



Research article

Plant growth promoting *Streptomyces* strains are selectively interacting with the wheat cultivars especially in saline conditionsAlireza Akbari ^a, Shahrokh Gharanjik ^a, Parisa Koobaz ^{b,**}, Akram Sadeghi ^{c,*}^a Department of Plant Breeding and Biotechnology, Faculty of Agricultural Engineering, Shahrood University of Technology, Shahrood, Iran^b Department of Molecular Physiology, Agricultural Biotechnology Research Institute of Iran (ABRII), Education and Extension Organization (AREEO), Karaj, Iran^c Department of Microbial Biotechnology, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

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ABSTRACT

Plant growth promoting (PGP) effect of *Streptomyces* on wheat growth in different conditions has been mostly reported although mechanisms which caused wheat cultivars differently response to a PGP *Streptomyces* has been less studied. In this study, the effect of two *Streptomyces* strains, previously reported as PGPR, on the growth of four salt-sensitive commercial wheat cultivars under normal and saline conditions was investigated. Strain C-2012 differently affected the growth of the cultivars in the normal and stress conditions. Cultivars Gonbad with the highest (63%) and Zarin without increased dry biomass upon C-2012 treatments were selected for further study. Salinity significantly decreased seedling fresh and dry weight, K⁺ and chlorophyll content and glutathione S-transferase activity. Moreover, the stress increased proline and Na⁺ content and peroxidase (POX) and ascorbate peroxidase (APX) activity in both cultivars. Strain C-2012, generally, ameliorated the negative effect of the stress with increased chlorophyll and carotenoid and reduced Na⁺ content and APX and SOD activity in both cultivars, however, its effect on biomass was different. Increase in SOD, APX and POX activities in bacterial inoculated-Zarin, but not Gonbad, under normal conditions suggested that this cultivar may recognize strain C-2012 as a gentle stressor and not as a PGPR. These results showed that the responses of the wheat cultivars to a defined PGPR is different in the physiological, phenotypic and molecular level. Based on the results, the evaluation of the effect of a bio-fertilizer on each wheat cultivar is necessary prior to use in a commercial field.

1. Introduction

Wheat (*Triticum aestivum*) is a very important crops grown and whose yield potential is usually constrained by salt stress. Salinity affects plant physiology depending on its genotype (Gilles et al., 2001; Rubio-Casal et al., 2003). Plants growing in salinity experience osmotic stress leading to an ionic imbalance in tissues, inhibition of nutrient uptake and oxidative damages (Chatzigianni et al., 2019; Hasegawa et al., 2000). To balance the oxidative state, plant cells use enzymes and non-enzymatic antioxidant mechanisms (Hossain et al., 2017). Antioxidant enzymes have an important role in increasing plant tolerance to various stresses, e.g., salinity. Also, plant employ the selective ion uptake or exclusion to maintain an appropriate K⁺/Na⁺ balance and synthesis of osmolytes (e.g., proline and glycine betaine) to accomplish the osmotic adjustment (Soleimanzadeh et al., 2010; Mittler, 2002). Plant growth-promoting

rhizobacteria (PGPR) improve plant growth and health. Growth stimulation could be provided by fixing nitrogen (Etesami and Maheshwari, 2018), soluble phosphate (Ahmad and Khan, 2012) production of plant hormones (Tank and Saraf, 2010) and iron chelators (Jahanian et al., 2012). Overall, PGPRs promote plant growth, development, and produce and could alleviate the damages of different stresses (Saleem et al., 2007; Lugtenberg and Kamilova, 2009). There are some commercial PGPRs which are used for agricultural purposes (Berg, 2009). *Streptomyces* are gram-positive PGPRs with a well-known potential to survive in various conditions including saline soils (Olanrewaju and Babalola, 2019). There are some reports confirming that *Streptomyces* species promote plant growth under normal and salinity (Palaniyandi et al., 2014; Abbasi et al., 2019) by producing plant growth regulators such as IAA (indole-3-acetic acid) (Aldesuquy et al., 1998) or through biosynthesis of iron chelators (Tokala et al., 2002).

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It is reported that reported that *Streptomyces* strains with high PGP activities significantly improved growth of wheat (var. Lokwan) (Jog et al., 2012). Although the effect of these PGP bacteria on growth of one wheat cultivar was significantly positive, it is unclear what effect these bacteria have on other wheat cultivars. Khan et al. (2019) showed that the levels of the effect of PGP strains on different wheat genotypes were not the same. It is well known that plants by secreting different compounds are partly involved in the attract beneficial bacteria or excretion of pathogens (Olanrewaju et al., 2019), but sometimes they have trouble identifying and responding to beneficial bacteria. Although the interaction between beneficial bacteria and plants has been well studied under normal and abiotic stress conditions (Kumar and Verma, 2018), it is little known why some cultivars or plant species do not receive the most positive impact from the relationship with a beneficial bacterium. This will be problematic when farmers use a commercial biofertilizer product for cultivars that have not been studied before. In addition to cultivars, environmental stresses such as salinity and drought affect the efficiency of a PGPR. Recently, the effect of a commercial biofertilizer (Rizotech plus®) containing PGP microorganism (*Glomus* spp., *Pseudomonas* sp., *Bacillus* spp., *Streptomyces* sp. and *Trichoderma* sp.) on growth and product of four tomato cultivars under normal and drought stress has been reported (Inculet et al., 2019). The results showed that the efficiency of the biofertilizer application was dependent on the cultivar and the water regimes used for irrigation. Rizotech plus® inoculation significantly increased the yield of three cultivars as compared to the uninoculated controls, regardless of the water regimes. However, for Inima de Bou cultivar, no difference was observed between inoculated and uninoculated plants in both irrigation regimes. Understanding the physiology and genetic basis of how different wheat cultivars respond to a PGPR under normal and stress conditions is a prerequisite for wheat breeding programs. Here, we first analyzed the effects of two *Streptomyces* strains (previously reported as salt-tolerant and PGP) on the growth of four wheat cultivars under normal and saline conditions were. Then, the effect of one strain on maintains water and ionic balance and antioxidant enzymes activity and genes of two cultivars that in the first step had different responses to that was investigated.

2. Materials and methods

2.1. Bacteria, culture media and conditions and inoculum preparation

S. monomycini strain C 801 (KX020407) and *Streptomyces rimosus* strain C-2012 (JX839830) were used in this study. These PGPRs were in our previous studies (Sadeghi et al., 2012, 2017; Esmail Zade et al., 2019). Bacterial strains were grown on a solid medium (MYA) containing 10 g/L malt extract, 4 g/L yeast extract, 4 g/L glucose and 18 g/L agars, with a pH 7.2 and incubated at 29 °C for 5 days. To bacterial inoculum preparation, spores were harvested from the surface of the medium and suspended in a sterile NaCl (0.9%) solution. Spore suspension (100 µl from a 10⁶ cfu/ml suspension) was transferred to 50 mL MYB medium (MYA with no added agar) and incubated in a rotary shaker incubator with 150 rpm at 29 °C. After 4 days, the culture was centrifuged at 1000 × g for 10 min. Bacterial cells and spores added in 50 mL sterile solution (containing 0.9% NaCl) and mixed with 500 g autoclaved sand. Bacterial concentration adjusted to 10⁶ cfu/g sand.

2.2. Plant materials, growth conditions and experiments

Plant were grown in a greenhouse at temperatures of 30/25 ± 2 °C (day/night), 55% relative humidity, with day length of 14h and light intensity in the range of 600–1000 µmol photon m⁻² s⁻¹ (in addition to sunlight, light-emitting diodes (LEDs) were used for supplementary lighting). Seeds of four commercial wheat cultivars (*Triticum aestivum*,

also known as bread wheat), Chamran2, Pishtaz, Zarin, and Gonbad were kindly provided by the Seed and Plant Improvement Institute (SPII), Karaj, Iran.

Chamran2 (originated from SPII breeding program, pedigree Attila 50y//Attila/Bacanora), Pishtaz (originated from SPII breeding program, pedigree Alvand//Aldan/Ias58), and Gonbad (originated from SPII breeding program, pedigree ATRAK/WANG-SHUI-BAI) are spring type wheat cultivars and Zarin (originated from CIMMYT, pedigree PK15841) is a facultative type (Esmailzadeh Moghaddam et al., 2017).

Fifteen surface sterilized seeds of each wheat cultivar were grown in a plastic tray (50 × 35 × 15 cm) in an individual row. Before filling the trays, sterile soil (a mixture of equal proportions of field soil, coco peat and manure) was treated with one-gram of bacterial inoculum/seed. Autoclaved sand was used as a uninoculated control. Soil irrigated with tap water every 3 days. After 28 days, inoculated and uninoculated trays were separated into two groups normal and stress. Plants in the first group irrigated normally during the experiment. In the second group, plants were irrigated with a NaCl-containing (saline) solution at a concentration of 100 mM. Forty-two days after planting ten plants were harvested and shoot and root fresh and dry weight were measured. Relative water content (RWC) of shoots was determined according to the method of Turner (1986). Shoots of three plants from each replicate were pooled and frozen at -80 °C for the following analysis. For each treatment, there were three trays that were arranged in a complete randomized design.

2.3. Physiological parameters

Chlorophylls and Carotenoid contents were analyzed according to the method of Costache et al. (2012). Protein content was determined according to the Bradford method (Bradford, 1976). Protein extraction was carried out by homogenization of frozen sample (100 mg) in Na-Pi buffer containing 10 mg polyvinylpyrrolidone, followed by centrifugation at 20, 800 × g for 30 min at 4 °C. Supernatant was mixed with glycerol at the final concentration of 12.5% and was used to enzyme activity assays and to determine the total protein content (Koobaz et al., 2016). The method of Ringel et al. (2003) was used to extract and determine free proline content. To determine the intracellular cation content, 0.1 g of dried shoot was dissolved in 10 ml of 0.1 M hydrochloric acid (HCl). Aqueous solutions were then incubated at 80 °C for 24 h. One ml of filtered solution (Wattman filter, No. 3) was diluted in the ratio of 1:10 by deionized water and used for analysis of the intracellular cations potassium (K⁺) and sodium (Na⁺) by ion chromatography (IC; 850 Professional IC, Metrohm, Switzerland) with a Metrosep C2 250 column (Metrohm company, 2011).

2.4. Redox assessments

Frozen shoot samples (100 mg fresh weight) were homogenized in 3 ml of HEPES-KOH buffer containing 0.1 mM EDTA (pH 7.8). The homogenate was centrifuged at 15000 × g for 15 min at 4 °C and the supernatant was used as a crude enzyme extract (CEE) to determine the activities of ascorbate peroxidase (APX: EC 1.11.1.11), catalase (CAT: EC 1.11.3.6), peroxidase (POX: EC 1.11.1.7), superoxide dismutase (SOD: EC 1.15.1.1) and glutathione S-transferase (GST: EC 2.5.1.18). The SOD activity was determined by measuring its ability to photochemically reduce the p-nitrotetrazole blue (NTB) (Del Longo et al., 1993). One hundreds microliter of the CEE was added to 900 µl of the reaction buffer (30 µM riboflavin, 13 mM methionine, 75 µM NTB, 50 mM potassium phosphate buffer pH 7.8 and 50 mM of sodium carbonate pH 10.2). After 10 min of light exposure (under a 15W lamp at room temperature), the increase in absorbance was measured using spectrophotometer (Cary 300, Agilent, USA) at 560 nm. The reaction mixture (without CEE) kept

in darkness for 10 min and was used as control. Absorbance of the control was measured at 560 nm and the value obtained was subtracted from the value obtained for each sample. One unit of SOD was defined as the amount of enzyme necessary to inhibit NBT photo reduction by 50%. To measure CAT activity, the reaction mixture consisted of 100 mM potassium phosphate buffer (pH 7), distilled water and 70 mM H₂O₂ diluted in 100 mM potassium phosphate buffer (pH 7). The reaction was initiated after adding 20 µl of the CEE, and the enzyme activity was measured by the rate of H₂O₂ decomposition at 240 nm for 3 min at 25 °C. The specific activity was analyzed and was expressed as µmoles/min/mg of total protein (Cakmak and Horst, 1991). The APX activity was determined following the method of Cakmak and Marschner (1992). The reaction mixture (in a volume of 980 µl) composed of 100 mM potassium phosphate buffer (pH 6.8), 2mM H₂O₂, and 0.5 mM ascorbate was added to 20 µl of CEE to start the reaction. Ascorbate oxidation at 290 nm was measured to determine the APX activity. Enzyme activity was expressed as µmol/min/mg protein. The POX activity was assayed following the colorimetric determination of pyrogallol oxidation according to Hasan et al. (2011). The reaction mixture (in a volume of 980 µl) contained 100 mM potassium phosphate (pH 6.8), 20 mM pyrogallol, and 70 mM H₂O₂ was added to 20 µl of CEE to start the reaction. Enzyme activity was measured following record of absorbance of colored purpurogallin at 420 nm for 3 min at room temperature. Finally, POX activity was expressed as µmol of purpurogallin produced per minute per milligram of protein. The GST activity was determined according to the method of Gronwald and Plaisance (1998). One hundred microliter of the CEE was added to 700 µl of the mixture containing 100 mM potassium phosphate buffer (pH 7) and 10 mM reduced glutathione. At the final step, 100 µl of 10 mM 1-chloro-2,4-dinitrobenzene was added to initiate the reaction. After 4 min, absorbance was measured at 340 nm for 1 min. GST activity was expressed as µmol/min/mg protein.

2.5. Transcript levels of SOD, APX and GST genes

Total RNA was isolated from fresh shoots using RNasy plant mini kit (QIAGEN) according to manual description. One microgram RNA was used for synthesizing cDNA after treating with RNase-free DNase I (Invitrogen) using iScript cDNA synthesis kit (BioRad).

Gene expression was assayed using BioRad multicolor real time PCR detection system and iQ SYBR Green Supermix kit (BioRad), according to manual description. Transcription of each gene was studied by RT-PCR with 0.5 µL of 10 pM of each forward and reverse specific primer designed in this study (Table 1) and 1 µL of template cDNA. The following PCR profile was used: 4 min at 95 °C; 40 cycles (30 s at 95 °C, 40 s at 58 °C, 60 s at 72 °C); 5 min at 72 °C and recording melting curve. The transcription of the 18S rRNA gene was used as an internal control. Gene expression ratio was calculated using REST 2009 software (Pfaffl et al., 2002).

2.6. Statistical analysis

The experiment was carried out in a completely randomized design (CRD) and there were three biological replications. Statistical analysis was performed using analysis of variance (ANOVA) by SPSS windows

version 16.0 (SPSS Inc., Chicago, IL, USA). The significance of difference between treatments were analyzed using Duncan test at level of $P \leq 0.05$.

3. Results

3.1. The effect of PGPR strains and salt stress on wheat biomass

In soil inoculated with strain C 801, the fresh and dry weight of shoot and root of all wheat cultivars significantly increased compared to the uninoculated control (Figure 1). Among the cultivars treated with strain C-2012, only fresh and dry biomass of Gonbad increased. The total dry weight increase of the wheat plants by strain C 801 was 17–42 %. Strain C-2012 increased only the total dry weight of Gonbad by 49%. Both strain increased root dry weight of cultivars 26–60% and 63–85% respectively. Strain C 801 was more efficient in improving the fresh and dry weight of wheat cultivars than strain C-2012.

Under salt stress conditions, shoot fresh and dry weight of all four wheat cultivars significantly decreased compared to the normal conditions. Strain C 801 enhanced shoot fresh and dry weight of Gonbad and Zarin although, did not affect their root biomass (Figure 2). Strain C-2012 increased growth (whole plant fresh and dry weight) of Gonbad and dry weight of shoot and root of Chamran 2 and Pishtaz. In saline conditions, the highest increases in total dry weight were 63 and 31% recorded for Gonbad and Zarin in treatments of C-2012 and C 801 respectively.

In general, strain C-2012 had a selective effect on Zarin and Gonbad cultivars under normal and stress conditions (Figure 3).

3.2. The effect of strain C-2012 and salt stress on wheat RWC, proline intracellular cations, chlorophylls and carotenoid

Percentage (%) of RWC in Zarin and Gonbad declined significantly in saline conditions. Under stress conditions strain C-2012 increased RWC of Zarin by 59% but did not affect Gonbad cultivar (Figure 4 a). Salt stress increased proline and Na content and decreased K content and the ratio of K⁺/Na⁺ of Zarin and Gonbad. In saline soil inoculated with strain C-2012, Na⁺ and K⁺ content of Zarin and Gonbad decreased (Figure 4 b & c), although the ratio of K⁺/Na⁺ remained constant (Figure 4 d). Zarin and Gonbad differently accumulated proline in the response to the salt stress in soil inoculate with C-2012. In a way that, proline content of Zarin increased by 56% while in Gonbad its amount remained constant (Figure 4 b).

In salinity, chlorophyll a and b and total chlorophyll content of Gonbad and Zarin and carotenoid content of Gonbad decreased. Zarin carotenoid content increased unexpectedly under stressful conditions. Treatment with strain C-2012 significantly increased total chlorophyll (44%), chlorophyll a (30%) and carotenoid (37%) content of Zarin in the normal conditions, although, did not have a positive effect on Gonbad chlorophyll. Conversely, in salt stress, Gonbad and Zarin greatly increased total chlorophyll 90 and 117 %, chlorophyll a 175 and 132 %, chlorophyll b 121 and 115% and carotenoid 25 and 113 % respectively in the response to strain C-2012 (Figure 5).

Table 1. The list of primers used in this study.

Gene Name	Sequence	Amplicon size (bp)
GST	F: 5'ATCAGCTCTTGCTCTCACCG3' R: 5'CAAGAAGAACCGAACCGAAG3'	103
APX	F: 5'TTTGACGGTGCATGGACTCG3' R:5'GCGTCGAAATTCAGGATCAT3'	135
SOD	F: 5'GGGTGCATATCAACAGGTCC3' R: 5'CGCCACACCTTCAGCATTG3'	120
18S rRNA	F: 5'TTAACGAACGAGACCTCAGCC3' R:5'GGCATGACAGACCTGTTATTGCS'	124

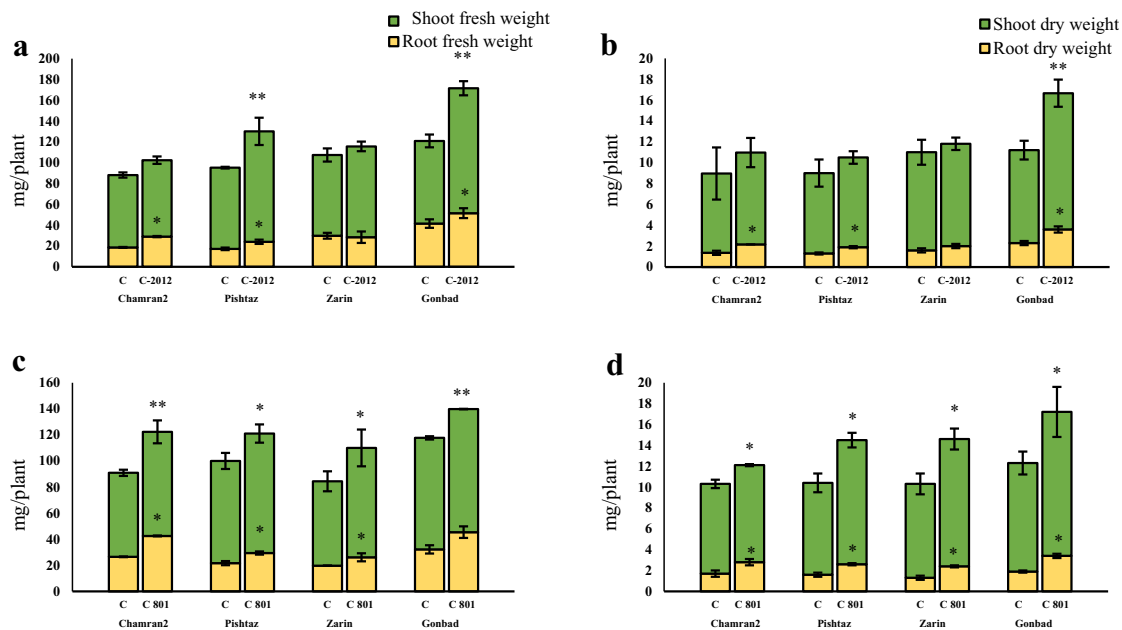


Figure 1. Effect of soil inoculation with *Streptomyces* strains on wheat biomass under normal conditions. Shoot and root fresh (a) and dry (b) weight of wheat cultivars inoculated with C-2012 and shoot and root fresh (c) and dry (d) weight of wheat cultivars inoculated with C 801. Error bars show the standard deviation of the mean values of three replicates. The values marked with an ($P < 0.05$) or two ($P < 0.01$) asterisks are significantly different from uninoculated control (C).

3.3. The effect of strain C-2012 and salt stress on CAT, POX, APX, GST and SOD activities

Zarin increased CAT activity under salt stress, while in Gonbad activity of the enzyme remained constant. In normal conditions, soil treatment with strain C-2012 did not affect the CAT activity of two wheat cultivars. Although, in salt stress Zarin decreased the activity of the enzyme in response to strain C-2012 (Figure 6 a).

Generally, POX (Figure 6 b) and APX (Figure 6 c) activity of both cultivars increased in saline conditions. In normal conditions, strain C-2012 slightly increased POX and APX activity of Zarin. In salt stress, as

observed for the CAT activity, strain C-2012 only reduced POX activity of Zarin. On contrary, the effect of strain C-2012 on reducing Gonbad APX activity was higher than Zarin. The PGP strain C-2012 reduced APX activity of Zarin and Gonbad 7 and 27% respectively compared to the uninoculated control in saline conditions. Salt stress differently affected levels of the SOD activity in Zarin and Gonbad. In Zarin, the level of enzyme activity remained constant but increased in Gonbad 1.7 times. In stress conditions, strain C-2012 reduced SOD activity of Zarin and Gonbad to 31 and 10 % respectively (Figure 6 d). GST activity significantly decreased to 74% upon salt stress in both examined cultivars. Gonbad cultivar decreased the activity of the enzyme to 32% in saline soil

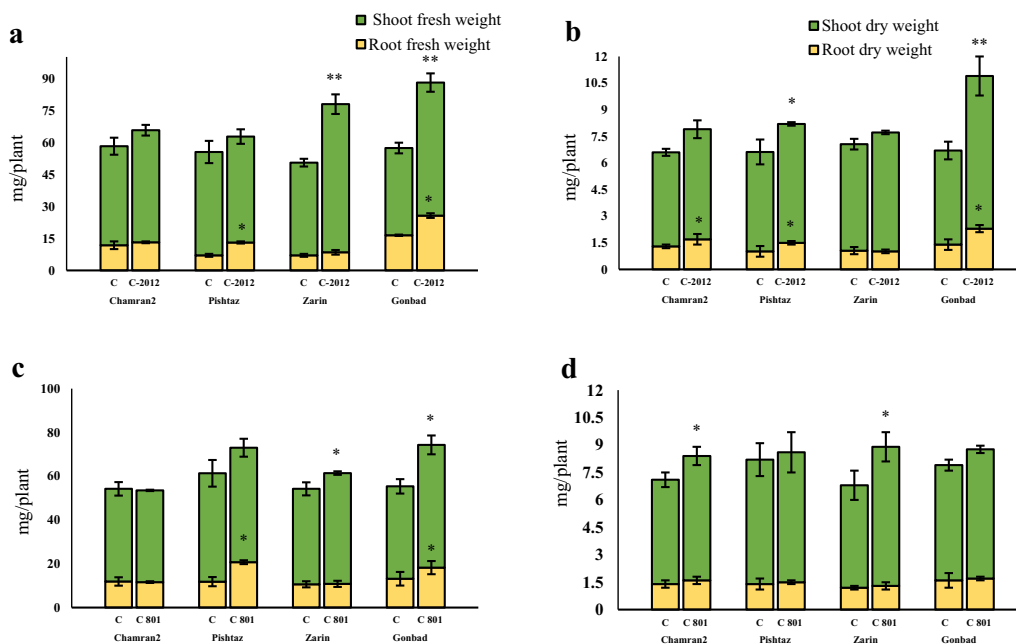


Figure 2. Effect of soil inoculation with *Streptomyces* strains on wheat biomass under salt stress conditions. Shoot and root fresh (a) and dry (b) weight of wheat cultivars inoculated with C-2012 and shoot and root fresh (c) and dry (d) weight of wheat cultivars inoculated with C 801. Error bars show the standard deviation of the mean values of three replicates. The values marked with an ($P < 0.05$) or two ($P < 0.01$) asterisks are significantly different from uninoculated control (C).

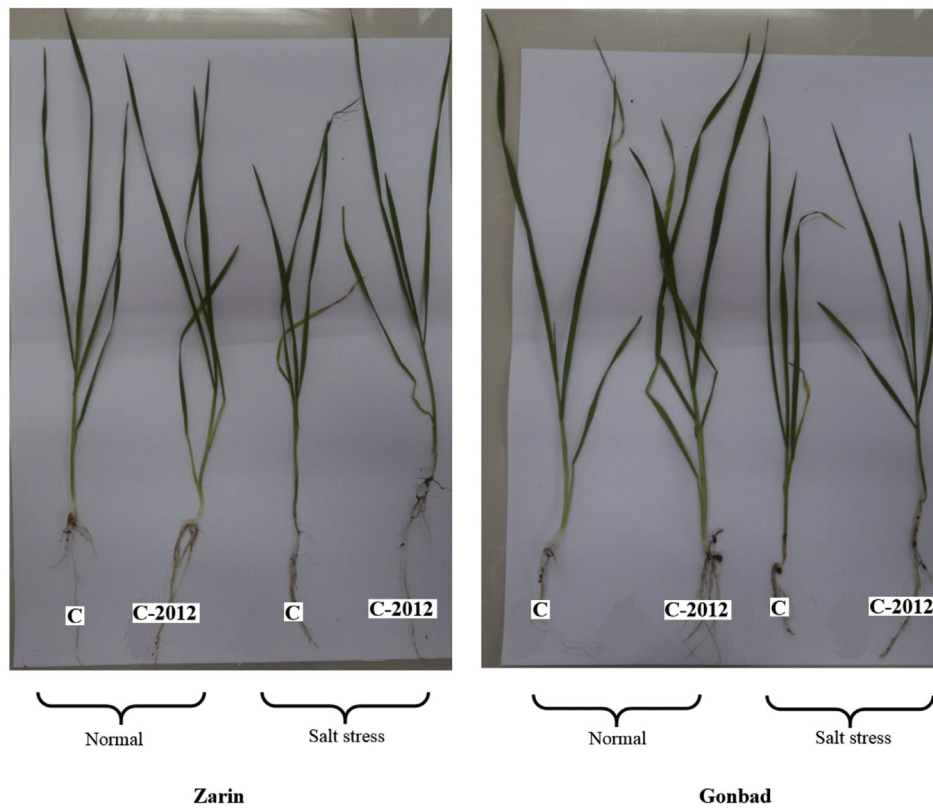


Figure 3. Effect of soil inoculation with *Streptomyces rimosus* strain C-2012 on wheat cultivars, Zrin, and Gonbad under normal and salt stress conditions.

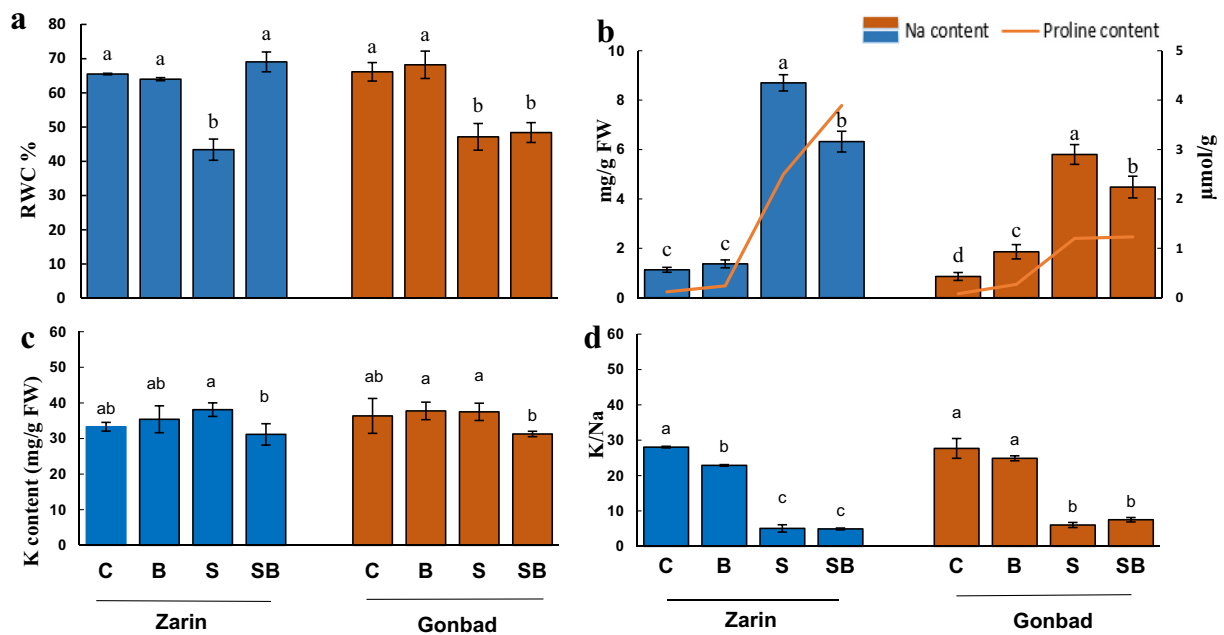


Figure 4. RWC (a), Na⁺ and proline (b), K⁺ (c) and the ratio of K⁺/Na⁺ (d) of Zarin and Gonbad in different treatments and conditions. Uninoculated (C) and inoculated with strain C-2012 (B) under normal conditions and uninoculated (S) and inoculated (SB) under salt stress conditions. Error bars show the standard deviation of the mean values of three replicates. Different letters indicate statistically significant (P < 0.05) differences between treatments and conditions of each wheat cultivar.

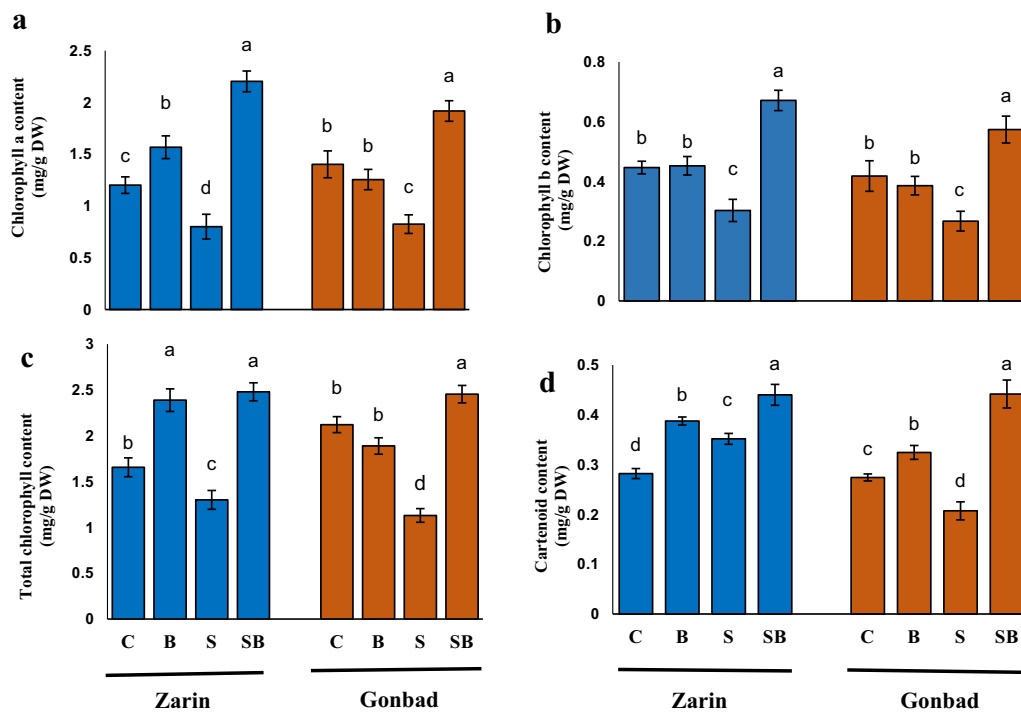


Figure 5. Chlorophyll a (a), chlorophyll b (b), total chlorophyll (c) and carotenoid (d) content of Zarin and Gonbad in different treatments and conditions. Uninoculated (C) and inoculated with strain C-2012 (B) under normal conditions and uninoculated (S) and inoculated (SB) under salt stress conditions. Error bars show the standard deviation of the mean values of three replicates. Different letters indicate statistically significant ($P < 0.05$) differences between treatments and conditions of each wheat cultivar.

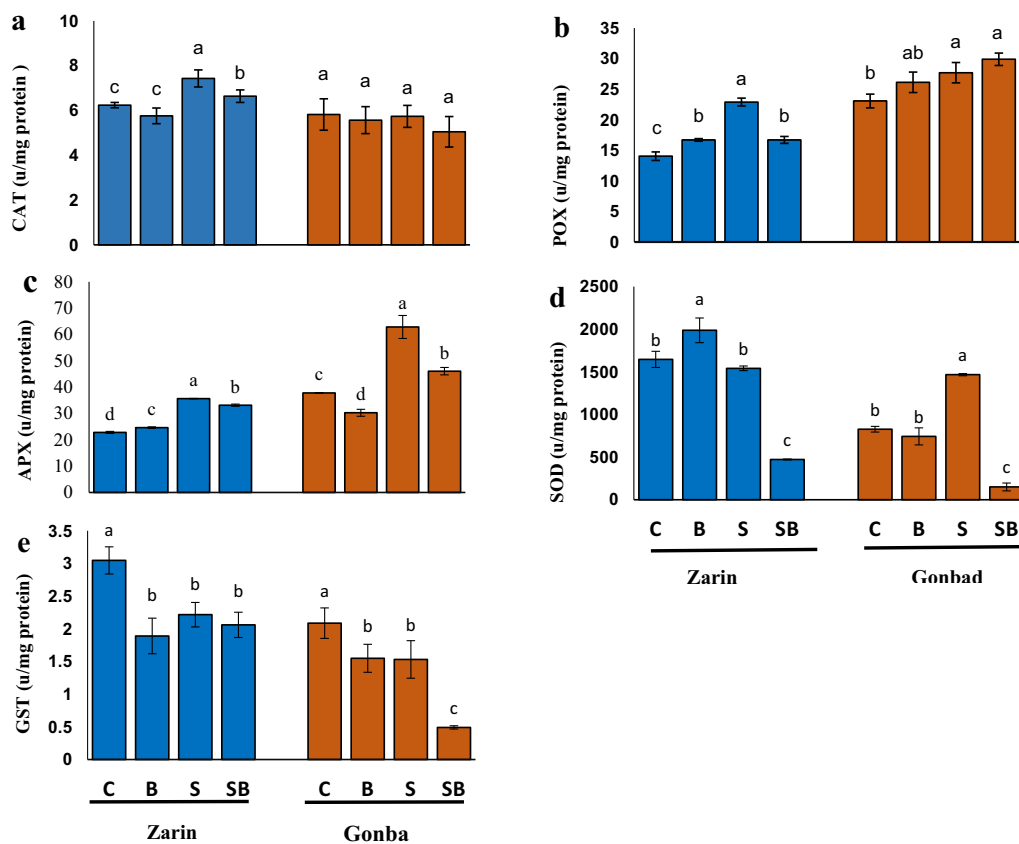


Figure 6. CAT (a), POX (b), APX (c), SOD (d) and GST (e) activity of Zarin and Gonbad in different treatments and conditions. Uninoculated (C) and inoculated with strain C-2012 (B) under normal conditions and uninoculated (S) and inoculated (SB) under salt stress conditions. Error bars show the standard deviation of the mean values of three replicates. Different letters indicate statistically significant ($P < 0.05$) differences between treatments and conditions of each wheat cultivar.

inoculated with strain C-2012. In response to the bacterial treatment, Zarin reduced the activity of the GST in normal conditions, while it did not change in salinity stress (Figure 6 e).

3.4. The effect of strain C-2012 and salt stress on regulation of SOD, APX and GST genes

In Zarin, salt stress did not change transcripts of SOD and APX compared to the normal conditions. Soil treatment with C-2012 or salinity significantly ($P \leq 0.05$) decreased expression level of GST. SOD decreased in Zarin inoculated with C-2012 only in saline soil. In Gonbad cultivar grown in stress conditions, only expression levels of GST significantly ($P \leq 0.05$) increased. Higher increased level of GST expression was observed in bacterial inoculated plant in saline stress (Figure 7).

4. Discussion

PGPRs has a positive effect on wheat growth in normal and stress conditions (Egamberdieva, 2010; Jog et al., 2012, 2014; Nadeem et al., 2013; Mukhtar et al., 2017). The effect of two *Streptomyces* strains *S. monocylini* C 801 and *S. rimosus* C-2012 on growth of plants (Sadeghi et al., 2012; Esmaeil Zade et al., 2019) and biological control of phytopathogenic agents by these bacteria (Karimi et al., 2012; Sadeghi et al., 2017) under normal and abiotic stress have been stated. In this study, the PGP activity of these isolates was investigated on four commercial wheat cultivars under normal and saline conditions. Chamran2, Pishtaz, Zarin, and Gonbad showed a sensitivity to salinity and decreased total fresh and dry weight 34, 42, 53 and 53% and 26, 26, 36 and 40% respectively. The strains had dissimilar effects on the wheat cultivars in normal and saline conditions. In soil irrigated with non-saline water, strain C 801 generally increased the growth of all four cultivars, but C-2012 only increased the growth of Gonbad. The effect of the PGPR strains in saline soil was selective, so the growth of Chamran2 and Gonbad increased in soil inoculated with each one of the strains but Pishtaz and Zarin increased the growth only in the response to C-2012 and C 801 respectively. To assess different modes of action of plants in increasing growth and ameliorating the salt effects in response to a PGP *Streptomyces*, Gonbad with the highest and Zarin without increased biomass upon C-2012 treatment were considered for further study. Physiological and molecular characteristics of the bacterial inoculated plants over the uninoculated control in normal and saline conditions were evaluated and the changes in the two cultivars were compared. The reduction in growth from high salinity is the result of both osmotic stress and Na^+ toxicity. Ion exclusion, compartmentalization of toxic ions into specific tissues, accumulating organic solutes (e.g. proline) and inorganic ions (e.g. K^+) and maintenance of shoot water status are the main mechanisms of salt tolerance (Munns and Tester, 2008). A negative significant correlation between salt tolerance and shoot Na^+ concentration of the wheat varieties was reported by Saqib et al. (2006). Akbari Ghogdi et al. (2012) and Hasan et al. (2015) also showed that salt-tolerant wheat varieties had lower levels of Na^+ , higher level of K^+ and greater K^+/Na^+ ratio and RWC in their leaf under saline conditions than the sensitive ones. Upon salinity stress, Na^+ content of Zarin and Gonbad leaf increased, potassium content did not change and consequently K^+/Na^+ ratios decreased. The percentage of RWC in both cultivars also decreased in saline conditions. The inability to maintain RWC, reduced vegetative growth and increased sodium content of the shoot tissue upon salinity, indicated that both cultivars were susceptible to the stress. In salinity, plants grown in soil inoculated with C-2012, decreased Na^+ and K^+ content compared to the uninoculated control. Under saline conditions, strain C-2012 increased RWC of Zarin, which resulted in an increased shoot fresh weight. By contrast, although C-2012 did not increase the RWC of Gonbad, but increased plant fresh weight. Under normal conditions, no increase in RWC of Zarin and Gonbad was observed due to treatment with C-2012. Reactive oxygen species (ROS) increase in stress conditions and result in structural and

functional damage of plant cells. To cope with the stress, plants use different mechanisms affecting the production and scavenging of ROS and keep their concentration at a minimal level. Proline is an osmoprotectant that plays a protective role by regulating the production of toxic ROS (Romero-Aranda et al., 2006). Siddiqui et al. (2017) reported that optimum growth of salt-tolerant wheat cultivars was associated with higher levels of proline accumulated in plant tissues. In the present study, Zarin and Gonbad showed 21 and 15 times increase in proline content under salt stress respectively. Sairam et al. (2002) showed that tolerant and moderately tolerant wheat genotypes differentially increased proline in salt stress. They stated that higher contents of proline in the tolerant genotype increased its RWC in stressful conditions. The increase, in proline content of Zarin and subsequent increase in its RWC in bacterial treatment upon salinity, were consistent with Siddiqui et al. (2017). By contrast, RWC and proline content of Gonbad did not change in the same conditions and treatment. Upadhyay and Singh (2015) experiments based on an evaluation of 9 PGPR strains, showed that there is generally no significant difference between proline content of PGPR- inoculated and uninoculated wheat in saline conditions. Besides, *Bacillus subtilis* strain SU47 which significantly increased grain yield did not increase proline content of plant in stress conditions compared to the uninoculated control.

Decreased chlorophyll and carotenoid content due to salinity has been reported for salt tolerant and sensitive genotypes (Khan et al., 2009; Dugasa et al., 2018). These reports also showed that the content of chlorophyll and carotenoid were higher in the tolerant cultivars compared to the moderately tolerant and susceptible ones in stress conditions. Although, exceptional cases have also been reported that do not follow this function. It is reported that only salt-sensitive genotypes, but not tolerant, significantly reduced chlorophyll content in salinity stress (Kumar et al., 2017). The chlorophyll and carotenoid content of Gonbad and chlorophyll content of Zarin influenced by salinity and significantly reduced. Zarin carotenoid content increased unexpectedly under stressful conditions. The different reaction of these two cultivars for maintaining carotenoid pigments showed that susceptible cultivars may be different in applying a mechanism to ameliorate the effects of stress. Although, the reaction of the two cultivars to bacterial treatment in normal and stress conditions was quite similar, and the carotenoid increase was observed for both. Treatment with strain C-2012 enhanced chlorophyll content of Zarin in the normal conditions, although, did not have a positive effect on Gonbad. Conversely, in the salt stress, the PGPR strain caused a great increase in chlorophyll content for both Gonbad and Zarin. One part of our results are consistent with Barnawal et al. (2017) who reported PGPR strains, *Arthrobacter protophormiae* and *Dietszia natronolimnaea* enhanced biomass of wheat seedling by increasing photosynthetic efficiency in normal and salt stress. The accumulation of sodium in the plant leaves reduces the content of photosynthetic pigments through the degradation of chlorophyll (Li et al., 2015) or decreases the accumulation of 5-aminolaevulinic acid (the precursor of chlorophyll) (Santos, 2004). Under salinity stress, strain C-2012 reduced sodium accumulation which was associated with the more chlorophyll content and was observed in both cultivars. Under these conditions, the increase in chlorophyll in Zarin, unlike the Gonbad, did not increase plant weight. It may come back to the higher photosynthetic efficiency of Gonbad compare to Zarin. These results confirm that factors affecting plant yield are not only limited to the chlorophyll content and photosynthetic efficiency is also related to a set of factors (Simkin et al., 2019) besides, wheat cultivars differ in this respect (Song et al., 2017).

In saline conditions, CAT, POX and APX activity of Zarin and POX, SOD and APX activity of Gonbad increased. ROS-scavenging enzymes CAT, POX, SOD, APX and GST are important components of the plant defense system and ameliorate damages of salinity (Das and Roychoudhury, 2014). Upadhyay et al. (2012) reported that the activity of antioxidant enzymes of wheat improved with increasing soil salt concentration. Siddiqui et al. (2017) showed that the induction levels of CAT, POX and APX activities under salt stress were higher in salt tolerant

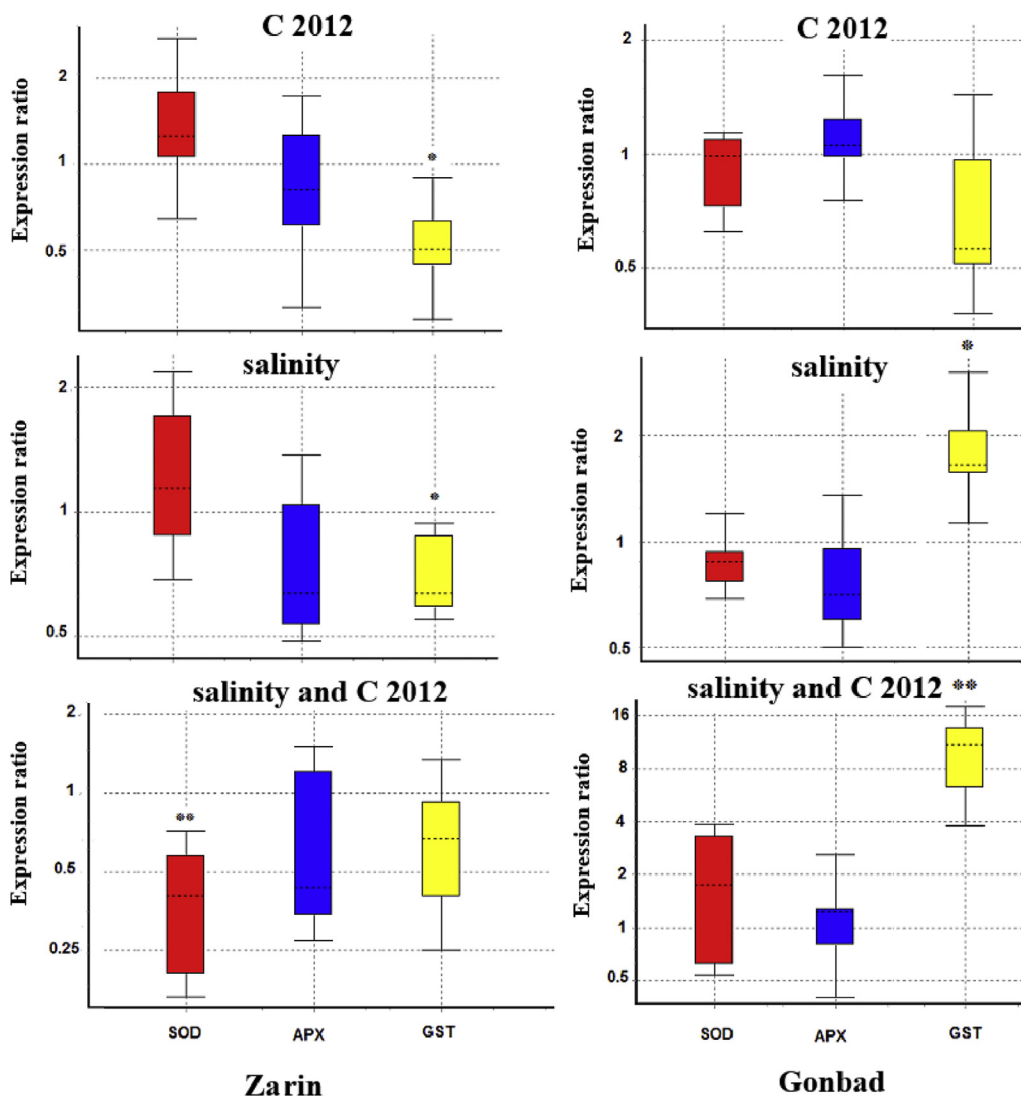


Figure 7. Expression ratio of *SOD*, *APX* and *GST* in Zarin and Gonbad in different treatments and conditions. Expression ratios were calculated using relative expression software tool REST 2009 (Pfaffl et al., 2002). Boxes represent the interquartile range, or the middle 50% of observations. The dotted line represents the median gene expression. Whisker-box plots represent the minimum and maximum observations. The values marked with one ($P < 0.05$) or two ($P < 0.01$) asterisks represent genes significantly overexpressed or suppressed relative to control (uninoculated plant grown in normal conditions).

cultivar than in salt sensitive. In soil treated with strain C-2012, SOD and APX activity of Gonbad and Zarin decreased upon salinity. POX activity increased in each treatment of C-2012 or salinity and also in PGPR treated -plants grown in saline conditions. In stress conditions, the bacterial influence was similar in reducing the activity of SOD and APX of both wheat cultivars. The reduction of the antioxidant enzymes, APX, CAT and glutathione reductase (GR) as a result of wheat treatment with *Bacillus subtilis* and *Arthrobacter* sp. strains under stress conditions have been reported by Upadhyay et al. (2012). Conversely, Singh et al. (2017) showed that inoculation of wheat with a PGPR strain of *Stenotrophomonas maltophilia* increased activities of SOD, CAT and POX in saline conditions. This information, though useful, is somewhat confusing. The best conclusion that can be drawn from these studies is that each PGPR strain uses its own special mechanism to ameliorate the effects of stress. In addition, a bacterium may have different effects on different plant genotypes in normal or stress conditions. Our result is in accordance with the results of Egamberdieva (2010) who suggested that inoculation of wheat with *Pseudomonas* strains can improve wheat growth, depending on the cultivar. GST was the only enzyme whose response to the PGPR in saline conditions completely dependent on the plant genotype. Gonbad GST activity was greatly reduced, but no significant difference was found in Zarin. Interestingly, GST activity of Zarin and Gonbad in soil treated with C 801 has been similarly increased in response to salinity (data not shown). We recall that under these conditions, strain C 801 increased

biomass of both cultivars similarly. GST conjugate xenobiotics to glutathione, the reduced form of glutathione and detoxify cellular environments. Although the protective role of GST against different stress has been shown in several plant species (Basantani and Srivastava, 2007), there are differences between the GST activity of plant genotypes in normal and stressful conditions. Gallé et al. (2009) reported induced GST activity of tolerant and susceptible wheat genotypes following drought stress and at different developmental stages. According to their experiments, GST induction was significantly earlier in resistant cultivars than in sensitive ones. In one of the sensitive cultivars, the activity of GST did not change under stress or over the time. Another sensitive cultivar, increased GST level only in the last stage when the plant was showing the negative effects of the stress. It seems that under salt stress, strain C-2012 caused a severe reduction in GST activity of Gonbad to inhibit its probable role in stress signaling. Highly increased level of GST expression in bacterial inoculated Gonbad in saline stress state that plant and bacterial efforts to ameliorate salt effects are not consistent with each other. Reduced GST transcripts upon salinity and reduced SOD transcripts in inoculated-stressed Zarin are consist with decreased GST and SOD activities in related conditions. Reduction of GST transcript in wheat plants inoculated with *Enterobacter cloacae* and treated with salt was reported (Singh et al., 2017). Although they did not point to an increase in plant growth in these conditions. Increase in SOD, APX and POX activities in bacterial inoculated-Zarin under normal conditions suggested that this

cultivar may recognize strain C-2012 as a gentle stressor and not as a PGPR. The increased activity of these enzymes was not observed for Gonbad in the same conditions.

In summary, since there is still no commercial bio-agent that can actually ameliorate salt stress in wheat fields, it is clear that the effect of PGPRs, although positive, are not uniform in different conditions and for all cultivars. Identification of different physiological and phenotypic responses of wheat cultivars to a defined PGPR, especially in the molecular level, provides valuable genetic materials for wheat tolerance improvement by the breeding program or genetic modification (GM). Based on our knowledge this is the first study focused to assess differences in modes of action of commercial wheat cultivars in response to a PGP *Streptomyces*.

Declarations

Author contribution statement

Alireza Akbari: Performed the experiments.

Shahrokh Gharanjik: Analyzed and interpreted the data.

Parisa Koobaz: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Akram Sadeghi: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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