). Therefore, it can be supposed that SA played a main role in regulating nitrate reductase activity and enhancing nitrite reductase synthesis through mobilizing intracellular NO^{3-} and providing protection to nitrite reductase degradation *in vivo* in the absence of NO^{3-} (Ghasemzadeh and Jaafar, 2013).

With respect to the weed extract priming treatments, the current results revealed a pronounced increase in the TSP of stressed wheat, and this finding is in accordance with Yasmeen *et al*., (2013), where *Moringa* extract application increased the TSPs of wheat plant under saline stress.

Plant seeds accumulate soluble carbohydrates throughout their development and maturation, which are involved in most of the basic physiological processes, and have important roles in seed germination and desiccation tolerance (Sinniah *et al*., 1998). Carbohydrate changes have a direct relationship with several physiological processes, including photosynthesis; transpiration, and respiration (Ahmad *et al*., 2006). In the present study, it was clear that 100 mM Pb treatment generally induced a major increase in the TSC, in agreement with Sewelam *et al*., (2017)

who tested wheat seedlings under drought stress. This may be attributed to the increased content of soluble sugars via an enhanced amylolytic activity (Mohamed and Hussein, 1994). High carbohydrate concentration has several roles, including decreasing the osmotic stress; contributes to avoiding the oxidative damage, and preserve the structure of proteins and membranes under stress conditions (Hoekstra *et al*., 2001).

The obtained results also showed a remarkable rise in TSC under *F. graminearum* inoculation, which might be a tolerance mechanism against oxidative stress. Regarding weed extracts; priming of wheat grains in purslane or chard extracts revealed a decrease in TSC, which might be attributed to the fact that plant extracts may provide an effective tool for the control of some fungi. High content of bioactive compounds of the plant extracts, including phenols; tannins, quinines, and flavonoids may have multifunctional roles against the phytopathogenic fungi (Baka and Rashad, 2016). Furthermore, priming of wheat grains in 5 mM Ca resulted in a significant decrease in the TSC of Pb and *F. graminearum* stressed grains, which was consistent with the previous findings recorded by Sewelam *et al*., (2017) on drought-stressed wheat.

The current results revealed a significant reduction of TSC under SA priming treatment when compared with Pb and *F. graminearum* inoculation treatments. In a previous study, SA pretreatment significantly accelerated the accumulation of glutathione (GSH) in eggplant and banana that have been subjected to cold stress (Chen *et al*., 2011). The results reported by Kang *et al*., (2013) also showed that application of SA significantly increased the GSH content in drought-stressed wheat seedlings, suggesting that the reduced lipid peroxidation and enhanced drought tolerance may be associated with the elevated content of GSH. Thus, the ameliorative roles of either Ca or SA priming exogenous treatments may be attributed to their roles in leaf area induction, osmotic adjustment through internal mineral increase, and increasing the plant water content; hence plant cell relief.

Conclusion

In this study, we concluded that the seed priming in natural plant extracts such as purslane and chard may be a promising method for mitigating the damaging effects caused by the abiotic and biotic stressors, such as Pb and *F. graminearum*. This was achieved through increasing the plant endogenous water content; total pigment, minerals, and up and down regulation of some genes. These increased parameters led to decreasing the total induced antioxidant capacity and enzyme activity. Furthermore, these plant extracts can compete with the chemical methods in obtaining less harmed plants using cheaper, safer, and ecofriendly alternatives.

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Conflict of interest

The author(s) declare that they have no competing interests.

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Ethical approval

Non-applicable

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