Effects of *Bacillus cereus* on physiological and biochemical characteristics of wheat under arsenic and cadmium stress: A biological agent to reduce heavy metal stress☆

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A B S T R A C T

B. cereus is an important plant growth promoting bacteria. It provides sustainable ways to increase plant yield as well as protection from environmental stress such as heavy metals. *B. cereus* can both increase plant nutrition and act as an osmoprotection agent. This study investigated seed priming of *B. cereus* into *T. aestivum* seeds under arsenic and cadmium stress. Growth parameters, gas exchange, photosynthesis efficiency, and antioxidant capacity were measured. As and/or Cd application caused 32 %, 18 % and 24 % decrease in the relative growth rate (RGR) in the As, Cd, and combined stress (As+Cd) groups, respectively. High amounts of reduction of photosynthetic parameters detected on *T. aestivum* under stress. *B. cereus* applications caused recovery in these parameters as well as increased proline accumulation. The increases in F_v/F_m and F_v/F_o indicated that photosynthesis system efficiency in PSII reaction center recovered. As and/or Cd stress caused ROS accumulation in *T. aestivum*. By *B. cereus* priming indicated ROS stress recovered with increased activity of SOD by 42.8 % in As+*B* group and 11.80 % in Cd+*B* group. With application of bacteria other antioxidant enzymes also showed boosted activity therefore reducing H_2O_2 content and preventing peroxidation. Bacteria application is also boosted AsA-GSH cycle in under both stresses. Therefore, seed priming with *B. cereus* is highly effective against heavy metal stress by reducing the availability of heavy metals and, ROS accumulation and increasing photosynthetic efficiency and antioxidant system for plants.

1. Introduction

Biological agents are known for their sustainability and eco-friendly structure. It is common to use microbes against heavy metal contaminated soil for bioremediation purposes (González Henao and Ghneim-Herrera, 2021; Gupta et al., 2023; Riseh et al., 2023). Different bacteria genera like *Bacillus* spp. (Jabeen et al., 2022; Khanna et al., 2019; Wrobel et al., 2023), *Acinetobacter* sp. (Qadir et al., 2021; Riseh et al., 2023) or *Enterobacteriaceae* sp. (Ajmal et al., 2022; Riseh et al., 2023) have some mechanisms to reduce the effects of heavy metals in

the environment as well as assist the phytoremediation process by stimulating plant growth, bioaccumulation, and reducing heavy metal concentration on the soil. The concept of Plant Growth Promoting Bacteria (PGPB) is well-established and remains a focal point in contemporary research. To be considered a PGPB, bacterial species must possess specific characteristics, including phosphate solubilization, nitrogen fixation, indole acetic acid (IAA) production, cytokines and gibberellin production, bio-fertilization, and stress tolerance (Denaya et al., 2021; Ruiu, 2020). Microbes have demonstrated a positive impact on plant health, alleviating stress effects in various plants such as

Triticum aestivum (Ajmal et al., 2022), *Stevia rebaudiana* (Hajar et al., 2014), *Secale montanum* (Rahnama et al., 2023), and *Lycopersicon esculentum* (Khanna et al., 2019). The mechanisms of PGPB are diverse, and each bacterial genus exhibits both adverse and unique effects on different plants (El-Esawi et al., 2018; Khan et al., 2020; Zhou et al., 2022).

B. cereus, a member of *Bacillus* spp., specifically selected for alleviating heavy metal stress in plants, was utilized in this study due to its origin in soil, enabling it to thrive in diverse environments, while offering optimal growth conditions for plants (Wrobel et al., 2023). *B. cereus* is a gram-positive, motile, aerobic, and endospore-forming bacterium (Friedman, 2023). *B. cereus* uses different mechanisms to reduce the stress of plants. The first of these mechanisms is metal accumulation in the cells and the reduction of the available heavy metal stress in the environment (Sharma and Shukla, 2021). By using different metal transporters and resistance genes, *B. cereus* can resist and adsorb different metals and reduce heavy metal concentration from the soil therefore reducing the stress effects on plants (Ayangbenro and Babalola, 2020; Huang et al., 2024; Jabeen et al., 2022). For these reasons using *B. cereus* is common against heavy metal stress alleviation especially for hyperaccumulators (Mishra et al., 2017).

Heavy metals (HMs) are characterized by their high atomic weight and density, posing toxicity to living organisms due to their impact on proteins and enzymes (Sadak, 2023). They are categorized as essential and non-essential HMs, with essential HMs, also known as micronutrients, required for various biological processes like growth and metabolism (Raychaudhuri et al., 2021). Despite being necessary in trace amounts, exceeding threshold values for essential HMs can have toxic effects on both plants and the environment (Narayanan and Ma, 2023). Heavy metal toxicity disrupts chemical messengers such as phytohormones, directly affecting the health and therefore yield of plants (Sharma et al., 2022). Each plant has a unique threshold concentration for heavy metals, determined by its coping mechanisms and tolerance levels for individual heavy metals. Once this threshold is surpassed, plants activate mechanisms to cope with heavy metals, a condition known as heavy metal stress. Heavy metal stress in plants is one of the highly focused and investigated areas in modern research. In the world currently around 500-million-hectare area soils are contaminated with different heavy metals (González Henao and Ghneim-Herrera, 2021; Li et al., 2019). Several countries suffer from different amounts and different types of heavy metals and some of these heavy metal contaminations are at very serious levels (Chao et al., 2014; Li et al., 2019). Under heavy metal stress, different plants, and even different genotypes of the same species, are affected distinctively (Salinitro et al., 2020; Shafiq et al., 2020; Skiba et al., 2020). However, it is known that heavy metal stress causes reactive oxygen species (ROS) accumulation in plant cells. These ROS led to the oxidization of lipids, carbohydrates, chlorophylls, nucleic acids, and proteins.

Plants have evolved different kinds of mechanisms to deal with ROS both enzymatically and non-enzymatically (Rajput et al., 2021). Non-enzymatic defense mechanisms include some water/lipid-soluble antioxidants such as ß-carotene and α-tocopherol (Mishra et al., 2023). Enzymatic mechanisms include cascades and individual enzymes working together to scavenge different ROS elements like singlet oxygen ($^1O_2$), Hydrogen peroxide (H_2O_2), and superoxide ($O_2^{\bullet-}$). These enzymes show different physiological responses, structural changes, or degradation of molecules (Mansoor et al., 2022). There are several enzymes to deal with ROS, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), glutathione reductase (GR), glutathione S-transferase (GST), glutathione peroxidase (GPX) and many more enzymes (Dvorak et al., 2020; Hasanuzzaman et al., 2020; Rajput et al., 2021; Zhao et al., 2021).

T. aestivum is one of the most consumed and produced plants by humans. According to FAO, in 2022%8 of total agricultural products is wheat and about%90–95 of those wheat is *T. aestivum* (Giraldo et al., 2019; Padilla-Torres et al., 2022; *World food and agriculture – statistical yearbook,* 2022). *Triticum aestivum* can undergo substantial adverse effects due to heavy metal toxicity, resulting in diminished plant growth, enzyme activity, and metabolism (Abedi and Mojiri, 2020; Saeed et al., 2021). The transfer of heavy metals within plant tissues poses risks to both human and animal health (Shi et al., 2015). Consequently, it is imperative to mitigate heavy metal stress in plants, particularly in *T. aestivum*, through biological approaches to contribute to the attainment of global sustainability goals. The aim of this study is to see how ROS scavenging enzymes changed after *B. cereus* application of plants with heavy metal stress. The study hypothesis is *B. cereus* might protect the *T. aestivum* by adsorbing some of heavy metals from the environment therefore making it less accessible to the plants and reducing the oxidative stress caused by heavy metal stress. As a result, increasing our knowledge about bacteria and plant interaction in stressed environments and reveals a way to deal with heavy metal in more sustainable ways.

Using biological organisms to improve the efficiency of agriculture and plant health is an important pathway to achieve global sustainability. PGPB has unique mechanisms that improve both plant and soil health through changing environmental stresses and global climate. To boost plant antioxidant system before the stress occurrence as well as increasing plant growth, bacterial seed priming is one of the sustainable approaches. Against different stresses, *B. cereus* can act as both bioprotector and fertilizer for plants. *B. cereus* can reduce both heavy metal related stress via antioxidant enzyme pathways as well as increase nutrient uptake of plants by boosting available ions in the environment. Scientific knowledge about the photosynthetic mechanisms together with microorganisms under multiple heavy metal stresses are unclear. For that reason, in this study, which pathways of antioxidant enzyme systems by *Bacillus cereus* priming are changed positively or negatively under the stress of As and/or Cd are investigated. One other aim of this study is to determine changing fluorescence kinetics as well as the effect on growth rate, water content, and gas exchange inside wheat (*T. aestivum*) seeds primed with *B. cereus* under toxic levels of As and/or Cd stress.

2. Materials and methods

2.1. Materials

The strain of *Bacillus cereus* ATCC 11,778 was obtained from the Microbiology Research Laboratory of Vocational School of Health Services.

2.2. Bacteria storage and incubation method

The maintenance of the strain was performed on Luria Bertani Agar. For this purpose, bacterium was inoculated by single colony method and incubated on LB agar medium at 37 °C for 18–24 h.

2.3. Determination of arsenic and cadmium resistance

In order to determine of resistance levels of bacterium against heavy metals, the method described by Ozfidan-Konakci et al. (2023) was carried out with some modification. The heavy metal salts were prepared at a concentration of 0.0192 M as a stock solution. The Mueller-Hinton Broth medium was distributed to all wells of plate as 100 μL. The stock heavy metal salts (100 μL) were transferred to first well of plate. After mixing of medium and solutions, 100 μL amount of mix transferred to second wells. This process continued sequentially for other wells and serial dilutions were made by multi-channel pipette. After dilutions, the concentration in the wells ranged from 4800 to 37.5 μM. The inoculum of bacterium was prepared from the fresh overnight culture grown in Brain-Hearth infusion broth. The culture was adjusted as 0.5 McFarland turbidity. The last concentration of bacterial inoculum

was diluted to 5×10^5 cfu/ml. Then 100 μL bacterial inoculum transferred to whole wells of plate. The micro plate incubated at 37 °C for 18–24 h. At the end of the incubation period 2,3,5 TTC was added to all wells for monitoring of microbial growth and determination of minimum inhibitory concentration (MIC) (Nibras Qader et al., 2022). It was defined that *Bacillus cereus* strain was resistant to sodium arsenate up to the 2400 μM concentration. Also, cadmium resistance levels were determined for this strain. Our study revealed that *B. cereus* was combated with cadmium up to the 300 μM dose (Fig. S1).

2.4. Adjusting of bacterial inoculum for seed priming

One single colony of *B. cereus* was picked from the LB Agar medium then it was inoculated into the Nutrient Broth No: 2 (Oxoid) and it was incubated with the condition of agitation by shaker incubator at 150 rpm for 18 h. Nearly $2–3 \times 10^8$ cfu/mL of bacterial inoculum was prepared by adjusting of the inoculum as 1 Mc Farland turbidity in the sterile sucrose solution (3 %). Disinfection of the seeds were performed in sodium hypochlorite (1 %). The seed priming process were carried out according to method described by Ozfidan-Konakci et al. (2023). The seeds exposed to bacterium and sterile distilled water (for control group) were placed into the petri plates onto the sterile filter papers. Then these plates were incubated for seven days under the controlled conditions.

2.5. Identifying sidephore production for bacteria

For sidephore production detection assay, Chrome Azurol S (CAS) (agar) and CAS (liquid) media is used. In CAS agar assay, formation of orange zone accepted as positive for siderophore production, whereas in CAS liquid assay color change in media accepted as positive for siderophore production (Himpsl and Mobley, 2019).

2.6. Experimental methodology

Wheat (*T. aestivum*) seeds were surface sterilized with sodium hypochlorite and alcohol and after seed priming, seedlings were transferred to Hoagland solution under controlled conditions. The hydroponic medium was refreshed every other day during the 21-day growth period. For stress treatment, 100 μM As and/or 100 μM Cd was applied to wheat seedlings by adding to Hoagland solution. Plants were harvested after one weeks treatment period. For these applications, sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) and cadmium chloride ($CdCl_2 \cdot 5H_2O$) is used. Heavy metal dose selection is done in accordance with previous studies for arsenic (da-Silva et al., 2017; Kumari et al., 2018; Singh et al., 2021) and selected cadmium (Cd) dose is also accordance with previous studies (Hasanuzzaman et al., 2018; Ozfidan-Konakci et al., 2018; Ur Rahman et al., 2021; Yotsova et al., 2020).

2.7. Elemental analysis

The endogenous contents of As and Cd were analyzed by ICP-AES (Varian-Vista) and the measurements were completed for 0.1 g dry leaf samples in triplicates (Nyomora et al., 1997).

2.8. Analysis of physiological parameters

The suggested procedure by Hunt et al. (2002) used to determine the relative growth rate (RGR). Six leaves per group were harvested after treatment, and their relative water content (RWC) was determined using Maghsoudi et al. (2019) formula. The LCpro+ portable device was used to measure gas exchange variables on the leaves of three plants in every treatment group (ADC, Hoddesdon, UK). The stomatal limitation value was derived as the C_i/C_a ratio deviation from 1 (L_s). Proline content was determined in 0.5 g fresh samples followed by Chandrakar et al. (2016).

2.9. Recording data for chlorophyll a fluorescence parameters

A portable fluorometer (Handy PEA, Hansatech Instruments Ltd., Norfolk, UK) was used to determine the maximal quantum yield of PSII photochemistry (F_v/F_m), physiological state of the photosynthetic apparatus (F_o/F_m) and potential photochemical efficiency (F_v/F_o). Many parameters showing the structure and function of photosynthetic apparatus were detected by Handy PEA (Plant Efficiency Analyser, Hansatech Instruments Ltd.).

2.10. Oxidative stress biomarker assays and confocal laser scanning microscopy

Determination of H_2O_2 content was measured according to Velikova et al. (2000). Leaves were homogenized in cold acetone and centrifuged. The supernatant was mixed with titanium reagent and then ammonium hydroxide was added to precipitate the titanium-peroxide complex. The reaction mixture was centrifuged. The pellet was washed with cold acetone and dissolved. The absorbance of the solution was measured at 410 nm. H_2O_2 concentrations were calculated using a standard curve prepared with known concentrations of H_2O_2. Lipid peroxidation (thiobarbituric acid reactive substances (TBARS) content) was determined according to Rao and Sresty (2000). TBARS concentration of leaf samples was calculated from the absorbance at 532 nm, and measurements were corrected for nonspecific turbidity by subtracting the absorbance at 600 nm. The concentration of TBARS was calculated using an extinction coefficient of 155 mM cm^{-1}.

2.11. Identification of enzyme/non-enzyme compositions related to the antioxidant system and asa-gsh cycle

For protein and enzyme extractions, 0.5 g of each leaf sample was homogenized in 50 mM Tris–HCl (pH 7.8) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.2 % Triton X-100, 1 mM phenylmethylsulfonyl fluoride and 2 mM dithiothreitol (DTT). The total soluble protein content of the enzyme extracts was determined (Bradford, 1976). Superoxide dismutase (SOD) isozyme/enzyme activity was defined (Beauchamp and Fridovich, 1971; Laemmli, 1970). The activity of catalase (CAT) isozyme/enzyme was determined using the procedure suggested by Woodbury et al. (1971) and Bergmeyer (1970). The isozymes/enzyme capacity of peroxidase (POX) was measured according to the method suggested by Seevers et al. (1971) and Herzog and Fahimi (1973). The enzyme/isozyme activities of glutathione S-transferase (GST) and glutathione peroxidase (GPX) were determined (Hossain et al., 2006; Ricci et al., 1984). The isoforms and total NADPH oxidase (NOX) activity were calculated (Jiang and Zhang, 2002; Sagi and Fluhr, 2001).

Ascorbate peroxidase (APX) and glutathione reductase (GR) were spectrophotometrically and electrophoretically carried out (Mittler and Zilinskas, 1993; Nakano and Asada, 1981). The contents of ascorbate (AsA) and oxidized ascorbate (DHA) were estimated (Dutilleul et al., 2003). The procedure for monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) was performed (Dutilleul et al. (2003). Glutathione (GSH) was assayed according to Paradiso et al. (2008), utilizing aliquots of supernatant neutralized with 0.5 M K-P buffer. Based on enzymatic recycling, glutathione is oxidized by DTNB and reduced by NADPH in the presence of GR, and glutathione content is evaluated by the rate of absorption changes at 412 nm. Oxidized glutathione (GSSG) was determined after removal of GSH by 2-vinylpyridine derivatization. Standard curves with known concentrations of GSH and GSSG were used for the quantification. GSH redox status was obtained (Shi et al., 2013).

Gels stained for SOD, POX, APX, GR, GST, and NOX activities were photographed with the Gel Doc XR+ System and then analyzed with Image Lab software v4.0.1 (Bio-Rad, California, USA). Known standard amounts of enzymes (0.5 units of SOD and CAT and 0.2 units of POX)

were loaded onto gels. For each isozyme set/group, the average values were significantly different at $p < 0.05$ using Tukey's post-test.

2.12. Statistical analysis

The experiments were repeated thrice independently, and each data point was the mean of six replicates. All data obtained were subjected to a one-way analysis of variance (ANOVA). Statistical analysis of the values was performed by using SPSS 20.0. Tukey's post-test was used to compare the treatment groups. Comparisons with $p < 0.05$ were considered significantly different. In all figures, the error bars represent the standard errors of the means.

3. Results

3.1. Characterization and identification of probiotic characteristics and heavy metal resistance of B. cereus

B. cereus is identified through both biochemical and MALDI-TOF analysis. The bacterial culture incubated on Nutrient Agar (NA).Bacteria stroked on the MALDI-TOF-MS device after the addition of necessary chemicals showed a score above 2. Agar plate morphology confirmed these results. For probiotic characteristics, a CAS agar plate was used, which exhibited positive results by showing a zone area in the petri dish. After confirmation with the agar plate, the culture was also passaged to CAS liquid media to demonstrate siderophore production. Regarding heavy metal resistance, the bacterial culture cultured on NA and passaged to a 96-well plate with known concentrations of cadmium (Cd), arsenic (As), and mercury (Hg) heavy metals showed resistance to As up to a concentration of 2400 μM, Cd up to 300 μM (Fig. S1).

3.2. The effects of B. cereus on growth, water relations, proline content As and Cd content in leaves

SEM images presented in Fig. 1 reveal bacterial adhesion at the end of the experimental period in plant roots inoculated with *B. cereus*, next to control plant roots. A 32 %, 18 % and 24 % decrease in the relative growth rate (RGR) was detected in the As, Cd and combined stress (As+Cd) groups, respectively (Fig. 2A). RGR exhibited an increasing pattern in bacterial-inoculated groups compared to the control plants. The most significant increase was observed in the As+B group, with a 66.67 % rise compared to only As, followed by the As+Cd+B group with a 40 % increase compared to As+Cd. The B and Cd+B groups showed an elevated rate of 26.41 % and 23.25 %, respectively, compared to the control and Cd groups. In Fig. 2B, it is evident that relative water content

(RWC) is affected by bacterial application. In the experimental groups (As+B, Cd+B, and As+Cd+B), a rise of 8.11 %, 13.76 %, and 19.42 %, respectively, was observed compared to the non-bacterial stress groups. No significant change was observed between the bacteria-applied control group and the control group. While As stress caused a 50 % decrease in the proline content of wheat leaves, an approximately 20 % decrease was detected in the Cd and As+Cd groups compared to the control (Fig. 2C). A 45 % increased proline accumulation was observed only in the As+B group compared to the stress-alone. Further decreasing proline content was noted in plants inoculated with bacteria under control, Cd, and As+Cd conditions. In Fig. 2D, with bacteria application As content severely dropped in the leaves with both combined stress and As stress alone as well as in Fig. 2E, Cd content in the leaves dropped with bacteria application for both Cd stress alone and combined stress group.

3.3. The effects of B. cereus on gas exchange parameters

While the stomatal conductance (g_s) of the As and As+Cd groups decreased compared to the control, g_s increased by 35 % in plants exposed to Cd stress (Fig. 3A). As+B group showed the most significant rise at a 3.8-fold increase in comparison to only As, followed by the bacterial control group at 2.16-fold, compared to the control. *B. cereus* was affected less in the Cd+B and As+Cd+B groups, with increases of 23.81 % and 27.27 %, respectively, to the non-bacterial groups. The rate of carbon assimilation (A) in wheat leaves under stress decreased significantly (Fig. 3B). A 45 % decrease in A was determined in the As and As+Cd groups and a 59 % reduction in the Cd group. *B. cereus* substantially increased the A. In the As+B, Cd+B, and As+Cd+B groups, an increment of 2.2-fold, 2.41-fold, and 79.42 %, respectively, was observed with respect to the non-bacterial applications. In the control bacterial group increased the A, but not as much as in the stress groups, with only a 24.31 % increase. Fig. 3C demonstrates intercellular CO_2 concentration (C_i), with the As+B and B groups showing increases of 15.33 % and 29.68 %, but the Cd+B and As+Cd+B groups showing decreases of 29.56 % and 28.62 % with measuring against their non-bacterial counterparts. Exposure to As stress reduced the transpiration rate (E) in leaves by 16 %, while an increase of 60 % and 21 % was noted in the Cd and As+Cd treatment groups, respectively (Fig. 3D). *B. cereus* boosted E in the stress groups, with the most significant boost in the As+B group at 3.1-fold, followed by the B group at 2.3-fold. Cd+B and As+Cd+B groups showed less boost at 39.01 % and 20.88 %, respectively all compared to the non-bacterial groups. Fig. 3E displays stomatal limitation values (L_s), and again, the As+B and B groups differ from the Cd+B and As+Cd+B groups. However, this time, the As+B and B groups show declines of 37.96 % and 58.06 %, while the Cd+B and
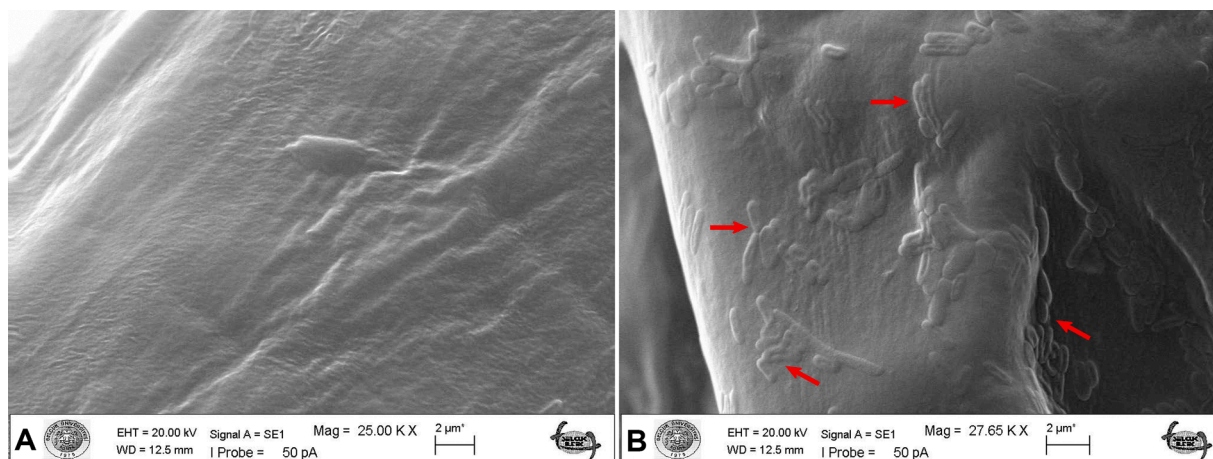

Fig. 1. SEM images of bacterial adhesion at the end of the experimental period in plant roots inoculated with *B. cereus* (**A**), SEM images of control group in the roots of *T. aestivum* (**B**).
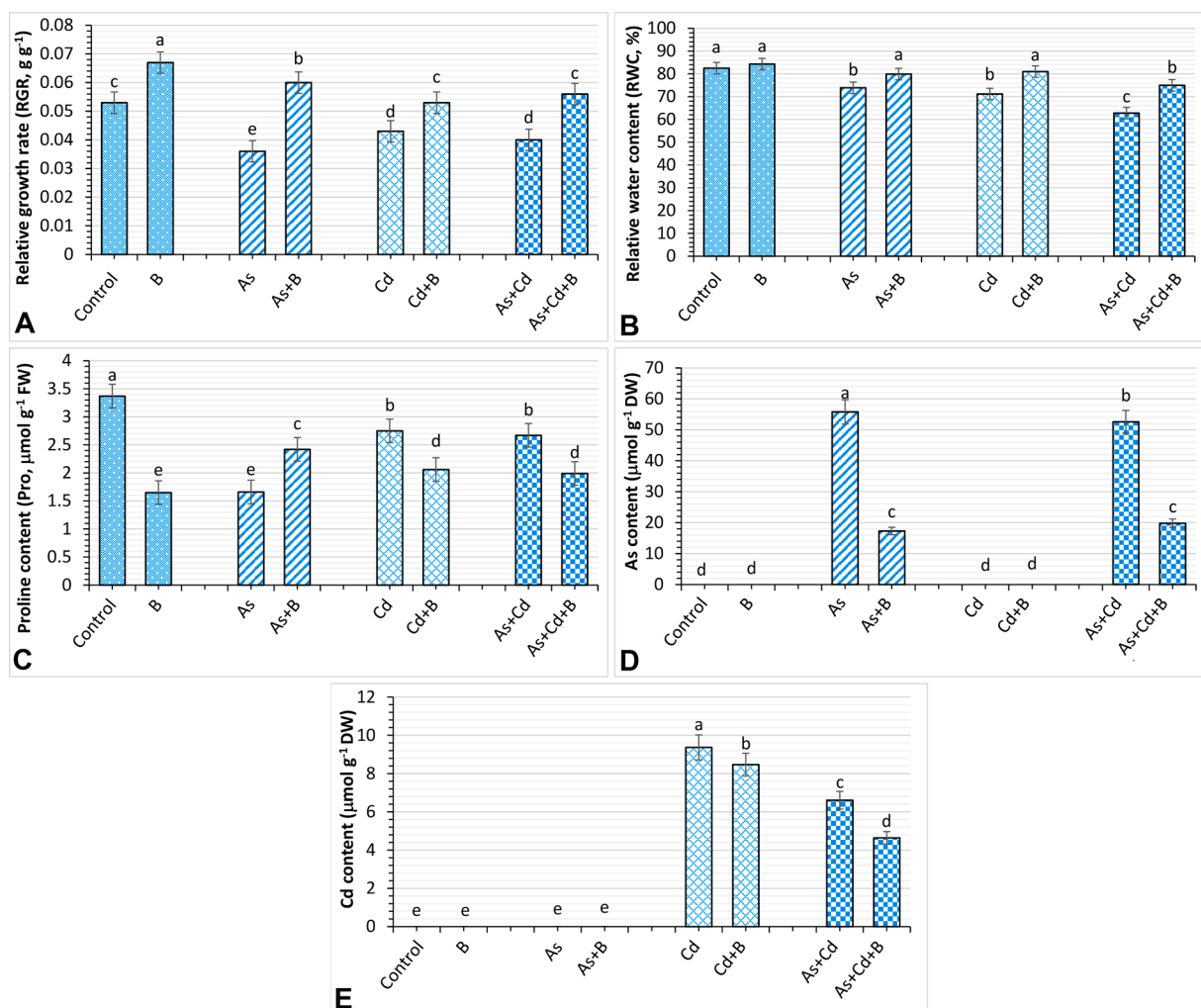
Fig. 2. The relative growth rate (RGR, **A**), relative water content (RWC, **B**), proline content (Pro, **C**), arsenic content (As, **D**), cadmium content (Cd, **E**), in the leaves of *T. aestivum* treated with bacteria under arsenic (As, 100 μM) and/or cadmium (Cd, 100 μM) stress. All data obtained were subjected to a one-way analysis of variance (ANOVA). Differences were considered to be significant at $p < 0.05$.

As+Cd+B groups show augmentations of 57.65 % and 104.29 % again with comparison of their non-bacterial peers. Fig. 3F showed a decline in the carboxylation efficiency in stress-applied plants 42.14 %, 65.58 % and 35.63 % for As, Cd and As+Cd groups respectively. However, the bacteria inoculation enabled the recovery of carboxylation levels to the control levels under stress conditions.

3.4. The effects of B. cereus on photosynthetic efficiency and photosynthetic machinery

In Fig. 4, slightly increased F_v/F_m rates were observed with bacterial application in stress groups, with the highest increase in the Cd+B group at 12.59 %. F_v/F_o also heightened with bacterial application in the As+B, Cd+B, and As+Cd+B groups, with increases of 16.13 %, 43.08 %, and 38 %, respectively, in contrast with non-bacterial groups. F_o/F_m is especially lowered with the curative application of *B. cereus* in the As+B, Cd+B, and As+Cd+B groups, with decreases of 10.81 %, 19.72 %, and 22.4 %, respectively.

3.5. The effects of B. cereus on the contents of H₂O₂ and TBARS

Fig. 5A presents confocal microscope images obtained from the treatment groups, indicating ROS accumulation in stomata. While the control and B group showed no significant difference in H₂O₂ content,

with the application of *B. cereus*, stress groups had significant drops in H_2O_2 content (Fig. 5B). Also, there is a sharp boost in H_2O_2 content in stress groups compared to the control group. H_2O_2 content is increased in the As group by 15.09 %, in the Cd group by 24.81 %, and in the As+Cd group by 19.05 %. H_2O_2 content dropped in the As+B, Cd+B, and As+Cd+B groups, with decreases of 13.67 %, 22.23 %, and 10.41 %, respectively compared to the only stress groups. The same conditions also occurred with TBARS content (Fig. 5C). When stress was applied, TBARS content gradually elevated in As, Cd, and As+Cd groups respectively 22.62 %, 69.97 %, and 77.26 %. TBARS content descended in the As+B, Cd+B, and As+Cd+B groups, with decreases of 18.21 %, 28.30 %, and 9.21 %, respectively with comparison to their non-bacterial groups.

3.6. The effects of B. cereus on the antioxidant enzyme/isoenzyme activities

Fig. 6A reveals a total of four bands of SOD isoenzymes (all of them are Mn-SOD) in *T. aestivum* leaves. Except for combined stress, individual stresses boosted SOD activity with reference to the control group. All bacteria-inoculated groups showed increased SOD activity compared to non-bacterial groups, with the highest increase in the B group at 72.8 % (Fig. 6B). Increased band intensity supports the increased activity of individual stressed groups. In native gel analysis, CAT had only one
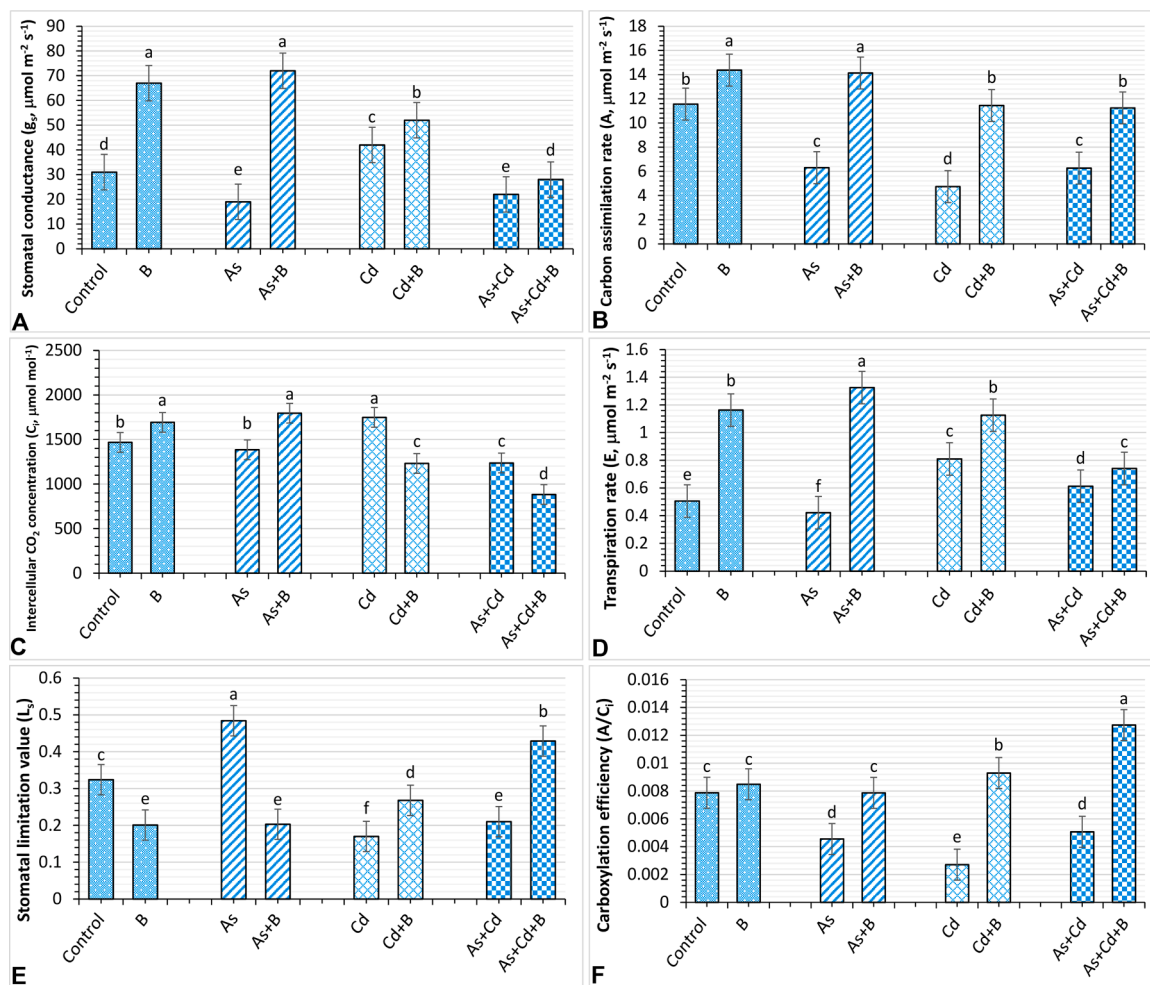
Fig. 3. Stomatal conductance (g_s, **A**), Carbon assimilation rate (A, **B**), intercellular CO_2 concentrations (C_i, **C**), transpiration rate (E, **D**), stomatal limitation rate (L_s, **E**), and carboxylation efficiency (**F**) in the leaves of *T. aestivum* treated with bacteria under arsenic (As, 100 μM) and/or cadmium (Cd, 100 μM) stress. All data obtained were subjected to a one-way analysis of variance (ANOVA). Differences were considered to be significant at $p < 0.05$.

Fig. 4. The maximal quantum yield of PSII photochemistry (F_v/F_m), potential photochemical efficiency (F_v/F_o), and physiological state of the photosynthetic apparatus (F_o/F_m) in the leaves of *T. aestivum* treated with bacteria under arsenic (As, 100 μM) and/or cadmium (Cd, 100 μM) stress. All data obtained were subjected to a one-way analysis of variance (ANOVA). Differences were considered to be significant at $p < 0.05$.
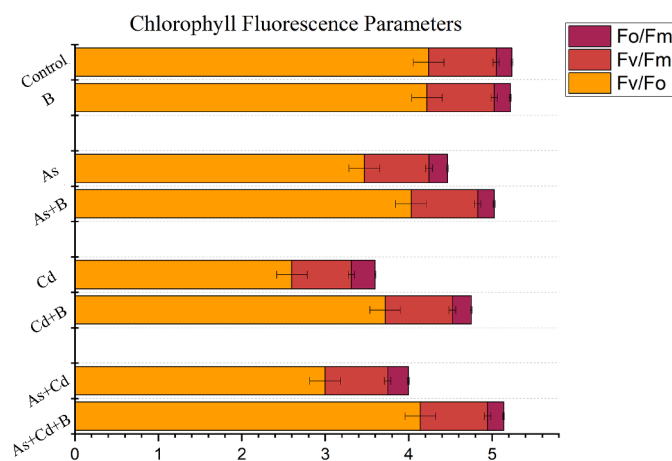
isozyme (Fig. 6C). CAT activity showed a significant increase in the bacterial control group; however, bacterial application with stress showed a decrease in CAT activity in all bacteria+stress groups compared to only stress groups, with the lowest in the As+Cd+B group with a 49.46 % reduction (Fig. 6D).

As can be seen in Fig. 7A, five POX isoenzymes were detected in wheat leaves (POX1–5). POX activity declined gradually in every stress group, and the lowest was with the combined stress group with an 86 % decrease (Fig. 7B). POX activity is boosted with bacterial application, especially in the As+*B* and As+Cd+B groups, with increases of 44.64 % and 2.19-fold, but interestingly showed a fall in the Cd+B group with 24.65 %. In Fig. 7C, NOX enzyme showed three isoenzymes (NOX1–3). NOX enzyme activity showed a rise in all stress groups compared to the control group, with the highest in the Cd group with a 2-fold increase (Fig. 7D). With bacteria application, stress groups showed a slight fall in NOX activity (except As+B), with the lowest in the As+Cd+B group at 16.29 % with compared to combined stress group.

Isoenzyme profiling showed that GST has seven isoenzymes (GST1–7) (Fig. 8A). GST activity is heightened with all stress application groups compared to control, and the sharpest increase is in the As group at 61.68 % (Fig. 8B). Bacterial application on stress groups also boosted the GST activity in the As+B, Cd+B, and As+Cd+B groups, with increases of 54.07 %, 28.22 %, and 33.41 %, respectively. Native PAGE gel analysis revealed that GPX has 3 isoenzymes in wheat leaves (GPX1–3) (Fig. 8C). While a 14 % increase in GPX activity was detected in plants exposed to As stress, an 8 % and 29 % decrease was detected in the Cd
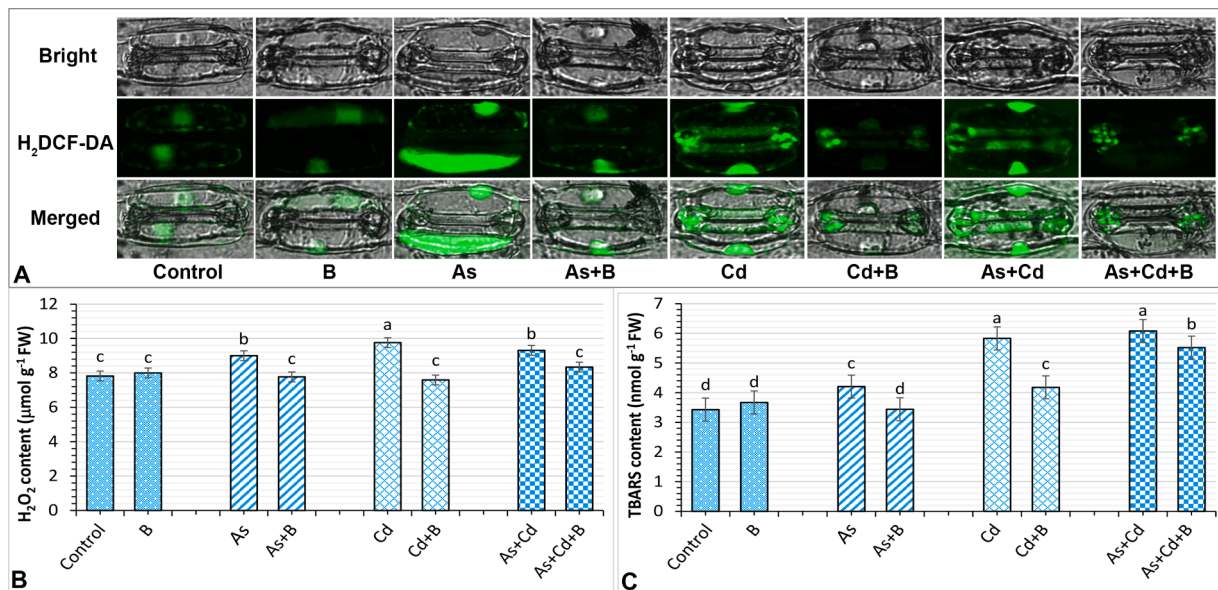
Fig. 5. Accumulation of H_2O_2 in guard cells identified with the 2,7-dichlorofluorescein diacetate (H_2DCF-DA) under confocal microscopy (**A**), the changes of hydrogen peroxide content (H_2O_2, **B**) and lipid peroxidation level (TBARS content, **C**) in the leaves of *T. aestivum* treated with bacteria under arsenic (As, 100 μM) and/or cadmium (Cd, 100 μM) stress. All data obtained were subjected to a one-way analysis of variance (ANOVA). Differences were considered to be significant at *p* < 0.05.

Fig. 6. The relative band intensity of different types of superoxide dismutase isoenzymes (SOD, **A**), total SOD activity (**B**), catalase isoenzymes (CAT, **C**) and total CAT activity (**D**) in the leaves of *T. aestivum* treated with bacteria under arsenic (As, 100 μM) and/or cadmium (Cd, 100 μM) stress. All data obtained were subjected to a one-way analysis of variance (ANOVA). Differences were considered to be significant at *p* < 0.05.
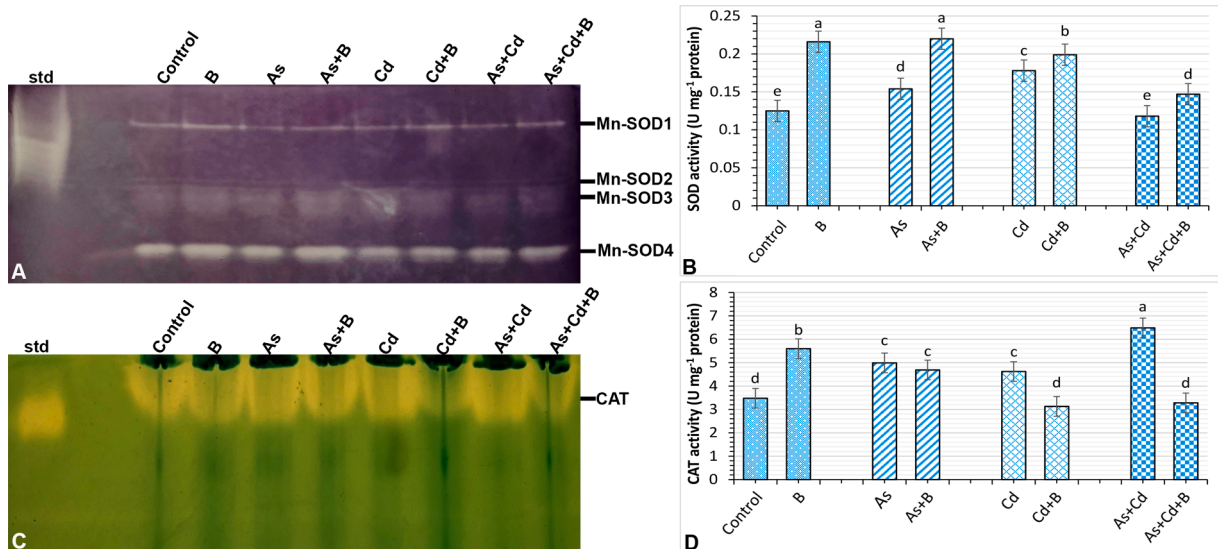
and As+Cd groups, respectively (Fig. 8D). It was observed that GPX activity was suppressed in all treatment groups inoculated with bacteria. Except, a 42 % increase was determined in the As+Cd+B group compared to the combined stress group.

Examining the APX native PAGE gel showed three different isoenzymes (APX1–3) (Fig. 9A). While individual stress groups showed a rise in APX activity, combined stress showed lower APX activity than the control group (Fig. 9B). APX activity increased in As+Cd+B with 67.98 % while Cd+B has 27.37 % boost than non-bacterial counterpart. GR showed six isoenzymes in native PAGE gel (GR1–6) (Fig. 9C). GR activity had increased with all stress groups by around 75 % comparison to the control group (Fig. 9D). However, when bacteria were applied, the As+B group showed 37.03 % decrease but Cd+B and As+Cd+B groups risen 55.46 % and 75.20 % in GR activity compared to non-bacterial

groups.

3.7. The effects of B. cereus on the enzyme/non-enzyme capacity related to the AsA-GSH cycle

MDHAR activity didn't change much between the control and bacteria control group; however, it fluctuates in bacteria+stress groups, such as in As+*B* group, decreased slightly (39.8 %), but Cd+B and As+Cd+B groups showed heightened MDHAR activity with 3.4-fold and 62.79 %, respectively compared to their non-bacterial counterpart (Fig. 10A). DHAR activity slightly diminished in all stress groups (Fig. 10B) comparison to the control group. On the other hand, with bacteria application, all the groups have boosted DHAR activity than only stress groups, with the highest increase in As+Cd+B with 42.52 %.
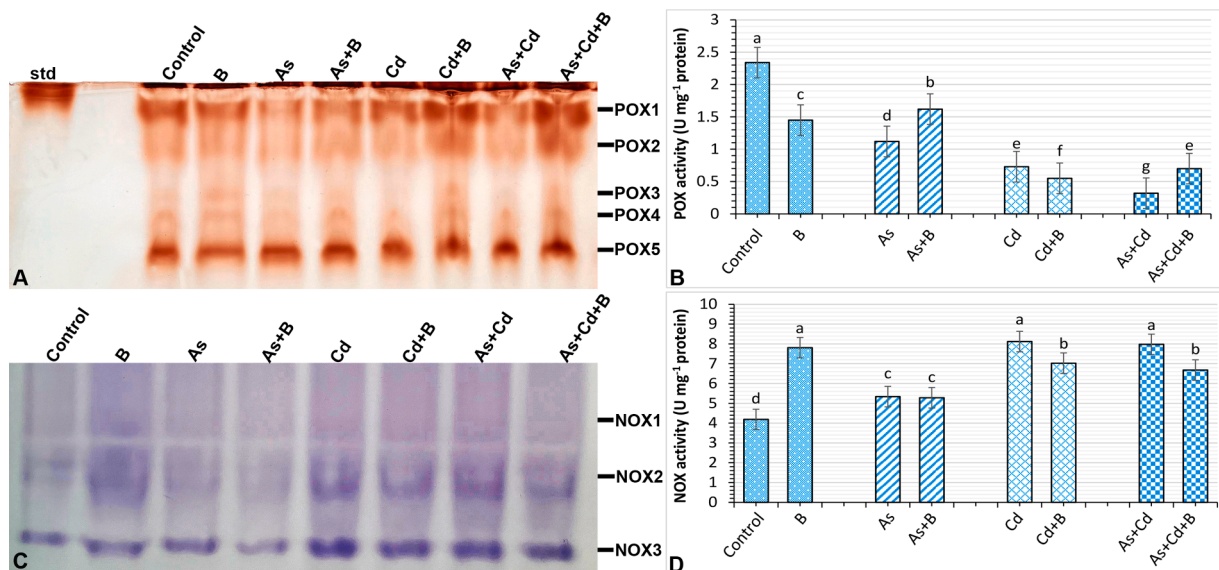
Fig. 7. The relative band intensity of different types of peroxidase isoenzymes (POX, **A**) and total POX activity (**B**), NADPH oxidase isoenzymes (NOX, **C**) and total NOX activity (**D**) in the leaves of *T. aestivum* treated with bacteria under arsenic (As, 100 μM) and/or cadmium (Cd, 100 μM) stress. All data obtained were subjected to a one-way analysis of variance (ANOVA). Differences were considered to be significant at $p < 0.05$.

Fig. 8. The relative band intensity of different types of glutathione S-transferase isoenzymes (GST, **A**) and total GST activity (**B**), glutathione peroxidase isoenzymes (GPX, **C**), and total glutathione peroxidase activity (**D**) in the leaves of *T. aestivum* treated with bacteria under arsenic (As, 100 μM) and/or cadmium (Cd, 100 μM) stress. All data obtained were subjected to a one-way analysis of variance (ANOVA). Differences were considered to be significant at $p < 0.05$.
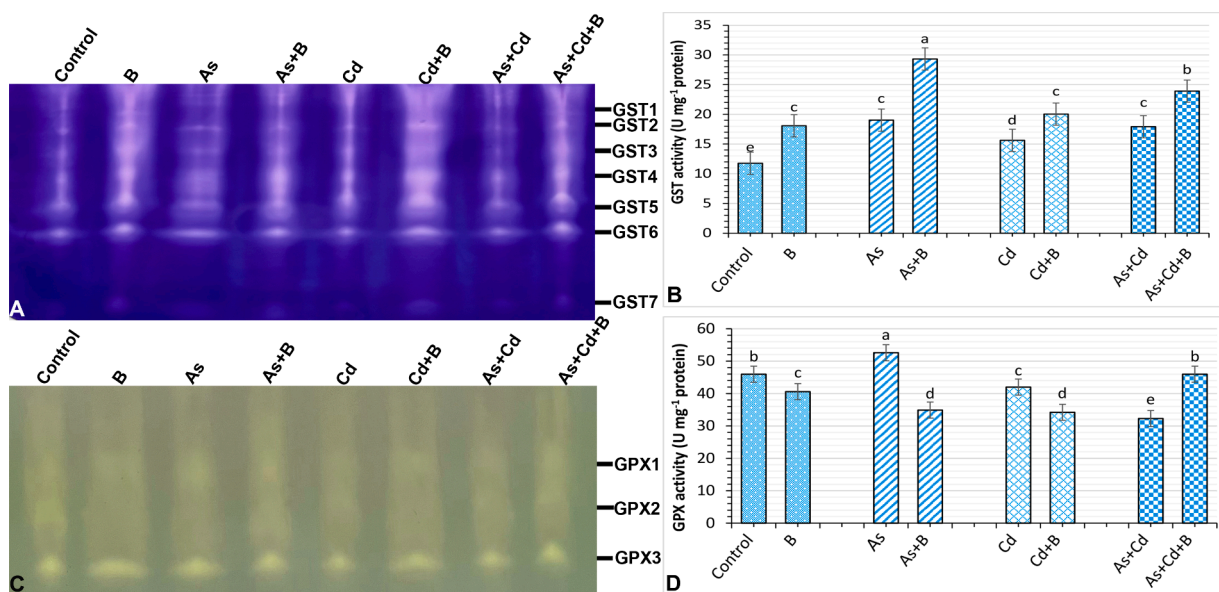
It is seen that total AsA content is decreased in stress groups by nearly 25 % with the control group taken as reference (Fig. 10C). With bacteria application, AsA content increased in all groups, with the strongest increase in Cd+B with 93.84 %. In all stress conditions, higher DHA content than the control group is evident (Fig. 10D). The highest DHA content among them is the Cd group with a 2.8-fold increase. Except for the As+B group, bacteria application lowered the DHA content compared to only stress groups severely, such as 70 % for Cd+B and 48.63 % for As+Cd+B. For the As+B group, DHA content grew by 31.92 %. GSH content is lowered in all stress groups compared to the control (Fig. 10E). Bacteria application dropped the GSH content by 18.17 % in the As+*B* group, but in Cd+B and As+Cd+B groups, it escalated by 17.56 % and 15.93 %, respectively compared to their non-bacterial groups. GSSG content had risen in all stressed groups in comparison to

the control group, with the top being 39.82 % in the As+Cd group (Fig. 10F). With bacteria, all GSSG content decreased slightly, with the lowest in As+B with 12.65 %. Exposure to heavy metal stress significantly reduced the tAsA/DHA ratio in all treatment groups (Fig. 10G). On the other hand, in bacterial inoculated plants, it increased 6.4-fold in the Cd+B group and 2.5-fold in the As+Cd+B plants. In the As+B group, no change was observed in the tAsA/DHA ratio compared to the As group. In Fig. 10H, the GSH redox state is critically crashed in stress groups compared to the control. In Cd+B and As+Cd+B groups, *B. cereus* application slightly recovered the redox state, but it has a negative effect on the As+B group.
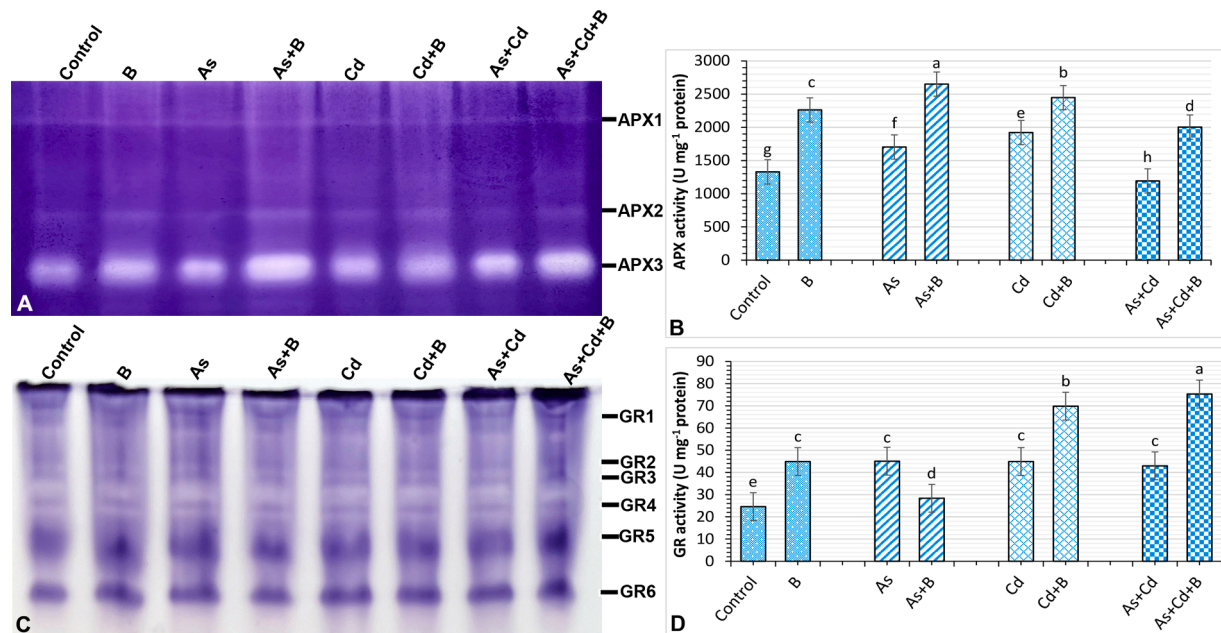
Fig. 9. The relative band intensity of different types of ascorbate peroxidase isoenzymes (APX, **A**) and total APX activity (**B**), glutathione reductase isoenzymes (GR, **C**) and total GR activity (**D**) in leaves of *T. aestivum* treated with bacteria under arsenic (As, 100 μM) and/or cadmium (Cd, 100 μM) stress. All data obtained were subjected to a one-way analysis of variance (ANOVA). Differences were considered to be significant at $p < 0.05$.

4. Discussion

4.1. Bacterial characteristics

MALDI-TOF analysis indicated that *B. cereus* showed more than score two and this result combined with NA plate agar morphology and SEM visualizations, proves that used bacteria is indeed *B. cereus* (Manzulli et al., 2021; Ramarao et al., 2020). Also, probiotic characteristics of *B. cereus* are shown by forming zone in CAS agar plate as well as color change in CAS liquid media tests positive for siderophore production with the supporting of heavy metal resistance tests (Fig. S2). This study shows parallel results with previous research on *B. cereus* (Ayangbenro and Babalola, 2020; Enis, 2014; Körçoban, 2020).

B. cereus is known to be a growth-promoting factor for plants (Khan et al., 2019; Magar et al., 2022). *B. cereus* uses different coping mechanisms to deal with arsenic (As), such as arsenite methylation and arsenite oxidation (Magar et al., 2022). As resistance proteins as well as cadmium (Cd) resistance proteins also present in most of *B. cereus* (Ayangbenro and Babalola, 2020; Banerjee et al., 2021). With similar mechanisms, *B. cereus* can also resist some level of Cd and act as a PGPB in Cd-contaminated soils (Jabeen et al., 2022). In this study, *B. cereus* also showed resistance against As and Cd which correlates with recent studies (Noreen et al., 2020; Sahile et al., 2021).

4.2. Effects of B. cereus priming on wheat seedlings exposed to heavy metal stress

Heavy metals especially As and Cd heavily disrupt the plant cell redox reactions, photosynthesis pathways and cause oxidative damage to lipids and proteins (Özyiğit et al., 2021; Saeed et al., 2021; Singh et al., 2018). In the case of As contamination, plants show symptoms of phosphorus (P) deficiency, because of competition between P ions and As(V) ions (Zhang et al., 2021). P deficiency is a big limitation for plants in case of yield. P plays a role in regulating physical responses, promoting heavy metal remediation, resistance to drought, heat, and salinity by increasing energy metabolism, cell division, and phospholipid production (Isidra-Arellano et al., 2021; Khan et al., 2023). In Cd contamination, plants can suffer from severe symptoms such as

producing reactive oxygen species (ROS) which causes an increase in cell permeability, also since Cd can directly permeate into the nucleus, it can disrupt DNA replication and repair mechanisms (Asare et al., 2023; Hussain et al., 2022; Özyiğit et al., 2021). Plants have some mechanisms to evade heavy metal stress in the environment, especially for oxidation caused by heavy metals. Cd heavy metal stress include several enzymatic and non-enzymatic pathways to increase antioxidation efficiency by plant hormones and several non-enzymatic proteins. These defence mechanisms activate when heavy metals present and can be boosted by different outside and inside factors (Rai et al., 2023).

By seed priming bacteria into the *T. aestivum* seeds, *B. cereus* can act as a phosphate binder and might increase phosphate phytoavailability (Soares et al., 2023).

Relative growth rate (RGR) is an important physical measure of heavy metal effect on plants. In this study by As exposure, plants suffered severe RGR loss related to a drop in relative water content (RWC), and photosynthetic parameters. RGR loss can be related to decreased photosynthesis and protein production because of reduced P availability and oxidative damage caused by ROS (Zhang et al., 2021). As also reduces chlorophyll content therefore limits photosynthesis (Praveen et al., 2020). Reduced photosynthesis causes RGR loss directly since organic material production is lowered. However, in bacteria inoculated arsenic group (As+B), RGR significantly increased. This result proves that under stress conditions, priming seeds with *B. cereus* by boosting plant radical oxygen scavenging systems and can alleviate the stress effects. This result was also displayed by other researchers, in different conditions. Liu et al. (2023) reported that in walnut seedlings under drought conditions *B. cereus* increases physical resistance of seedlings and accelerates the remediation of plants. Other reports also support these findings in other plants such as groundnut (Bhatt et al., 2020), *Arabidopsis thaliana* (Zhang et al., 2022), and *Glycyrrhiza uralensis* (Wang et al., 2022). RGR loss might also be caused by distortion of soil salt and nutrition balance by abiotic stresses. When priming occurs, bacteria may produce siderophores for plants and helps the plant survivability. Increasing RGR by priming in this study is mainly caused by bacteria related reduction in heavy metal concentration in the environment and stabilizing salt and pH balance in soil (Akhtar et al., 2021; Sahile et al., 2021).
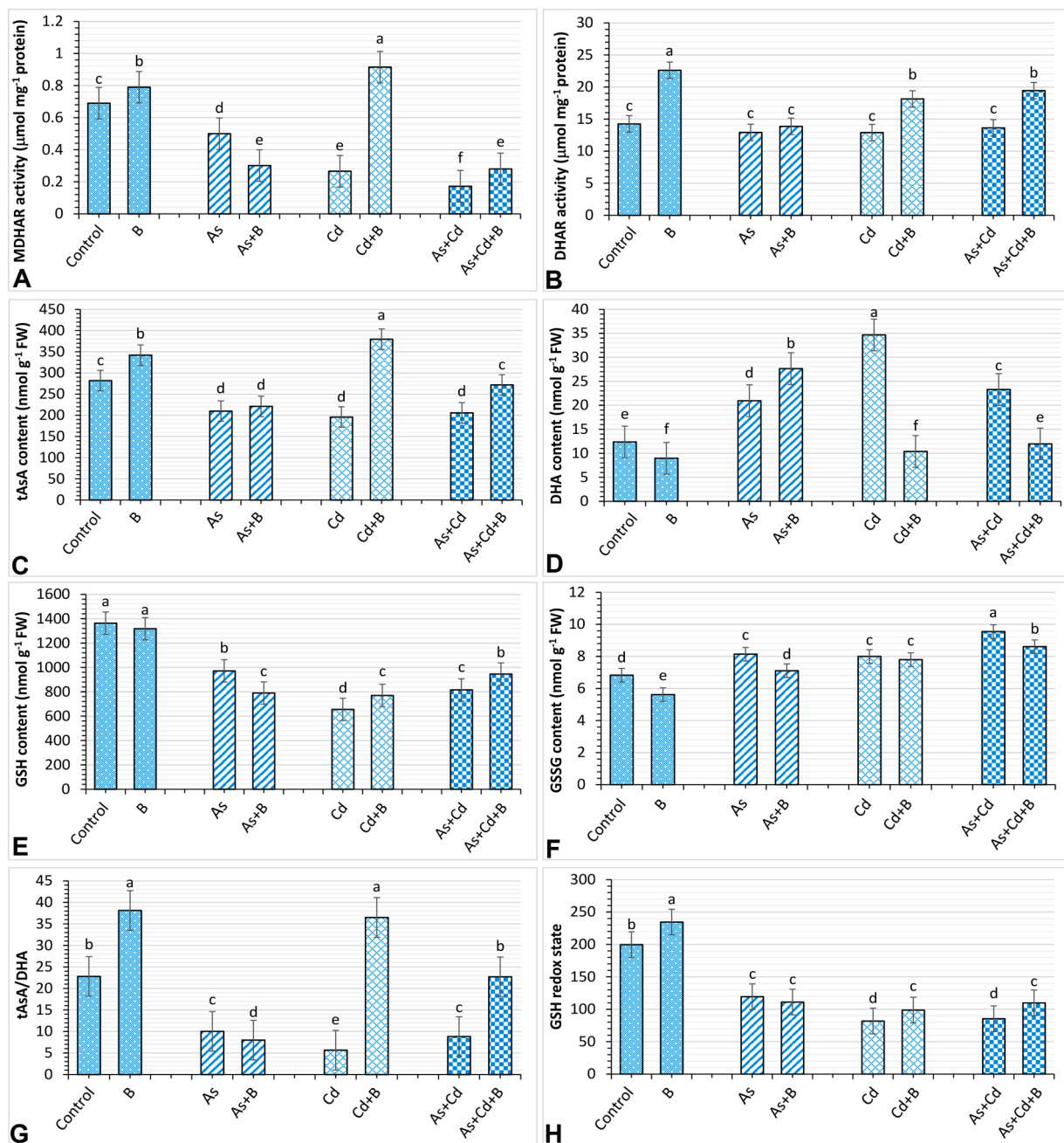
Fig. 10. The monodehydroascorbate reductase activity (MDHAR, **A**), dehydroascorbate reductase activity (DHAR, **B**), total ascorbate content (tAsA, **C**), dehydroascorbate content (DHA, **D**), glutathione content (GSH, **E**), oxidized glutathione content (GSSG, **F**), AsA/DHA (**G**) and GSH redox state (**H**) in the leaves of *T. aestivum* treated with bacteria under arsenic (As, 100 μM) and/or cadmium (Cd, 100 μM) stress. All data obtained were subjected to a one-way analysis of variance (ANOVA). Differences were considered to be significant at $p < 0.05$.

Proline is one of the frontline defenses of plants against harsh environmental stresses especially against heavy metals (Zulfiqar and Ashraf, 2022). Proline is both an energy source and osmoprotectant for plants especially during embryogenesis (Spormann et al., 2023). Another important role of proline is metal chelation and signal transduction therefore under heavy metal stress most plants overproduce the proline (Guan et al., 2020; Zulfiqar and Ashraf, 2022). In heavy metal contaminated environments, proline acts as a stabilizing agent and prevents electrolyte leakage (Lozano-Montaña et al., 2021). In this study stress application caused a severe drop in proline content. However, with priming with *B. cereus* in As stress, proline recovered to its control state. This result showed that the application of bacteria to the seeds might protect osmolyte balance by increasing proline content as well as

covering the surface area of root to decrease osmolyte loss (Akhtar et al., 2021). Peng et al. (2023) showed that seed priming with *B. cereus*, increased salt stress tolerance in *Glycyrrhiza uralensis* by boosting the proline and glycine content. Also in tobacco plants Tian et al. (2022) proved that, priming with *B. cereus* increases both reactive oxygen scavenging enzyme activity and proline content to decrease salt stress effects in plants. Another example of augmented proline content under stress is on strawberry plants by *B. cereus* is stated by Koç et al. (2016). In strawberries when heavy metal stress applied proline content is severely elevated due to stress related water loss. Proline content generally increases when water content is decreased.

Undeniably most important mechanism in plants to survive and adapt is photosynthesis. With heavy metal stress, photosynthesis is

heavily disrupted due to unbalanced stomatal movement (Guo et al., 2023). Cd can cause disruption in stomata structure as well as distorted morphology therefore reducing photosynthesis in *Pennisetum* sp. as Yang et al. (2021) stated. With As application, both chlorophyll production and photosynthesis-related pathways can be disrupted as well as g_s can be decreased (Fatima et al., 2021; Nahar et al., 2022).

With bacteria application both As and Cd content in the leaves are dropped significantly. However As content in the leaves dropped much more than Cd content. This result showed that even though bacteria application reduces the heavy metal stress effects for both As and Cd, the mechanisms behind these amelioration are different. For As stress bacteria might act as an protective agent to prevent arsenic uptake (Shah et al., 2021). However for Cd stress it can be suspected that bacteria act as immunobooster for plants to prevent heavy metal oxidation effect. PGPR can boost ROS scavenging activity of enzymatic and non- enzymatic antioxidants which in turn decline excessive ROS accumulation in metal stressed plants (Khan et al., 2023)

4.3. Changed photosynthetic parameters under As and/or Cd stress

In this study, Cd and combined stress application severely reduced photosynthetic parameters such as carbon assimilation rate and carboxylation efficiency. These results are expected and correlated with similar studies (Younis et al., 2016; Zhang et al., 2019). This inhibition is related to a reduction in g_s with the conclusion of decreased photosynthesis. However, seed priming with *B. cereus* showed a significant boost in carbon assimilation rate and carboxylation efficiency as well as g_s. This proves that bacteria with increased availability of ions through electron transport channels and supported stomatal structure protection. By covering the surface of plants and stomata *B. cereus* can adsorb Cd and reduce the ion loss therefore reducing the heavy metal effects on plants. With the help of these mechanisms *B. cereus* might successfully reduce the negative effects of Cd on stomata (Khanna et al., 2019).

Under As stress, *T. aestivum,* in this study, stomatal conductance, carbon assimilation rate, and carboxylation efficiency were heavily reduced. This result is parallel with other researches. Bano et al. (2022) showed that with As application, the photosynthetic rate dropped severely on *Brassica napus*. Sehar et al. (2022) reported that under As stress, *Brassica juncea* L. has lowered photosynthesis activity with a combination of low stomatal conductivity and low chlorophyll activity. In the current study, *B. cereus* priming prior to As stress significantly reduced the heavy metal stress effect on photosynthesis caused by As. This might be related to bacteria providing an improved surface area to increase nutrient uptake for plant as well as reducing the As in the environment (Pandey et al., 2023). Another mechanism might be that bacteria might be reducing As(III) to less toxic As(V) to alleviate the As toxicity for plants (Thongnok et al., 2022). Also, bacteria by reducing the available As in environment, enables the much-needed phosphorus to bind more efficiently to the plant cells (Soares et al., 2023).

Under both heavy metal stress, the photosynthesis of *T. aestivum* plants was significantly reduced. Reduction in maximum efficiency of PSII photochemistry (F_v/F_m) indicates heavy metal stress (Chen et al., 2019; Faseela et al., 2020). In another study, Shi, Duan, et al. (2020) showed that F_v/F_m rates dropped under copper stress in co-cultured duckweeds (*Lemna aequinoctialis* and *Spirodela polyrhiza*). Kaya et al. (2020) also displayed that, F_v/F_m rates dropped under Cd stress in *T. aestivum*. Heavy metals disrupt photosynthesis by penetrating inside the chloroplast and oxidizing the critical subunits of photosynthesis (Souri et al., 2019). F_v/F_o rates also significantly dropped in every stress group. Photo-inhibitory damage to the PSII reaction center can cause these kinds of changes in F_v/F_o. (Xu et al., 2017). Other researchers supported the reduction in PSII reaction center inhibition with heavy metal such as Faseela et al. (2020) proved that, with the application of heavy metal F_v/F_o decreased in rice plants. In the current study, F_v/F_m rate also dropped with all groups and recovered with *B. cereus* priming. Application of heavy metals reduces the F_v/F_m as indicated by other

studies. Giannakoula et al. (2021) stated that applying Pb and/or Cu reduced the F_v/F_m rate as well as other photosynthetic parameters in *Citrus aurantium*. Huihui et al. (2020) displayed applications of Pb and/or Cd reduced the F_v/F_m rate as well as other photosynthetic parameters in *Morus alba*. This indicates that bacteria might be indirectly related with reduction of the ROS species by the reducing the heavy metal in environment. Bacteria can also activate of reactive oxygen scavenger enzymes and/or boost the photosynthesis in *T. aestivum*.

4.4. Mitigation of oxidative stress in wheat seedlings via B. cereus priming

In the present study, *B. cereus* effectively reduced the H_2O_2 content inside As stress applied *T. aestivum* cells, by reducing the binding capacity of heavy metal in the environment with the help of siderophores (Jan et al., 2019). These results suggest that the increase in APX and POX (also known as POD) activity lowered the H_2O_2 content therefore reducing the reactive oxygen stress in As+B group (Pandey et al., 2023; Zhang et al., 2023). This H_2O_2 content decrease by bacteria priming phenomenon, displayed in other *B. cereus* related studies such as, under salt stress in cucumber seeds (Zhou et al., 2022), under Cd stress in bananas (Zhang et al., 2023) and under cadmium stress in rice seeds (Jabeen et al., 2022). In the case of Cd application, H_2O_2 content also decreases with *B. cereus* priming. However, reduction mechanisms of H_2O_2 content might be different from As stress since As and Cd solubility is different than each other. Whilst Cd solubility much more dependent on pH level, As solubility doesn't change much in pH (Zhao and Wang, 2019). Therefore, decreasing the Cd phytoavailability is not only dependent on Cd concentration in soil but also pH changes the Cd solubility (Shi, Lu, et al., 2020). Therefore, it can be suspected that bacteria in the root of the plant, create a microenvironment for the plant to reduce the solubility around roots therefore reducing the Cd uptake inside the plant. Similar mechanisms to decrease As in the environment affects the Cd in the environment. But since Cd is more mobile in the environment (Gankhurel et al., 2020) and in plants (Shi et al., 2015, 2019), bacteria might reduce the mobility of Cd and reduce the toxic effect of heavy metal on plants. In the case of Cd stress SOD, GST, GR, APX, DHAR and MDHAR activities increased significantly. In the current study, SOD enzyme activity was increased. This is expected as other researchers also showed similar results (Bruno et al., 2021; Sahile et al., 2021; Zhang et al., 2023). *B. cereus* seed priming boosted antioxidant enzymes even further. *B. cereus* may reduce the translocation of Cd inside the plant and therefore reduce the toxicity caused by it (Mushtaq et al., 2020). Cd uptake by *B. cereus* might also reduce Cd concentration for plants and have affected plants in positive ways (Abdollahi et al., 2020). In contact with heavy metal stress, SOD acts as a scavenger for oxygen radicals caused by heavy metal (Zheng et al., 2023). Therefore, reduction in H_2O_2 content is directly related to SOD enzymatic activity. In As stress, like Cd stress, APX and SOD activities increased, however CAT activity didn't change much even with *B. cereus* seed priming. Bacteria boosted the APX and SOD activity in As stress with suspicion of increasing P availability for plants and therefore reducing the As binding to the proteins of plant (Wu et al., 2022). As and P are transported via same channels inside the plant cell therefore if P concentration is higher on environment because of the competition, As binding and uptake will be reduced (Das and Das, 2020).

AsA-GSH cycle is crucial to prevent and cure the oxidative stress inside the cells and stabilizing redox homeostasis. The main components for this cycle include APX, GR, DHAR, and MDHAR and by direct effects of these enzymes, AsA and GSH. Bacteria priming increased APX activity in both the Cd and As groups. In As groups with or without bacteria DHAR activity didn't change significantly and MDHAR and GR activities dropped minimally. These results suggest that the application of bacteria accelerated the first-line defenses against ROS such as POX, SOD, and APX. However, stabilized other cycles and might not necessarily increase the activity of downward pathways. These might be related to plants successfully evading the effect of As in the cell and therefore did

not need to increase downward pathways. Another explanation for this phenomenon might be that As penetration inside plants is very low due to bio adsorption of As in *B. cereus* so that plants only avoided the surface effect of As. In Cd stress application with seed priming, nearly all components of the AsA-GSH cycle were boosted. Therefore, the cycle is getting significantly boosted. Increased AsA content shows that high amounts of MDHAR activity as well as decreased DHA content and increased DHAR activity. Increased GSH content also shows increased GR activity. These results are also parallel to each other therefore it can be seen that all the AsA-GSH cycle components are boosted, and the cycle is activated. Increased AsA regeneration also shows increased heavy metal resistance by increasing ROS scavenging enzyme activities. Reduction of heavy metal stress might be related to bacteria adsorption of Cd in the environment (Muzammil et al., 2021). Zhang et al. (2022) showed that the application of *B. cereus* like bacteria increased antioxidant enzyme activity (APX, POX, SOD and GSH) in *Solanum nigrum*. In another study, Daraz et al. (2023) showed that the effect of Cd was significantly reduced when bacteria were inoculated. In our study, bacteria might increase the available nutrition for plants therefore reducing the heavy metal stress.

4.5. Bacterial mechanisms to evade heavy metal stress

In this study, bacteria application significantly increases photosynthesis parameters as well as reduces the stress agents. These results suggest that bacteria application increases plant survivability via different mechanisms. Some of the mechanisms that might be activated by bacteria priming could be investigated with the help of other relevant articles. For instance, *Bacillus* spp. can accumulate heavy metals from the environment with a process called biosorption. Nickel (Babar et al., 2021), Zinc (Khan et al., 2021) and Arsenic (Altowayti et al., 2019) can be absorbed inside bacteria cells as well as accumulate around the cell surface. This decreases the plant toxicity by heavy metals and therefore boosts the plant survivability. Another mechanism is extracellular polymeric substances. These substances can act as metal binders as well as emulsifier agents for heavy metals therefore reducing the heavy metal availability for plants (Casentini et al., 2019; Nadda et al., 2021; Raghunandan et al., 2018). As a result, reducing the heavy metal caused plant stress. Besides these factors, another mechanism is that bacteria act as a protection barrier to decrease osmotic pressure on plant roots caused by metal imbalance.

Application of *B. cereus* especially under As and Cd stress should increase the yield of *T. aestivum* under field conditions. Especially because of the fact that *B. cereus* effectively reduced heavy metal stress as well as boosted the RGR and RWC conditions of *T. aestivum* plants. These results suggest that applications of *B. cereus* and also closely related species should be effective in increasing production rates (Sorokan et al., 2023).

5. Conclusion

Current study shows the effects of plant-microbe interaction between *B. cereus* and *T. aestivum* seedlings under heavy metal stress. As and/or Cd contamination of *T. aestivum* causes significant losses in RGR and RWC. As and/or Cd stress reduced the stomatal conductance and as a result, decreased the transpiration rate and carbon assimilation rate. *B. cereus* application successfully recovered both RGR and RWC rates and increased photosynthesis. As stress caused increased H_2O_2 content as well as increased TBARS. Cd stress is also responsible for the same increased stress patterns. With bacteria priming, under both stresses, H_2O_2 and TBARS content dropped. Reduction in H_2O_2 and TBARS contents related to activity increase in SOD, GST, and APX enzymes. Under Cd stress, bacteria acted as a booster for the AsA-GSH cycle and reduced the negative effects caused by ROS. Under both conditions, bacteria effectively reduced the oxidation-related stresses and helped plants to develop resistance under stressed conditions. With these results, increasing yield and health for plants under heavy metal stress with the help of *B. cereus* might be a sustainable approach for a better future. In the future production of plants will be directly linked with the bacterial microbiome of soil therefore bacteria such as *B. cereus* will help to increase both the survivability and the productivity of plants. In the future other microbiome aspects of *T. aestivum* should be investigated and more pathways should be explored.

CRediT authorship contribution statement

Ahmet Direk: Methodology, Investigation. **Busra Arikan-Abdulveli:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Ceyda Ozfidan-Konakci:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Evren Yildiztugay:** Writing – review & editing, Writing – original draft, Resources, Investigation. **Ahmet Uysal:** Writing – review & editing, Writing – original draft, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2024.100458.

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