

Chromium(VI) Toxicity and Active Tolerance Mechanisms of Wheat Plant Treated with Plant Growth-Promoting Actinobacteria and Olive Solid Waste

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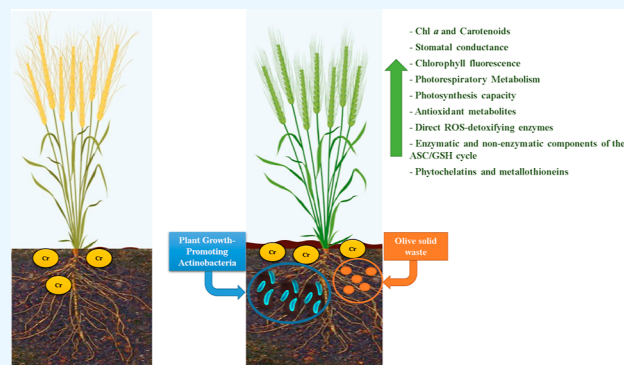
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ABSTRACT: The present study aimed to assess the potential of plant growth-promoting Actinobacteria and olive solid waste (OSW) in ameliorating some biochemical and molecular parameters of wheat (*Triticum aestivum*) plants under the toxicity of high chromium levels in the soil. With this aim, a pot experiment was conducted, where the wheat plants were treated with a consortium of four *Actinobacterium* sp. (Bf treatment) and/or OSW (4% w/w) under two levels of nonstress and chromium stress [400 mg Cr(VI) per kg of soil] to estimate the photosynthetic traits, antioxidant protection machine, and detoxification activity. Both Bf and OSW treatments improved the levels of chlorophyll a (+47–98%), carotenoid (+324–566%), stomatal conductance (+17–18%), chlorophyll fluorescence (+12–28%), and photorespiratory metabolism (including +44–72% in glycolate oxidase activity, +6–72% in hydroxypyruvate reductase activity, and +5–44% in a glycine to serine ratio) in leaves of stressed plants as compared to those in the stressed control, which resulted in higher photosynthesis capacity (+18–40%) in chromium-stressed plants. These results were associated with an enhancement in the content of antioxidant metabolites (+10–117%), of direct reactive oxygen species-detoxifying enzymes (+49–94%), and of enzymatic (+40–261%) and nonenzymatic (+17–175%) components of the ascorbate–glutathione cycle in Bf- and OSW-treated plants under stress. Moreover, increments in the content of phytochelatin (+38–74%) and metallothioneins (+29–41%), as markers of detoxification activity, were recorded in the plants treated with Bf and OSW under chromium toxicity. In conclusion, this study revealed that the application of beneficial Actinobacteria and OSW as biofertilization/supplementation could represent a worthwhile consequence in improving dry matter production and enhancing plant tolerance and adaptability to chromium toxicity.



1. INTRODUCTION

Potentially toxic element contamination is one of the leading concerns for the soil environment, which could expose a potential threat to crop production and consequently can be toxic for crops, animals, and humans when they surpass a threshold range.¹ Such a hazard can be more elevated for elements, such as chromium (Cr), which, relying on the soil redox conditions and the availability of organic matter, can change its oxidation condition, creating favorably mobile and harmful hexavalent species [Cr(VI)], as CrO_4^{2-} and $\text{Cr}_2\text{O}_7^{2-}$ anions.² Nevertheless, the stable and less toxic form of chromium in the soil is Cr(III), which is 10–100 times more abundant than Cr(VI) and exists in the form of complexes with NH_3 , SO_4^{2-} , Cl^- , F^- , OH^- , CN^- , and soluble organic ligands in soils.¹

The plant–microbial interaction in soils is one of the principal processes affecting the potentially toxic element

uptake by plants, especially those belonging to bacterial phyla Proteobacteria, Firmicutes, and Actinobacteria.^{1,3} Some of such soil bacteria are also known as plant growth-promoting bacteria (PGPB) and are used in crop production as biostimulants.^{3,4} Actinobacteria phylum contains some species strongly resistant under higher potentially toxic elements toxicity, also notably influencing plant root system colonizers and competent of sustaining adverse growth conditions by forming spores.⁵ These Gram-positive bacteria are free-living microorganisms in soil,⁶ which can affect the agronomic and

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physiological traits of crops^{7,8} and can also act as a primary antibiotic by producing specific metabolites.⁹ Such abilities in plant growth-promoting functions are considered one of the best strategies to manage soil fertility and crop nutritional status by replacing synthetic chemical fertilizers.^{10,11}

On the other hand, another main concern in agricultural sustainability has been the underscore on the recovery and recycling of food byproducts and wastes.¹² Producing organic fertilizers from olive solid waste (OSW), as a source of optimum nutritive value compounds, has been the subject of intense attention within the scientific community.¹³ OSW, generated by the olive oil manufacturing process, is known for its positive effects on plants, especially under heavy metal stress.¹² It has previously been reported that OSW not only has antimicrobial and antiviral capability¹⁴ but also contributes to enrich the organic matter content (e.g., fiber, lignin, uronic acids, and polyphenolic compounds; >1 mg g⁻¹), N (>5 mg g⁻¹), P (>3 mg g⁻¹), and K (>11 mg g⁻¹).^{12,15}

Despite the previous findings summarized above, there has been little discussion about the synergic effects of olive waste and beneficial bacteria in soils on the metabolites and biochemical composition of plants, especially with heavy metal pollutants. The present study aimed to address the following questions (i) whether oxidative stress caused by chromium toxicity can be alleviated in wheat (*Triticum aestivum*) treated with a consortium of plant growth-promoting Actinobacteria or OSW?, (ii) whether the improvement of metabolic parameters and biochemical compounds of plants in the simultaneous application of beneficial Actinobacteria and olive waste is more pronounced than their individual applications. We hypothesized that the application of plant growth-promoting Actinobacteria and OSW (synergistically or applied individually) could positively improve some photosynthetic and metabolic parameters and antioxidant defense mechanisms in chromium-stressed plants compared to the control plants.

2. MATERIALS AND METHODS

2.1. Plant Materials and Experimental Setup. The experiment was based on a completely randomized design with two factors and three replications. The first factor contained four levels of fertilization/supplementation, including (i) no fertilization control level (Co), (ii) soil treated with OSW, (iii) biofertilization with plant growth-promoting Actinobacteria (Bf), and (iv) a combined treatment of OSW and Bf (OSW + Bf). In the pot experiment, soils were supplemented with 4% w/w of solid olive wastes after collecting from a traditional and air-drying for 1 month before use.¹⁶ OSW was composed of 12.6% dry matter, 64.7% organic matter, 5.6 g L⁻¹ nitrogen (N), 3.2 g L⁻¹ phosphorus (P), 2.1 g L⁻¹ calcium (Ca), 1.6 g L⁻¹ magnesium (Mg), 1.6 g L⁻¹ iron (Fe), and 0.7 g L⁻¹ of zinc (Zn). pH was 5.7, and the electric conductivity (EC) was 18.6 dS m⁻¹. At a humidity (0.35 g water g⁻¹ dry soil), the soil originally contained 12.1 mg of nitrate-nitrogen, 9.2 mg of carbon (C), 1.0 mg of ammonium-N, and 9.6 mg of P g⁻¹ air-dry soil. The pH was 7.9, and the EC was 3.4 dS m⁻¹. After adding the OSW, the soil nutrient status was changed to 14.9 mg nitrate-nitrogen, 14.3 mg C, 1.6 mg ammonium-N, and 11.6 mg P per g of air-dry soil. The soil pH was 7.0, and EC was 6.7 dS m⁻¹.

The second factor was chromium (Cr) stress at two levels, including nonstress (control) and chromium stress at 400 mg Cr (VI) (from K₂CrO₄) per kg of soil. According to the

preliminary experiment of testing different concentrations (0–1000 mg of kg⁻¹ soil), we selected concentrations with a clear growth response of 50%. A fixed soil mass was used for each individual pot to which the Cr (VI) solution (400 mg 25 mL⁻¹) was added dropwise under continuous mixing with a mixer. After that, it was mixed for an extra 2 min to obtain a homogeneous Cr (VI) distribution.

Biofertilizer treatment was composed of four plant growth-promoting Actinobacteria strains, which were isolated from the Jazan mangrove shoreline (Saudi Arabia), identified as the genus *Saccharomonospora*.¹⁷ To prepare a bacterial suspension, Actinobacteria grown in a nutrient broth culture medium (at 29 °C for 24 h) were concentrated by centrifugation (at 5000 rpm for 15 min) and the obtained pellet was washed and resuspended in a sterile potassium chloride solution (0.9%, w/v).¹⁸ The density of Actinobacteria suspension was adjusted to 10⁻⁶ cfu mL⁻¹, corresponding to an optical density at 600 nm equal to 0.6–0.7, and was used to inoculate the soil before cultivation and add to pots every 3 weeks.¹⁸ Control pots were also treated with a sterile potassium chloride solution.

Wheat seeds were sterilized in a sodium hypochlorite solution (1% v/v) for 10 min and cultivated in a potting mix (Tref EGO substrates, Moerdijk, The Netherlands) in pots, which were filled with a mixture of loamy soil and organic compost (1:1, v/v). Plants were kept in a controlled environment chamber for 6 weeks, with a constant regime of 20 °C, 14/10 h day/night photoperiod, 150 μmol m⁻² s⁻¹ photosynthetically active radiation, and ~65% soil water content. Plant shoot tissues were harvested at 6 weeks after cultivation. A part of them was used to determine the fresh and dried weight of the shoot and the remaining for subsequent biochemical analysis.

2.2. Determination of Photosynthetic Related Parameters. Some photosynthetic pigments, including chlorophyll a, chlorophyll b, and carotenoid content in leaves, were determined according to the modified Porra¹⁹ method by reading the absorbance of the extracted samples at wavelengths of 665.2, 652.4, and 470 nm.¹⁸ The last developed leaves were also subjected to stomatal conductance (gs) and photosynthesis rate (PN) measurements using a LI-COR portable photosynthesis system (LI-COR 6400/XT, USA). The maximum efficiency of photosystem II in dark-adapted leaves (Fv/Fm) was also determined using a pulse amplitude modulated fluorometer (PAM-2500, Walz, Germany), in which Fm and Fv are the maximum fluorescence and the variable fluorescence, respectively.²⁰

2.3. Assessment of Stress Biomarkers. Then, oxidative damage caused by chromium stress was evaluated in leaves. In detail, samples were homogenized in ethanol (80% v/v), the extracted samples were tested using the thiobarbituric acid assay, followed by reading the absorbance at 440, 532, and 600 nm to measure the concentration of malondialdehyde (MDA).²¹ Hydrogen peroxide (H₂O₂) content in leaves was also quantified in trichloroacetic acid (0.1%) based on the xylenol orange method, which relies on peroxide-catalyzed oxidation of Fe²⁺.²¹ Protein oxidation (PO) parameter in leaves was assessed based on the spectrophotometrical measurement of protein carbonyl content at 360 nm.²²

2.4. Assessment of Antioxidant Metabolites and Enzymes. To attain a better in-depth knowledge of the biochemical strategies in plants in response to Bf and OSW treatments under Cr exposure, antioxidant metabolites and enzymes were assessed. To this aim, samples were homogen-

ized in 1 mL of buffer [50 mM potassium phosphate, pH 7.0, 10% (w/v) polyvinylpyrrolidone (PVP), 0.25% (v/v) Triton X-100, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 1 mM ASC] and centrifuged to get a clear supernatant for measuring the activity of the antioxidant enzymes. Accordingly, superoxide dismutase (SOD) was measured based on the inhibition of nitroblue tetrazolium (NBT) reduction at 560 nm.²³ Peroxidase (POX) activity was determined by determining the pyrogallol oxidation.²⁴ The breakdown of H₂O₂ at 240 nm was considered to measure catalase activity.²⁵ The estimation of ascorbate peroxidase (APX) and glutathione reductase (GR) activities was fully described by Sohel Murshed et al.²⁶ The reduction in NADPH absorption at 340 nm was recorded to determine glutathione peroxidase (GPX) activity.²⁷ Reduced glutathione (GSH), reduced ascorbate (ASC), and phytochelatin levels were assessed by high-performance liquid chromatography (HPLC).^{12,28} Glutathione S-transferase (GST) activity was determined using 1-chloro-2,4-dinitro-benzene as the substrate, based on the method of Habig et al.²⁹

Folin–Ciocalteu and aluminum chloride calorimetric assays were used to measure the contents of polyphenols and flavonoids, as fully described by Zhang et al.³⁰ and AbdElgawad et al.,³¹ respectively. Total antioxidant capacity (TAC) was quantified through the ferric-reducing antioxidant power method using Trolox as a reference base.³² Tocopherol content was measured using HPLC, in which Dimethyl tocol was used as an internal standard.³³ Quantification of organic acids in soil extracted from the rhizosphere of wheat plants was done using HPLC.³⁴

2.5. Measurement of Amino Acids. Amino acids were extracted from leaf samples (100 mg) by homogenization in ethanol, followed by centrifugation. The pellet was resuspended in chloroform after vacuum evaporation of ethanol traces. The supernatant obtained by centrifugation (14,000 rpm, 10 min) was filtered using a Millipore micro filter (0.2 M pore size). A Waters Acquity UPLC-tqd system (Milford, Worcester County, MA, USA) with a BEH amide column was used to measure amino acids quantitatively.³⁵

2.6. Assessment of Photorespiratory Metabolism. Glycolate oxidase (GO) activity was determined spectrophotometrically measuring the oxidation of O-dianisidine into a colored O-dianisidine radical cation.³⁶ The assessment of hydroxypyruvate reductase (HPR) in leaves was performed using NADH-HPR-NADH in the presence of hydroxypyruvate.³⁷

2.7. Measurement of Chromium Levels in Soil and Plant. The samples were digested overnight at 120 °C in a solution of HNO₃ (65% v/v) and HClO₄ (70% v/v) in a ratio 2:1, until the deep white fumes were released. Then, the flow injection hydride generation atomic absorption spectrophotometry (FI-HG-AAS, PerkinElmer Analyst 400, Waltham, MA, USA) was used to determine the concentration of Cr.³⁸ HCl (10% v/v) and NaBH₄ (0.4% v/v) were also used to obtain the maximum sensitivity.

2.8. Statistical Analysis. All statistical analyses, including a two-way analysis of variance (ANOVA) and Tukey HSD (honestly significant difference) test, as well as graphs drawing, were performed using SigmaPlot software. The results were expressed as the mean ± standard deviation.

3. RESULTS

3.1. Photosynthesis Parameters. Under nonstress conditions, either beneficial bacteria or OSW treatments had no significant effects on the photosynthetic pigments (chlorophyll a, b, and carotenoids) (Figure 1A), stomatal

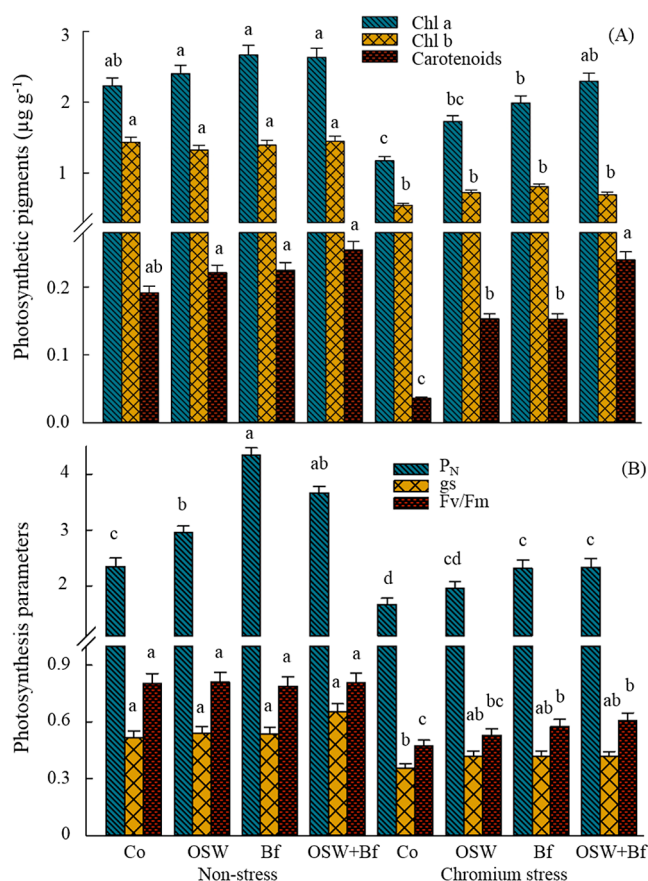


Figure 1. Effect of biofertilizer (Bf) and OSW on the photosynthetic pigments (A) and photosynthesis parameters ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for P_N and $\text{mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for g_s) (B), under nonstress and chromium stress conditions. Means in each parameter followed by similar letter(s) are not significantly different at 5% probability level (Tukey test). Chl a: chlorophyll a; Chl b: chlorophyll b; P_N : photosynthesis rate; g_s : stomatal conductance; and Fv/Fm: maximum efficiency of PSII photochemistry in dark-adapted leaves.

conductance (g_s), and the maximum quantum yield of photosystem II (Fv/Fm) (Figure 1B) as compared to the control. Nevertheless, the photosynthesis rate (P_N) under nonstress was recorded equal to 4.35, 3.67, and 2.95 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in Bf, combined treatment (Bf + OSW), and OSW treatments, respectively, which were significantly higher than those in the control (Figure 1B). These treatments also could maintain the photosynthesis rate in stressed plants at the same statistical level ($p > 0.05$) as those control nonstressed, however, were 18–40% higher than the stressed control plants. Similar results were found for Chl a and carotenoids under stress in response to Bf, OSW, and Bf + OSW, where the Chl a and carotenoids contents ranged from 80 and 78% (OSW) to 125 and 103% (Bf + OSW) as the content of those in unstressed control plants ($p > 0.05$), respectively. The values of g_s and Fv/Fm in stressed plants were improved under both Bf and OSW treatments, which were about 17–18 and 12–28% higher than those in stressed control plants and 19–20 and

24–34% lower than those in unstressed control plants, respectively.

3.2. Oxidative Markers and Antioxidant Components.

Strong evidence of the effect of chromium stress on oxidative markers ($p < 0.05$) was obviously recorded in the control plants (Figure 2A). In this regard, the levels of H_2O_2 , MDA,

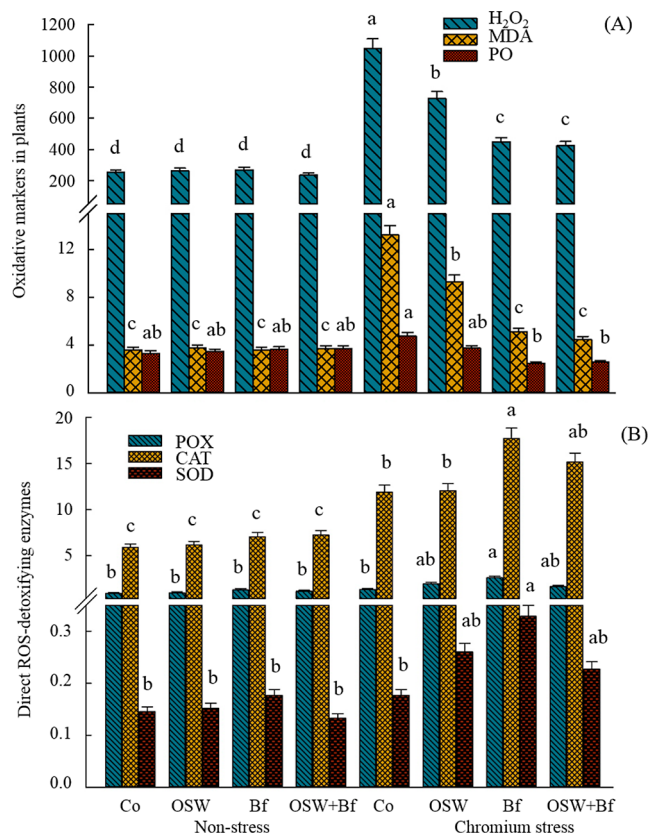


Figure 2. Effect of biofertilizer (Bf) and OSW on the oxidative markers (represented as $\mu\text{mol g}^{-1}$ FW for H_2O_2 , nmol g^{-1} FW for MDA, and nmol mg^{-1} protein for PO) (A) and antioxidant direct scavenging enzymes ($\mu\text{mol min}^{-1}$ mg^{-1} protein for POX and CAT, and mmol min^{-1} mg^{-1} protein for SOD) (B) under nonstress and chromium stress conditions. Means in each parameter followed by similar letter(s) are not significantly different at 5% probability level (Tukey test). H_2O_2 : hydrogen peroxide; MDA: malondialdehyde; PO: protein oxidation; POX: peroxidase; CAT: catalase; and SOD: superoxide dismutases.

and PO in stressed control plants were about +314, +267, and +44% higher than those under nonstress, respectively. Nevertheless, these markers showed a decreasing trend in response to Bf and OSW treatments under stress conditions. Accordingly, the lowest contents of H_2O_2 , MDA, and PO were found in the combined treatment (Bf + OSW) in the stressed plants, which were about 41, 34, and 53% of their content in stressed control plants ($p < 0.05$), respectively (Figure 2A).

To reveal the mechanisms of plants in decreasing the accumulation of oxidative markers under stress, the concentration of the direct reactive oxygen species (ROS)-detoxifying enzymes (CAT, POX, and SOD) and those enzymatic (APX, GPX, GR, DHAR, and MDHAR) and nonenzymatic (ASC and GSH) components of the ascorbate–glutathione (ASC–GSH) cycle were investigated, as shown in Figures 2B and 3. In this regard, although the levels of POX, CAT, and SOD were not significantly affected by treatments in unstressed plants, they

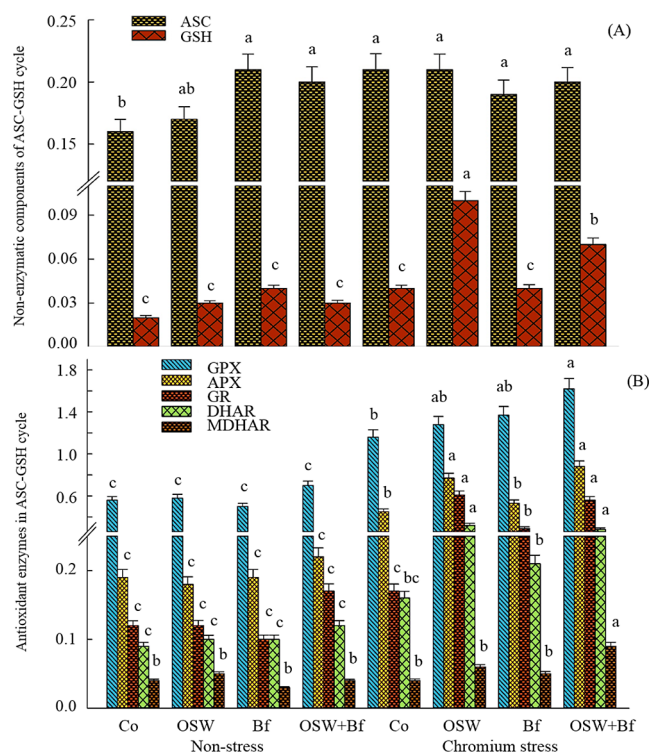


Figure 3. Effect of biofertilizer (Bf) and OSW on the nonenzymatic (represented as $\mu\text{mol g}^{-1}$ FW) (A) and enzymatic components ($\mu\text{mol min}^{-1}$ mg^{-1} protein) (B) of the ascorbate–glutathione (ASC/GSH) cycle under nonstress and chromium stress conditions. Means in each parameter followed by similar letter(s) are not significantly different at 5% probability level (Tukey test). ASC: ascorbate; GSH: glutathione; GPX: glutathione peroxidase; APX: ascorbate peroxidase; GR: glutathione reductase, DHAR: dehydroascorbate reductase; and MDHAR: monodehydroascorbate reductase.

were significantly higher in response to Bf and OSW under stress. The highest activity of POX, CAT, and SOD enzymes was obtained from the Bf treatment, equal to $2.6 \mu\text{mol min}^{-1}$ mg^{-1} protein, $17.7 \mu\text{mol min}^{-1}$ mg^{-1} protein, and $0.3 \text{ mmol min}^{-1}$ mg^{-1} protein, respectively, which were significantly ($p < 0.05$) 94, 49, and 86% higher than control treatment under stress conditions (Figure 2B). In contrast, the accumulation of ascorbate (nonsignificantly) and glutathione ($p < 0.05$), as the nonenzymatic components of ASC–GSH cycle, were higher in OSW-treated plants (OSW and Bf + OSW), under stress compared to those in the control and Bf-treated ones (Figure 3A). Such an increment in ASC and GSH in OSW-containing treatments resulted in higher activity of the enzymes involved in the ASC–GSH cycle. Accordingly, the highest accumulation of GPX, APX, and MDHAR was found in Bf + OSW treatment under stress, which were about 1.4, 2.3, and 2 times higher than in control treatment under stress and 2.9, 2.3, and 4.7 times higher than the control under nonstress conditions, respectively (Figure 3B). In addition, GR and DHAR were more accumulated in OSW treatment in stressed plants, being 3.6 and 2 times higher than control-stressed plants and 5.1 and 3.5 times higher than the control unstressed plants, respectively (Figure 3B).

3.3. Antioxidant Metabolites and Molecules in Plant and Soil. The research also focused on antioxidant metabolites in plants and soil in response to the treatments and chromium stress conditions. The content of TAC reached values of $77.2 \mu\text{mol torolex g}^{-1}$ FW in Bf + OSW-treated plants

under stress, which, although placed at the same statistical group with other treatments in stressed plants ($p > 0.05$), was significantly ($p < 0.05$) higher than all treatments in unstressed plants (Figure 4A). Similarly, the greatest accumulation of

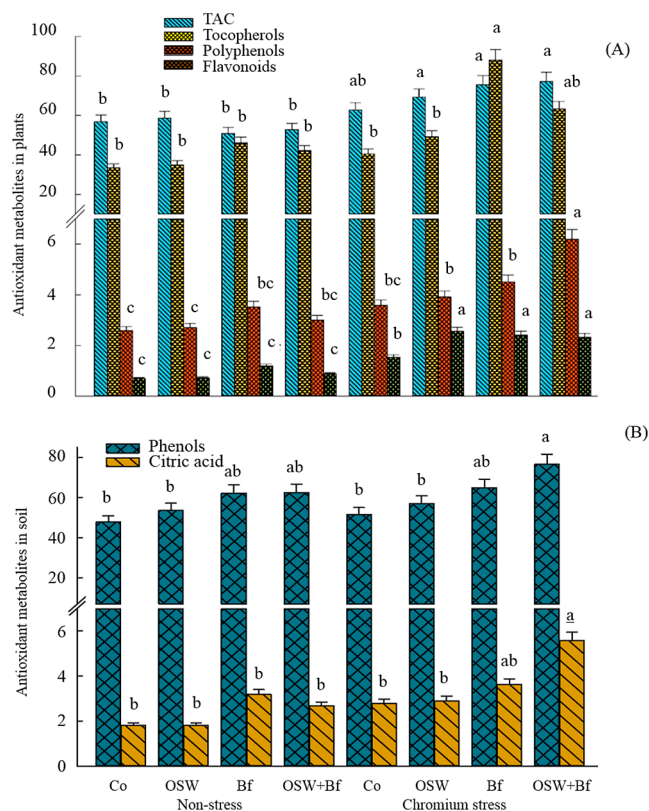


Figure 4. Effect of biofertilizer (Bf) and OSW on the antioxidant metabolites in plant (A) and soil (B) (μmol torolex g^{-1} FW for TAC; mg GAE g^{-1} FW for polyphenols; mg quercetin g^{-1} FW for flavonoids; ng g^{-1} for tocopherols) under nonstress and chromium stress conditions. Means in each parameter followed by similar letter(s) are not significantly different at the 5% probability level (Tukey test). TAC: total antioxidant capacity.

polyphenols in plants was observed in Bf + OSW treatments under stress, equal to $6.2 \text{ mg gallic acid equivalents (GAE)} \text{ g}^{-1}$ FW, which were significantly ($p < 0.05$) higher than all other treatments under both stress and nonstress conditions (Figure 4A). Moreover, Bf and OSW enhanced the accumulation of total tocopherols and flavonoids, equal to 88.1 ng g^{-1} and $2.56 \text{ mg Quercetin g}^{-1}$ FW, respectively, which were 2.2 and 3 times ($p < 0.05$) higher than those in control stressed plants (Figure 4A). Phenol and citric acid concentrations in soil were also significantly affected by Bf + OSW treatments under stress, in which they were more concentrated than control treatments under stress (+48 and +100%, respectively) and nonstress (+100 and +110%, respectively) conditions (Figure 4B).

3.4. Photorespiratory Metabolism. The analysis of photorespiratory metabolism in plants revealed an obvious increment in GO activity under stress ($p < 0.05$), which was also more active in response to Bf and OSW treatments under stress (+44–72%) in comparison with control treatment under stress (Figure 5A). Moreover, the glycine to serine ratio was significantly ($p < 0.05$) higher in stressed plants as compared to those unstressed, in which this ratio was greater in OSW-containing treatments (OSW and Bf + OSW) (Figure 5A). In

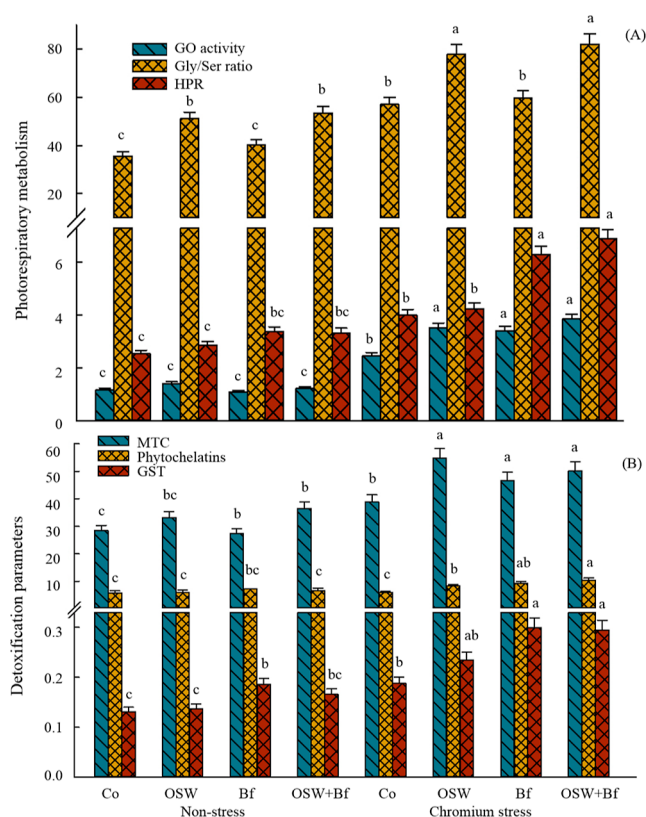


Figure 5. Effect of biofertilizer (Bf) and OSW on photorespiratory metabolism ($\mu\text{mol mg}^{-1}$ Chl min^{-1} for GO and HPR) (A) and detoxification activity ($\mu\text{g g}^{-1}$ FW) (B) under nonstress and chromium stress conditions. Means in each parameter followed by similar letter(s) are not significantly different at 5% probability level (Tukey test). GO: glycolate oxidase; HPR: hydroxypyruvate reductase; MTC: metallothioneins; and GST: glutathione-S-transferase.

contrast, Bf-containing treatments (Bf and Bf + OSW) had more effects on HPR activity in stressed plants and enhanced the HPR values up to 6.3 and $6.9 \mu\text{mol mg}^{-1}$ Chl min^{-1} , which were +57 and +72% higher than those of control stressed plants, respectively (Figure 5A).

3.5. Detoxification Activity. As reported in Figure 5B, detoxification parameters were affected by treatments and chromium stress. Accordingly, the highest phytochelatin content was found in the combined treatment (Bf + OSW) under stress, which was not significantly greater than Bf treatments under stress, while it was about two times ($p < 0.05$) higher than control unstressed plants (Figure 5B). Also, OSW and Bf treatments induced the highest content of metallothioneins (MTC) and glutathione-S-transferase (GST) activity, equal to 54.7 and $0.3 \mu\text{g g}^{-1}$, respectively. Although their values were not significantly higher than each other under stress, they were greater than the control stressed plants ($p < 0.05$), of about +41 and +59%, respectively (Figure 5B).

3.6. Plant Biomass and Chromium Content in Plant and Soil. As shown in Figure 6, the results indicated a significant decrement in plant biomass (dry and fresh weights) in control plants under chromium stress. In addition, the dry weight was significantly improved in response to the Bf and OSW treatments under stress. In this regard, the maximum dry weight was recorded in Bf-treated plants under stress, which had no significant differences with OSW- and Bf-OSW-treated

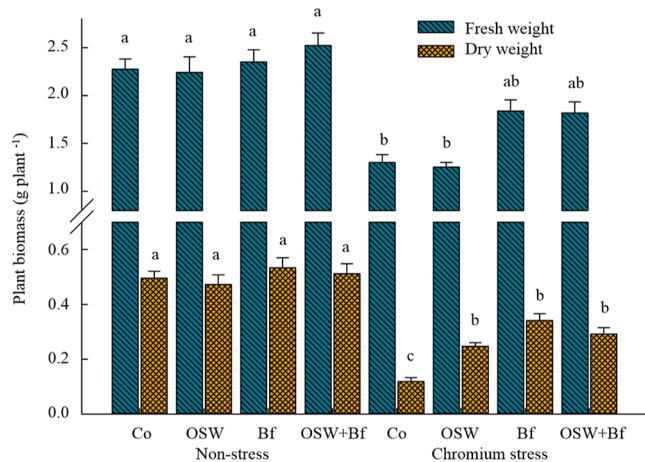


Figure 6. Effect of biofertilizer (Bf) and OSW on plant biomass under nonstress and chromium stress conditions. Means in each parameter followed by similar letter(s) are not significantly different at the 5% probability level (Tukey test).

plants, while was 187% higher than control stressed plants ($p < 0.05$) (Figure 6). Moreover, although the content of chromium in plants and soil was negligible, it was significantly more concentrated under chromium stress. Nevertheless, there were no significant differences among the treatments under stress (Figure 7).

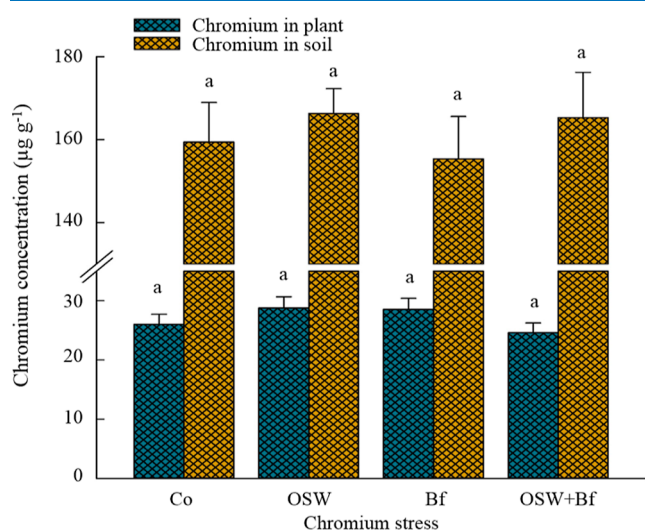


Figure 7. Effect of biofertilizer (Bf) and OSW on chromium concentration in plants and soil under chromium stress conditions. Means in each parameter followed by similar letter(s) are not significantly different at the 5% probability level (Tukey test).

4. DISCUSSION

One of the principal objectives discoursed in the present research was monitoring the biostimulation effects of the beneficial Actinobacteria and OSW on the plant photosynthesis pathway, especially under chromium stress. The decrements in photosynthetic pigments and the efficiency of photosynthesis rate in heavy metal-stressed plants was already reported by Li et al.³⁹ and Albqmi et al.,¹² who related this issue with the disruption of photosynthetic electron transport chain and the accumulation of ROS in chloroplasts, hereafter affecting photosynthesis pigment synthesis. As shown in Figure

1A, both biofertilizer and OSW treatments significantly improved chlorophyll and carotenoid contents in leaves of stressed plants. Such improvement in photosynthetic pigments resulted in maintaining the photosynthesis rate and stomatal conductance in chromium-stressed plants as the same level ($p > 0.05$) as the control plants under nonstress (Figure 1B). This result conforms the findings of others, who reported improvement of the photosynthesis capacity and pigment content in response to olive waste and plant growth-promoting microorganisms in stressed plants.^{12,40–41,42} Also, the Fv/Fm ratio in the present study ranged from 0.79 to 0.81 and was not influenced by fertilization and supplementation (Figure 1B). It has already been reported that Fv/Fm ratios, as the indication of the highest efficiency of photosystem II (PSII) in dark-adapted leaves, usually varied from 0.79 to 0.85 in unstressed plants.²⁰ Similar to our results in fertilized plants, current evidence points that the Fv/Fm ratio is less sensitive under nonstress conditions and can stay unaffected.¹⁸ Nevertheless, the higher Fv/Fm ratio in fertilized plants under chromium stress, as compared to those in control plants, could confirm the potential of beneficial Actinobacteria and OSW in handling the portion of excitation energy reaching the reaction centers in PSII and bypassing photodamage in leaves under stress.⁴³ Similar reports pointed out that the PGPB-treated plants can be systematically more tolerated against stress,⁴⁴ due to some direct and indirect mechanisms, including the modification of soil–plant system capacity in supplying/uptaking nutrients from soil,⁴⁵ production of indole acetic acid,¹¹ affecting 1-aminocyclopropane-1-carboxylate (ACC) deaminase⁴⁶ activity, and regulation of the expression of specific genes.⁴⁷

The photorespiration pathway, as a major source of ROS in peroxisomes of stressed plants,²² was also monitored by assessing GO and HPR activity, and gly/ser ratio under heavy metal stress. Accordingly, a clear increase in GO activity ($p < 0.05$) was recorded under both stress and fertilization treatments (Figure 5A). Such an improvement in the content of this key photorespiratory enzyme was in agreement with another study under heavy metals,⁴⁸ proposing that photorespiration could contribute to the conservation of photosynthetic components against over-reduction.²² Bf-containing treatments (Bf and Bf + OSW) and chromium stress had more impact on the HPR content, as a peroxisomal enzyme, in stressed plants (Figure 5A). This can be a piece of evidence of the potential of beneficial Actinobacteria in modulating the photorespiratory pathway to scavenge produced ROS under chromium stress and protects the photosynthetic apparatus from stress damages through the excess energy dissipating process in PSII.^{22,49} Moreover, the higher Gly/Ser ratio in OSW-containing treatments compared to Bf, indicated the higher impacts of OSW in regulating leaf N metabolism and stimulating the fixation of C into amino acids,^{49,50} which is consequently crucial in handling leaf allocation of excitation energy under stress.^{49,51}

Higher levels of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in the present research revealed oxidative stress induced by chromium, in which these oxidative markers demonstrated a clear decreasing trend in response to fertilization/supplementation treatments under stress, especially in PGPB-treated plants (Figure 2A). This result is in agreement with previous studies that documented an increment in the levels of H_2O_2 and MDA in various plant tissues under heavy metal-induced stress and resulted in intense oxidative damage.^{52,53} In addition, a decrement in H_2O_2 and

MDA accumulation in plants treated with PGPB and OSW under heavy metal stress was previously reported.^{11,12} One explanation for the higher detoxification of H₂O₂ and MDA in fertilized plants under chromium-induced stress can be the higher stimulation of antioxidant enzymes and metabolites involved in the ASC/GSH pathway (ASC, GSH, APX, DHAR, MDHAR, GR, and GPX) in response to OSW-containing treatments (Figure 3A,B) and direct ROS-detoxifying enzymes (POX, SOD, and CAT) in PGPB-treated plants (Figure 2B). The association between detoxifying excess ROS in plant cells and improving the antioxidant protection mechanisms was previously reported.⁵⁴ Nevertheless, the different reactions of antioxidant enzymes to Bf and OSW treatments must be interpreted with caution because the interaction among these antioxidant pathways and other signaling molecules and metabolites under oxidative stress is not well understood.⁵⁵ In addition, the susceptibility of the components of the ASC/GSH cycle and other antioxidant pathways to oxidative stress should be considered because it can thereupon influence their antioxidant potential under stress, despite their defensive function in preserving the cellular components from oxidative damage.^{12,55}

We also focused on antioxidant molecules from different antioxidant pathways to debate whether antioxidant capacity in fertilized plants can be improved under chromium stress. Our findings provide more clearance for the improvement of the levels of the main antioxidant molecules, including vitamins (tocopherols), polyphenols, flavonoids, and TAC, in plants treated with PGPB and OSW treatments (Figure 4A).^{11,12,56} These molecules are reported to play a crucial role in acclimating the plant to the stress conditions,^{11,12} especially by protecting the photosynthesis machine.⁵⁷ Consequently, it seems that the accumulation of antioxidant molecules, detected in the current research, especially in PGPB-treated plants, is one of the most important preservation strategies under chromium stress.

Although the accumulation of chromium in the plant shoots and rhizosphere did not decrease in response to applied fertilization/supplementation treatments (Figure 7), due to the increase of detoxification marker parameters, especially under chromium stress conditions (Figure 6B), their possible positive effect in boosting plant tolerance cannot be ignored. Increments in the contents of phytochelatin and metallothioneins (MTC) in the plants treated with Bf and OSW under chromium stress (Figure 6B) can support previous studies, which documented the activation of a complex network of detoxification mechanism in plant cells under heavy metal stress, through chelating of metal ions with phytochelatin and MTC in the cytosol, and subsequently, sequestering into the vacuole.^{58,59} Moreover, higher accumulation of glutathione S-transferases (GST) in PGPB- and OSW-treated plants under chromium stress is consistent with other studies, in which GST was proposed as a protective agent against oxidative damage by quenching the ROS molecules with the addition of GSH in heavy metal-induced stress.⁶⁰ The increase of enzymatic and nonenzymatic antioxidants, metabolites, and detoxification activity in stressed plants, especially those treated with beneficial Actinobacteria in our study led to an improvement in dry matter production in plants (Figure 6).

It seems possible that the improvement of Cr tolerance of wheat is due to utilizing mechanisms of toleration and detoxification of heavy metals and still producing chelating

agents that bind metals and lessen their toxicity by Actinobacteria.^{61,62} It was earlier reported that metal accumulation/biotransformation is an alternative strategy employed by beneficial bacteria for metal detoxification.⁶³ The ability of olive waste in improving plant tolerance against oxidative stress has been suggested to be linked with its higher levels of metabolites, in particular, phenolic compounds including caffeic acid, *p*-coumaric acid, vanillic acid, syringic acid, gallic acid, etc.^{64,65} Further, the protection mechanism, based on antioxidant activities, such metabolites, have been spotlighted for their scavenger effects against free radicals and ROS, as well as the capability to function as chelators of heavy metals and the ability to inhibit lipoxygenase.⁶⁵

The improvement of some parameters in the combined treatment of OSW and beneficial Actinobacteria might have something to do with the higher levels of aerobic bacteria activity (e.g., Actinobacteria) in soil.⁶⁶ In this regard, however, the current investigation was limited by not measuring soil biological parameters, it has already been proved that olive waste can significantly improve soil respiration and soil enzyme activity, which in turn could improve the efficiency of beneficial bacteria.^{66,67}

5. CONCLUSIONS

The current study was based on understanding the active mechanisms of the wheat plant under chromium stress when they were treated with plant growth-promoting Actinobacteria and OSW. Increments in the content of oxidative markers and alterations in numerous physiological and biochemical parameters in stressed plants indicated severe oxidative damage in the control plants under chromium-induced stress. Referring to the hypothesis mentioned above, it is now conceivable to declare that the wheat plants have benefited from both PGPB and OSW treatments (individually or in combination); however, their synchronous impact was more noticeable under stress. The great potential of PGPB and OSW was represented as stimulated biomass production, improved photosynthetic pigments and capacity, accumulated secondary metabolites in plants, and activated antioxidant pathways. Therefore, the application of beneficial Actinobacteria and OSW as biofertilization/supplementation is expected to have a worthwhile consequence in enhancing plant tolerance and adaptability to chromium toxicity.

Nonetheless, it is unfortunate that the study did not include some soil biological and physicochemical parameters, such as the status of soil nutrient content, microbial activity and diversity, cation exchange capacity, water-holding capacity, pH, etc. Therefore, the generalizability of the findings of the present research is subject to certain limitations and consequently, further investigations using the same experimental setup are strongly recommended.

■ ASSOCIATED CONTENT

Data Availability Statement

The data presented in this study are available upon request from the corresponding author.

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.3c02447>.

Tow-way ANOVA analysis of the effect of plant growth-promoting Actinobacteria and OSW on studied physiological and biochemical parameters (PDF)

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Notes

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