



Potassium Silicate and Plant Growth-promoting Rhizobacteria Synergistically Improve Growth Dynamics and Productivity of Wheat in Salt-affected Soils



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Salinity is one of the most brutal environmental factors limiting the productivity of crop plants. Thus, there is an urgent need for environmentally friendly techniques to enhance growth and productivity of wheat (*Triticum aestivum* L.) growing in saline soils. In our 2-year field experiments, we evaluated the effect of two beneficial plant growth-promoting rhizobacteria (PGPR; *Azospirillum brasilense* and *Bacillus circulans*) and the foliar application of potassium silicate (K_2SiO_3 ; PS) to improve growth and yield of three cultivars of wheat, namely Misr 1, Gemmeza 12 and Sakha 95, under salt-affected soils ($EC=7.71$). The results supported our hypothesis that the combined application of PGPR+PS significantly ($P<0.05$) improved growth and yield, nutrients (N, Na^+ and K^+) uptake, photosynthetic pigments, proline content, and total soluble sugars content compared to the individual application of PGPR or PS and the untreated (control) plants. In addition, the combined application significantly ($P<0.05$) increased peroxidase and catalase activities, scavenging the damage effects of the reactive oxygen species. Our data revealed that the combined application could activate the soil key enzymes, mainly dehydrogenase and urease, and boost soil microbial activity. Overall, the combination of PGPR and PS applications, as a simple and low-cost biological method, has shown a positive effect in terms of improving soil properties, enhancing plant growth, and increasing element contents of wheat under salinity stress.

Keywords: Salinity, proline, ascorbate peroxidase, catalase, soil enzymes.

1. Introduction

Worldwide, rapid population growth exerts great pressure to increase wheat production. Consumers will require 60% more wheat by 2050 than today (Asseng et al. 2018), especially under the climate

changes in the recent decades, which have significantly affected the productivity of agricultural crops (He et al., 2017; Abdoulaye et al. 2017; Ning et al. 2019; Gao et al. 2020a; AbdElgawad et al., 2022). Globally, wheat (*Triticum aestivum* L.) is one of the strategic crops in human life, as it is used for food, feed and biofuel security (Naveed, et al. 2014). The cultivated area of wheat in Egypt was 1370235 hectares in 2020 with a production of 9 million tons,

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accounting for nearly 42.85% of total consumption (FAOSTAT, 2020). Productivity of wheat can be improved by successful management of soil and water, especially in salt-affected soils (estimated by 809371 hectares) which account for 35% of the total cultivated area in Egypt. Soil salinization is increasing globally as well as in Egypt, for many reasons including suboptimal irrigation, desertification and excessive fertilization. Worldwide, more than 800 million hectares of soil are affected by salt stress (Ramadoss et al. 2013). Salt stress can affect plants in different ways, including osmotic stress and ion homeostasis, affecting nutrient imbalance, increasing concentrations of Na^+ and Cl^- , and reducing plant growth and productivity (Shrivastava and Kumar 2015).

Application of the beneficial bacteria strains or plant growth-promoting rhizobacteria (PGPR) is a cost-effectively and eco-friendly strategy that reduces the toxic effects of high salinity (Sagar et al., 2021). PGPR are capable of promoting health of soils and growth dynamics of crops (Viscardi, et al. 2016). Seed treatment with PGPR can improve the rate of seed germination (Ali et al. 2014), increase plant growth and N_2 fixation (Shilev, 2020; Ibrahim and El-Sawah, 2021), synthesize beneficial hormones of plant *i.e.* auxins, cytokinins, and gibberellins (Chen et al. 2018; Gao et al. 2020b; Sheteiwy et al. 2021a), improve the ratio of K^+/Na^+ , scavenging reactive oxygen species (ROS) and improving soil enzymes activities (Yadav and Verma 2014), and improving different properties of soil (Denton et al. 2012). In addition, foliar spraying of PGPR is an efficient approach in agriculture used to enhance growth of plants and ameliorate the effect of salt stress (Gomaa et al. 2021, Sheteiwy et al., 2021b; Hu et al., 2016).

Foliar application with potassium silicate (K_2SiO_3) is another important approach that can be used as a bio-stimulant for plant as well as a source of K and Si (Rodriguez, 2009). As an essential element, K plays a central role in forming sugars, carbohydrates, protein, cell division, and quality of seeds. In addition, K^+ stimulates vegetative growth of plants, regulates osmosis, enhances physiological processes, and improves ionic balance and enzymatic antioxidant activity (Ahmad et al. 2016 and Hasanuzzaman et al. 2018). Thus, it can mitigate the negative effects of salt stress in plants. In addition, Si is one of the most important elements in crop production that reduces the negative effects of salt stress by reducing the uptake of Na^+ and improving the uptake of K^+ in leaves, oxidative stress as well as improving root structure, vegetative growth, photosynthesis and water relations (Liang et al. 2003 and Yaghubi et al. 2016). Some studies have shown that K_2SiO_3 positively results plant growth, production, and

quality as well as improves the activities of enzymatic antioxidants (Liang et al. 2007; Ahmad et al. 2019; Salim et al. 2019 and Hafez et al. 2021). Although several studies have assessed the effects of PGPR (Bharti et al., 2016; Habib et al., 2016; Ilangumaran and Smith, 2017; Egamberdieva et al., 2019) and K_2SiO_3 (Yaghubi et al., 2016; El-Akhdar et al. 2018; El-Ramady et al. 2019; Yaghubi et al., 2019) in alleviating salinity stress in salt-affected soil, very few have determined the synergistic effect of the two factors (Hafez et al., 2021). Accordingly, we hypothesize that the combined application of PGPR (*Azospirillum brasilense* and *Bacillus circulans*) and K_2SiO_3 synergistically mitigate the negative effects of salt stress in different cultivars of wheat. Our results may help understand possible morphological, physiological, and biochemical mechanisms underlying synergistic effects of PGPR and K_2SiO_3 in inducing growth and yield of wheat plants under saline stress conditions.

2. Materials and Methods

2.1. Experimental site description

In salt-affected soils, field trials were conducted during 2019/2020 and 2020/2021 seasons at the Sakha Agricultural Research Station (SARS), Kafr El-Sheikh, Egypt (31.1107° N, 30.9388°). The metrological data for the two growing seasons shown in Figure 1. The soil at the experimental site was salt-affected (EC = 7.71), with a clayey texture. Randomly, from 0-30 cm depth, soil samples were collected, air-dried, and stored for analyses of various physicochemical and biological properties during both growing seasons, as shown in Table 1

Plant growth-promoting rhizobacterial strains (PGPR)

Two bacterial strains of *Azospirillum brasilense* SARS 1001 and *Bacillus circulans* NCAIM B.02324 were provided from Agricultural Microbiology Department, SWERI, ARC, Egypt. The cultures of *Azospirillum* strain were maintained on a Semi-Solid Malate medium (Döbereiner et al., 1976) while, *Bacillus* strain was maintained on a Nutrient Broth medium (Atlas, 2010). The mixture of the two bacterial strains (1:1) was prepared as peat-based inoculums at a rate of 1400 g ha⁻¹, which mixed with seeds of wheat before sowing.

Potassium silicate (PS)

Potassium silicate (K_2SiO_3) was obtained from Merck, Germany (CAS: 1312-76-1). Using a hand atomizer, K-Silicate was used as a foliar application treatment at 30 and 50 days from sowing with the rate of 300 mg L⁻¹.

Table 1. Some physical, chemical, and biological properties of the experimental soil.

Soil properties	2019/2020	2020/2021
Clay %	55.00	53.00
Sand %	11.00	17.00
Silt %	34.00	30.00
Soil texture (%)	Clayey	Clayey
pH (1: 2.5 water suspension)	8.28	8.04
EC (dSm ⁻¹)	7.67	7.76
Organic matter (g Kg ⁻¹)	17.5	16.4
Available P (mg Kg ⁻¹)	11.00	10.33
Available NH ₄ (mg Kg ⁻¹)	15.5	14.30
Available K (mg Kg ⁻¹)	336	366
Cations (mmolc L ⁻¹)		
Ca ⁺⁺	6.30	7.00
Mg ⁺⁺	2.40	2.00
Na ⁺	11.00	12.30
K ⁺	1.51	0.50
Anions (mmolc L ⁻¹)		
HCO ₃ ⁻	6.80	6.00
Cl ⁻	11.00	13.00
SO ₄ ⁻⁻	3.50	2.80
CO ₃ ⁻	0.00	0.00
TCB (CFU ×10 ⁶ g ⁻¹ dry soil)	106	134
TCF (CFU ×10 ⁴ g ⁻¹ dry soil)	57	79
TCA (CFU ×10 ⁵ g ⁻¹ dry soil)	81	92

TCB, total count of bacteria; TCF, total count of fungi; TCA, total count of actinobacteria; CFU, colony forming unit.

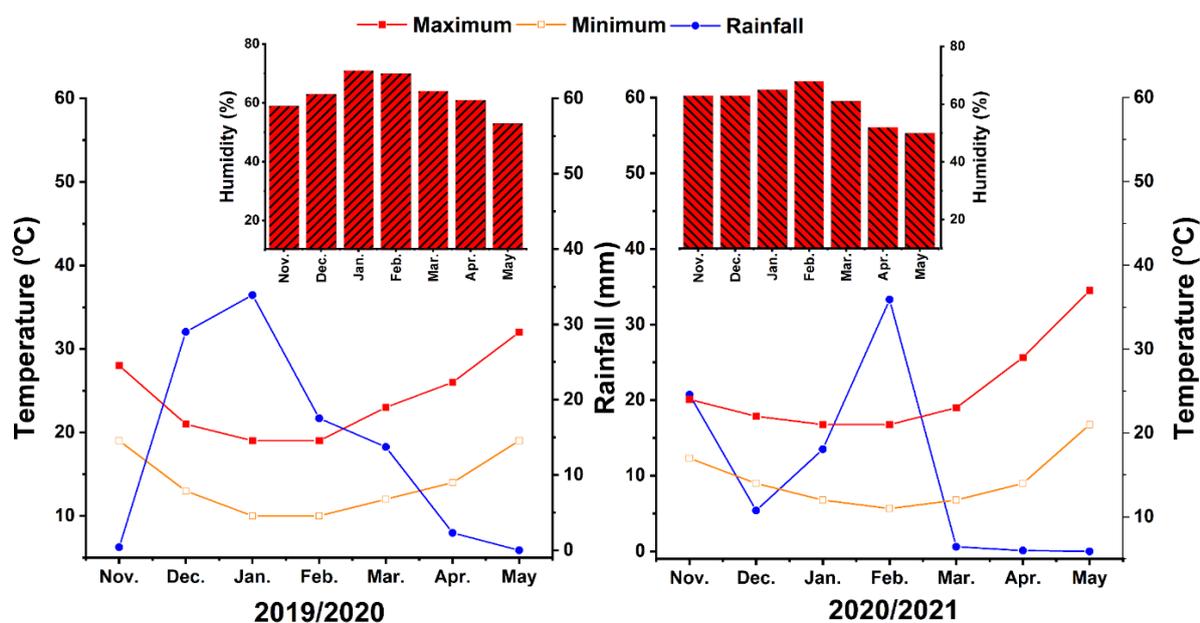


Fig. 1. Meteorological data of temperature (°C), rainfall (mm), and relative humidity (%) during 2019/2020 and 2020/2021 growing seasons.

Plant materials

Three cultivars of wheat (*Triticum aestivum* L.), namely Misr 1, Gemmeza 12, and Sakha 95 were provided by the Wheat Research Department, Sakha Agricultural Research Station, Egypt.

Experimental design, treatments, and field trial

In triplicates, the experiment was conducted in a split-plot design. Different cultivars of wheat plants were considered the main plots, and non-treated plants (control), plants inoculated with PGPR, potassium silicate, and the combination treatment of PGPR + PS were considered the sub-plots. Using a drilling method, wheat seeds were sown on 9th November during the first growing season (2019/2020) and 12th November during the second growing season (2020/2021). Each plot measured 3 x 3.5 m with a rate of 140 kg ha⁻¹. During soil tillage, The phosphorus fertilizer was added as calcium superphosphate (15.5% P₂O₅) at a rate of 360 kg ha⁻¹, and potassium fertilizer as potassium sulfate (48% K₂O) at a rate of 120 kg ha⁻¹, respectively. Nitrogen fertilizer was added as urea (46.5% N) into two equal doses (prior to 1st and 2nd irrigations) with a rate two-thirds of 240 kg ha⁻¹ as the full dose for PGPR treatments and 360 kg ha⁻¹ as the full dose for all other treatments.

Measurements

Ten plants were randomly selected from each replicate after 70 days from sowing to measure vegetative growth, nutrient content, physiological modifications, antioxidant enzymes, and soil enzyme activity. Characteristics of the yield (grain yield, straw yield and biological yield (ton ha⁻¹)), as well as, harvest index (%) were calculated at the end of the experiment.

Vegetative parameters

Plant height (cm plant⁻¹), dry weight (g plant⁻¹) and root length (cm plant⁻¹) were measured. The plant height and root length were measured by tape, while the dry weight was measured by using an electronic balance.

Nutrients content in plant

Nitrogen percentage was determined according to Jones *et al.* (1991) methods. In brief, 0.5 g of the ground plant was digested by concentrated sulfuric acid and hydrogen peroxide (30%) on a hot plate, and nitrogen was estimated using micro-Kjeldahl (Peters, *et al.* 2003). In addition, potassium, sodium, and the potassium/sodium ratio were measured by a Flame photometer (Cottenie, *et al.* 1982).

Photosynthetic Pigments in plant

The total chlorophylls and carotenoids were estimated in 0.1 g of fully expanded flag leaves' samples from each treatment that were ground in 5

mL of acetone (80%), and extracted. Using a UV spectrophotometer, the pigments were determined then expressed as mg g⁻¹ FW (Lichtenthaler, 1987).

Proline

Based on a standard curve of proline, the concentration of proline was determined by a UV spectrophotometer at 520 nm (Bates, *et al.* 1973). Briefly, 0.5 g of the flag leaves was homogenized with five mL of ethanol (95%). After centrifugation at 5000 x g for 10 min, one mL of each alcoholic extract and distilled water and two mL of each ninhydrin and glacial acetic acid were mixed with the supernatant at 100 °C. After 60 min, the reaction was stopped (cold water), and then mixed with four mL of toluene. Proline concentration was calculated and expressed as mol g⁻¹ FW.

Total Soluble Sugars (TSS)

Based on a standard curve of glucose, total soluble sugars' concentration was determined by a UV spectrophotometer at 620 nm (Ibragimova *et al.* 2006). In a water bath, 0.5 g of the flag leaves sample was homogenized in five mL of 80% ethanol, then placed for 30 min at 80 °C. After centrifuging at 10000 x g for 10 min, the supernatants were collected and measured. The concentration was expressed as mg g⁻¹ FW.

Activity of Antioxidant Enzymes in plant

A mixture of 50 mM Na⁺ of PO₄⁻³ buffer (pH 7.0), 1 mM H₂O₂ and 20 µL/mL enzymatic extract was used to assess catalase (CAT) activity at 240 nm, according to the methods of (Aebi, 1983). At 290 nm, the ascorbate peroxidase (APX) activity was determined according to the methods of (Nakano and Asada, 1981). The reaction contained 200 µL of enzyme extract + 25 mM of PO₄⁻³ buffer (pH 7.0) + 0.1 mM of EDTA + 0.25 mM of ascorbic acid + 1.0 mM of H₂O₂. Enzyme activities were measured as µM H₂O₂ min⁻¹ g⁻¹ FW.

Soil Enzyme Activities

In the soil samples, dehydrogenase activity (DAH) was determined according to Casida *et al.* (1964). The reaction contained 10 g of soil sample + 0.2 g calcium carbonate + 1 mL of TTC (2,3,5-triphenyl tetrazolium chloride, 3%), + 1 mL of glucose (1%) + 8 mL of dH₂O which incubated at 30 °C for one day. Then filtered by Whatman No. 50 after adding 10 mL of methanol. DAH was measured at 485 nm using a UV spectrophotometer and expressed as the mg TPF g⁻¹ soil day⁻¹.

In addition, urease activity was determined according to (Pancholy and Rice, 1973). Five grams of soil sample was mixed with 0.5 mL of toluene for 15 min. Then added 10 mL of PO₄⁻³ buffer (pH 7.6) + 10 mL

of urea (solution 10%) and shaken for 5 min then incubated at 30 °C for one day. After filtered by Whatman No. 42, one mL was mixed with one mL (10%) of $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ and one mL of gum acacia (solution 1%) and five ml of Nessler's reagent was added (Campbell and Plank, 1998). At 410 nm, spectrophotometer was used to measure the urease activity as $\text{mg NH}_4^+ - \text{N g}^{-1} \text{ soil day}^{-1}$.

Yield and its attributes

At maturity, each plot was harvested, and grains were separated, dried at standard moisture of 14%, weighed, and calculated as ton ha^{-1} . In addition, the straw yield was weighted and calculated as ton ha^{-1} .

The harvest index (HI) was calculated as a percentage of produced grains and whole plants' weight (straw and grains).

Statistical Analysis

Data were analyzed using split-plot analysis of variances (NOVA). The different wheat cultivars were considered the main plots, non-treated plants (control), plants inoculated with PGPR, PS, and the combination of PGPR + PS were considered as sub-plots. According to Duncan (1955), Duncan's multiple range test was used to compare the treatment means.

Table 2. Effects of PGPR soil application, PS foliar application, and their combination on plant height (cm), root length (cm) and dry weight (g plant^{-1}) of three wheat cultivars at 70 days post seed sowing during two growing seasons 2019/2020 and 2020/2021.

Year	cultivars (C)	Treatment (T)	Height (cm plant^{-1})	Root length (cm plant^{-1})	Dry weight (g plant^{-1})
2019/2020	Misr 1	T1	70.36 ± 0.84 f	20.10 ± 0.24 e	3.51 ± 0.05 g
		T2	63.54 ± 1.37 g	18.17 ± 0.42 f	3.17 ± 0.07 h
		T3	77.91 ± 0.08 bc	22.26 ± 0.04 bc	3.89 ± 0.01 bc
		T4	79.63 ± 0.57 a	22.73 ± 0.21 a	3.98 ± 0.03 a
	Gemmiza 12	T1	74.21 ± 0.91 e	21.18 ± 0.26 d	3.71 ± 0.05 f
		T2	74.96 ± 1.06 e	21.27 ± 0.38 d	3.74 ± 0.06 f
		T3	74.78 ± 1.06 e	21.35 ± 0.30 d	3.76 ± 0.04 ef
		T4	79.03 ± 0.29 ab	22.59 ± 0.08 ab	3.95 ± 0.02 ab
	Sakha 95	T1	69.58 ± 0.79 f	19.80 ± 0.26 e	3.47 ± 0.04 g
		T2	77.70 ± 0.50 bcd	22.15 ± 0.13 c	3.88 ± 0.02 bcd
		T3	76.48 ± 0.65 d	21.85 ± 0.17 c	3.82 ± 0.03 de
		T4	77.25 ± 0.94 cd	22.10 ± 0.26 c	3.86 ± 0.05 cd
2020/2021	Misr 1	T1	70.48 ± 0.84 f	20.22 ± 0.24 e	3.56 ± 0.05 e
		T2	63.63 ± 1.37 g	18.27 ± 0.44 f	3.24 ± 0.07 f
		T3	78.02 ± 0.08 bc	22.35 ± 0.04 bc	3.94 ± 0.02 b
		T4	79.72 ± 0.58 a	22.79 ± 0.21 a	4.06 ± 0.03 a
	Gemmiza 12	T1	74.33 ± 0.91 e	21.30 ± 0.26 d	3.76 ± 0.05 d
		T2	75.05 ± 1.06 e	21.35 ± 0.38 d	3.81 ± 0.06 cd
		T3	74.89 ± 1.06 e	21.46 ± 0.30 d	3.80 ± 0.04 cd
		T4	79.11 ± 0.29 ab	22.65 ± 0.08 ab	4.03 ± 0.02 a
	Sakha 95	T1	69.70 ± 0.79 f	19.92 ± 0.26 e	3.52 ± 0.04 e
		T2	77.79 ± 0.50 bcd	22.23 ± 0.13 bc	3.96 ± 0.02 b
		T3	76.59 ± 0.65 d	21.96 ± 0.17 c	3.86 ± 0.03 c
		T4	77.33 ± 0.94 cd	22.16 ± 0.26 c	3.94 ± 0.05 b
F-test					
Cultivar (C)			***	***	***
Treatment (T)			***	***	***
Interaction (C X T)			***	***	***

Means of the same growing season designated with different letters indicate significant differences among treatments according to the Duncan's test ($P < 0.05$). Values are means ± standard deviation (SD) from 3 replicates. PGPR, plant growth-promoting rhizobacteria; PS, potassium silicate; T1, control; T2, PGPR; T3, PS; T4, PGPR+PS.

3. Results

Vegetative parameters

The two-way ANOVA indicated significant effects of plant treatment (PGPR, PS and PGPR+PS), cultivar and their interactions on plant height, root length, and dry weight after 70 days from seed soaking ($P < 0.001$, Table 2). During the two seasons, the applied of

PGPR, PS and their combination had significantly ($P > 0.001$) improved the growth parameters of wheat cultivars grown in salt-affected soils (Table 2). In general, the combined treatment enhanced the growth parameters over the control. Although, the individual application of PGPR or PS increased the growth parameters over the control, we did not notice a significant reduction in growth parameters. For

example, plants simultaneously treated with PGPR and PS attained greater height than in control (non-treated) plants by 13.1% in Misr 1, 6.5% in Gemmiza 12 and 11.0% in Sakha 95 cultivars in the 2019/2020 season. Plant heights with PGPR treatment only was greater than control plants by 11.7% in Sakha 95 and 1% in Gemmiza 12, but lower than control by 9.7% in Misr 1. Similar direction was noted in the 2020/2021 season (Table 2). Such results indicate that the response to different treatments in salt-affected soil is dependent on the cultivar.

Nutrient contents in wheat leaves

Under different treatments of soil and foliar application, the combined treatment (PGPR+PS) led to increases in N, K⁺%, and K⁺/Na⁺ ratio as well as decreased Na% in leaves of different wheat cultivars with significant ($P<0.05$) differences at 70 days after sowing (Table 3). By comparing the cultivars, the highest percentage of N and K was the PGPR+PS treatment (T4) which attained an increased rate of 13.24%, 7.34% and 10.77% for N, 11.15%, 6.22% and 9.05% for K in 2019/2020, and 12.34%, 6.29% and 9.54% for N, and 9.83%, 4.53% and 7.09% for K in 2020/2021, compared with control treatment (T1), respectively (Table 3). In addition, the greatest reduction of Na⁺% was noted in leaves from 2.49% (Control) to 2.18 % (PGPR+PS) for Misr 1 cultivar, 2.47% (Control) to 2.29% (PGPR+PS), for Gemmiza 12, and 2.41% (Control) to 2.16 % (PGPR+ PS) for Sakha 95 in 2019/2020 season. Similar pattern of Na⁺% in the three cultivars was followed in the second season. The PGPR+PS had the highest K⁺/Na⁺ ratios which followed the descending order of Misr 1 > Sakha 95 > Gemmiza 12 during the first and second growing seasons compared with all other treatments (Table 3).

Physiological Characteristics

At 70 days from sowing, physiological characteristics included (total chlorophyll, carotenoids, proline and TSS) of the three wheat cultivars showed significant differences ($P<0.05$) for soil and foliar applications of T1 (control), T2 (PGPR), T3 (PS) and T4 (PGPR+PS) (Figure 2). During the growing seasons, the maximum contents of total chlorophyll 3.29 and 3.44 mg g⁻¹ FW were measured in Misr 1; 3.27 and 3.43 mg g⁻¹ FW in Gemmiza 12; 3.21 and 3.37 mg g⁻¹ FW in Sakha 95 when wheat plants were treated with PGPR+PS, respectively (Figure 2a). Under different treatments, T4 treatment also recorded the highest carotenoids of 1.09, 1.07 and 1.01 µg g⁻¹ FW; proline of 6.90, 7.87 and 8.77 µmol g⁻¹ FW; and TSS of 3.98, 4.45 and 4.82 for µg g⁻¹ FW in Misr 1, Gemmiza 12 and Sakha 95 in 2019/2020 season compared with other treatments (Figure 1b-d). We also observed the same trend in the growing season of 2020/2021 (Figure 2).

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Antioxidant enzymes

In general, the antioxidant enzymes activity, CAT and APX, in wheat cultivars were significantly ($P<0.05$) different due to the application of PGPR, foliar application by PS, and their combination during the two growing seasons (Figure 3). At 70 days from sowing, all treatments significantly increased the enzymatic activity of CAT and APX in leaves as comparison to the control (Figure 3). During the first season, APX activity (µM H₂O₂ min⁻¹ g⁻¹ FW) significantly increased from 20.03, 16.98 and 12.52 in the control treatment to 23.83, 21.92 and 17.61 in PGPR+PS of Misr 1, Gemmiza 12 and Sakha 95 cultivars, respectively. There was a similar trend in APX activity in the second season (Figure 3a). Among the studied treatments, PGPR+PS efficiently increased the CAT content by 413.00, 405.00 and 389.67 (µM H₂O₂ min⁻¹ g⁻¹ FW) in 2019/2020 season and 444.00, 436.00 and 420.67 (µM H₂O₂ min⁻¹ g⁻¹ FW) in 2020/2021 season in Misr 1, Gemmiza 12 and Sakha 95, respectively (Figure 3b). From the previous-mentioned results, the descending order of Misr 1 > Gemmiza 12 > Sakha 95 during the first and second growing seasons compared to other treatments.

Soil Enzyme activities

In the rhizosphere of wheat plants, PGPR+PS combined treatment showed significant ($P<0.05$) increases in the soil enzymes activity of dehydrogenase and urease compared to the single PGPR, PS or control treatments at 70 days from sowing (Table 4). Thus, PGPR+PS had the highest activity of dehydrogenase (mg TPF g⁻¹ soil day⁻¹) was 102.61 in Misr, 116.36 in Gemmiza 12 and 134.17 and Sakha 95 followed by PGPR which was 97.54 in Misr, 105.90 in Gemmiza 12 and 116.17 Sakha 95 in 2019/2020. In the same way, the second season (2020/2021), 114.63, 133.33 and 151.19 mg TPF g⁻¹ soil day⁻¹ were attained by PGPR+PS; followed by PGPR attaining 111.52, 120.94, and 131.07 mg TPF g⁻¹ soil day⁻¹ in Misr 1, Gemmiza 12 and Sakha 95, respectively (Table 4). For urease activity, PGPR+PS was the best among all treatments with 95.33, 100.63 and 105.65 in the first growing season and 106.67, 113.63, and 118.60 NH₄⁺-N g⁻¹ soil day⁻¹ in the second growing season in Misr 1, Gemmiza 12 and Sakha 95, respectively (Table 4).

Grains yield and yield-related parameters

Under salt-affected soil conditions, yield and yield-related parameters of the three wheat cultivars were significantly enhanced by inoculation with PGPR combined with PS during the trial period (Table 5). T4 treatment (combined) of PGPR +PS caused the maximum grain yield (ton ha⁻¹), recording 7.75, 7.86, and 8.10 in the 1st growing season; and 7.88, 7.98 and 8.22 in the 2nd growing season for Misr 1, Gemmiza

12 and Sakha 95, respectively, compared to the other individual and control treatments. In respect to straw yield (ton ha⁻¹), biological yield and harvest index (%), control treatment had the lowest values, reaching to 9.23, 14.33, and 35.60 for Misr 1; 9.42, 14.58, and 35.40 for Gemmiza 12; 9.59, 14.73 and 34.92 for Sakha 95, respectively, in 2019/2020. For

the 2nd growing season (2020/2021), data were similar to those noted in the 1st season (Table 3). From these results, the descending order for the different cultivars of wheat plants Sakha 95 > Misr 1 > Gemmiza 12, while the descending order for soil and foliar application PGPR+PS > PS > PGPR > Control.

Table 3. Effect of soil application by PGPR, foliar application by PS, and their combination on N, Na⁺, K⁺ and K⁺/Na⁺ percentage in the leaves of the three wheat cultivars at 70 days post seed sowing during two growing seasons 2019/2020 and 2020/2021.

Year	cultivars (C)	Treatment (T)	N	K ⁺	Na ⁺	K ⁺ /Na ⁺
2019/2020	Misr 1	T1	2.34 ± 0.04 f	2.78 ± 0.04 f	2.49 ± 0.02 a	1.11 ± 0.03 e
		T2	2.11 ± 0.04 g	2.55 ± 0.04 g	2.42 ± 0.04 bc	1.05 ± 0.01 f
		T3	2.58 ± 0.02 bc	3.02 ± 0.02 bc	1.95 ± 0.02 g	1.54 ± 0.02 a
		T4	2.65 ± 0.02 a	3.09 ± 0.02 a	2.18 ± 0.04 f	1.41 ± 0.01 b
	Gemmiza 12	T1	2.45 ± 0.05 e	2.89 ± 0.05 e	2.47 ± 0.01 ab	1.17 ± 0.02 e
		T2	2.49 ± 0.06 de	2.93 ± 0.06 de	2.34 ± 0.06 de	1.25 ± 0.01 d
		T3	2.50 ± 0.04 de	2.94 ± 0.04 de	2.33 ± 0.04 de	1.26 ± 0.02 d
		T4	2.63 ± 0.01 ab	3.07 ± 0.01 ab	2.29 ± 0.05 e	1.34 ± 0.01 c
	Sakha 95	T1	2.32 ± 0.02 f	2.76 ± 0.02 f	2.41 ± 0.02 c	1.14 ± 0.01 e
		T2	2.59 ± 0.02 bc	3.03 ± 0.02 bc	2.38 ± 0.02 cd	1.27 ± 0.03 d
		T3	2.54 ± 0.02 cd	2.98 ± 0.02 cd	2.34 ± 0.04 de	1.27 ± 0.03 d
		T4	2.57 ± 0.04 c	3.01 ± 0.04 c	2.16 ± 0.02 f	1.39 ± 0.01 bc
2020/2021	Misr 1	T1	2.43 ± 0.04 g	2.95 ± 0.03 f	2.64 ± 0.03 a	1.11 ± 0.03 e
		T2	2.17 ± 0.04 h	2.70 ± 0.06 g	2.35 ± 0.03 f	1.14 ± 0.02 de
		T3	2.68 ± 0.03 abc	3.19 ± 0.03 abc	2.59 ± 0.03 abc	1.23 ± 0.02 c
		T4	2.73 ± 0.03 a	3.24 ± 0.03 a	2.10 ± 0.06 g	1.54 ± 0.01 a
	Gemmiza 12	T1	2.54 ± 0.05 ef	3.09 ± 0.05 e	2.63 ± 0.05 ab	1.17 ± 0.01 d
		T2	2.55 ± 0.06 f	3.07 ± 0.06 e	2.49 ± 0.06 e	1.23 ± 0.01 c
		T3	2.61 ± 0.04 de	3.11 ± 0.04 de	2.51 ± 0.04 de	1.23 ± 0.03 c
		T4	2.70 ± 0.01 ab	3.23 ± 0.01 ab	2.47 ± 0.01 e	1.30 ± 0.03 b
	Sakha 95	T1	2.41 ± 0.02 g	2.96 ± 0.02 f	2.57 ± 0.02 bcd	1.15 ± 0.05 de
		T2	2.65 ± 0.02 bcd	3.17 ± 0.02 bcd	2.57 ± 0.02 bcd	1.23 ± 0.05 c
		T3	2.65 ± 0.02 bcd	3.15 ± 0.02 cd	2.55 ± 0.04 cd	1.23 ± 0.03 c
		T4	2.64 ± 0.04 cd	3.17 ± 0.04 bcd	2.36 ± 0.02 f	1.34 ± 0.04 b
F-test						
Cultivar (C)			***	***	***	***
Treatment (T)			***	***	***	***
Interaction (C X T)			***	***	***	***

Means of the same growing season designated with different letters indicate significant differences among treatments according to the Duncan's test ($P < 0.05$). Values are means ± standard deviation (SD) from 3 replicates. PGPR, plant growth-promoting rhizobacteria; PS, potassium silicate; N, nitrogen; Na⁺, sodium; K⁺, potassium; K⁺/Na⁺, potassium/sodium ratio; T1, control; T2, PGPR; T3, PS; T4, PGPR+PS.

Table 4. Effect of soil application by PGPR, foliar application by PS, and their combination on soil enzymes activity, DHA and urease, in the rhizosphere of the three wheat cultivars at 70 days post seed sowing during two growing seasons 2019/2020 and 2020/2021.

Year	Cultivars (C)	Treatment (T)	DHA (mg TPF g ⁻¹ soil d ⁻¹)	Urease (NH ₄ ⁺ - N g ⁻¹ soil day ⁻¹)
2019/2020	Misr 1	T1	80.37 ± 0.63 g	82.45 ± 2.11 i
		T2	97.54 ± 1.38 e	93.11 ± 0.21 f
		T3	91.65 ± 0.95 f	89.48 ± 0.74 g
		T4	102.61 ± 2.68 d	95.33 ± 1.11 e
	Gemmiza 12	T1	82.00 ± 1.39 g	76.42 ± 1.12 j
		T2	105.90 ± 1.39 cd	98.36 ± 1.59 d
		T3	90.18 ± 0.63 f	87.37 ± 0.79 h
		T4	116.36 ± 4.41 b	100.63 ± 0.90 c
	Sakha 95	T1	82.85 ± 1.06 g	73.85 ± 0.57 k
		T2	116.17 ± 1.63 b	103.10 ± 0.79 b
		T3	107.49 ± 1.47 c	102.07 ± 0.89 bc
		T4	134.17 ± 4.39 a	105.65 ± 0.80 a
2020/2021	Misr 1	T1	92.37 ± 0.62 g	91.45 ± 2.11 h
		T2	111.52 ± 2.04 d	106.11 ± 0.21 e
		T3	106.65 ± 0.95 e	99.14 ± 3.63 f
		T4	114.63 ± 2.68 d	106.67 ± 2.01 e
	Gemmiza 12	T1	94.00 ± 1.39 g	85.42 ± 1.12 i
		T2	120.94 ± 1.39 c	111.36 ± 1.59 cd
		T3	102.18 ± 0.63 f	95.37 ± 0.79 g
		T4	133.33 ± 4.41 b	113.63 ± 0.90 bc
	Sakha 95	T1	94.85 ± 1.02 g	82.85 ± 0.57 j
		T2	131.07 ± 1.66 b	116.10 ± 0.79 ab
		T3	119.49 ± 1.40 c	110.07 ± 0.89 d
		T4	151.19 ± 3.33 a	118.60 ± 0.80 a
F-test				
Cultivar (C)			***	***
Treatment (T)			***	***
Interaction (C X T)			***	***

Means of the same growing season designated with different letters indicate significant differences among treatments according to the Duncan's test ($P < 0.05$). Values are means ± standard deviation (SD) from 3 replicates. PGPR, plant growth-promoting rhizobacteria; PS, potassium silicate; DHA, dehydrogenase; T1, control; T2, PGPR; T3, PS; T4, PGPR+PS.

4. Discussion

Abiotic stresses, including salinity, have been widely reported to reduce wheat growth and productivity (Datir et al., 2020; Liu et al., 2020; Gui et al., 2020a & b; El-Ramady et al. 2021; Sheteiwy et al., 2021b). In our study, we adopted a sustainable approach to mitigate the negative effects of salinity and to enhance wheat growth and yield by coupling the application of PGPR and PS to alleviate salinity-induced inhibition in three wheat cultivars. Results revealed that the mixture application of PGPR and PS enhanced plant biomass, yield, essential elements uptake, physiological responses, antioxidant enzymes and soil enzyme activities compared to control treatment and those applied with either PGPR or PS (Tables 2 and 3).

The mixed application of PGPR and PS promoted the growth of root cells, which was reflected on the growth dynamics of the plants. This positive effect could be due to the role of PGPR, K and Si in cell transport systems that facilitate nutrients and water

mobility (Etesami and Adl, 2020). In addition, PGPR inoculation positively affected modifications in the growth parameters. This was evident in the phytohormones (auxins, cytokinins, and gibberellins) produced by PGPR to enhance root morphogenesis; thus, this can increase the length of lateral roots and density of root hairs (Seleiman et al. 2019; Hafez et al. 2020; Hafez et al. 2021). The detrimental effect of salt stress was attributed to the adverse effects on cell division and elongation, delayed cellular growth and reduced photosynthetic rate, which eventually reduced the productivity-related traits (Hafez et al. 2020). However, the application of PGPR and/or PS improved wheat yield and yield-related parameters under salinity stress (Table 3). Similar results have documented by Singh et al. (2017), who indicated that the PGPR strain, *Enterobacter cloacae* ZNP-3, can enhance stress tolerance of wheat plants via enhancing physiology, biochemical and growth dynamics of plant. Moreover, Karimi et al. (2018) have reported that wheat plants inoculated with *Azospirillum brasilense* and *Azospirillum zeae* have

recorded the highest values in total grain yield up to 18% compared with the non-inoculated plants. It has been concluded that PGPR combined with foliar

application of salicylic acid has led to improve the characteristics of the yield compared with control treatment (Hafez et al. 2019).

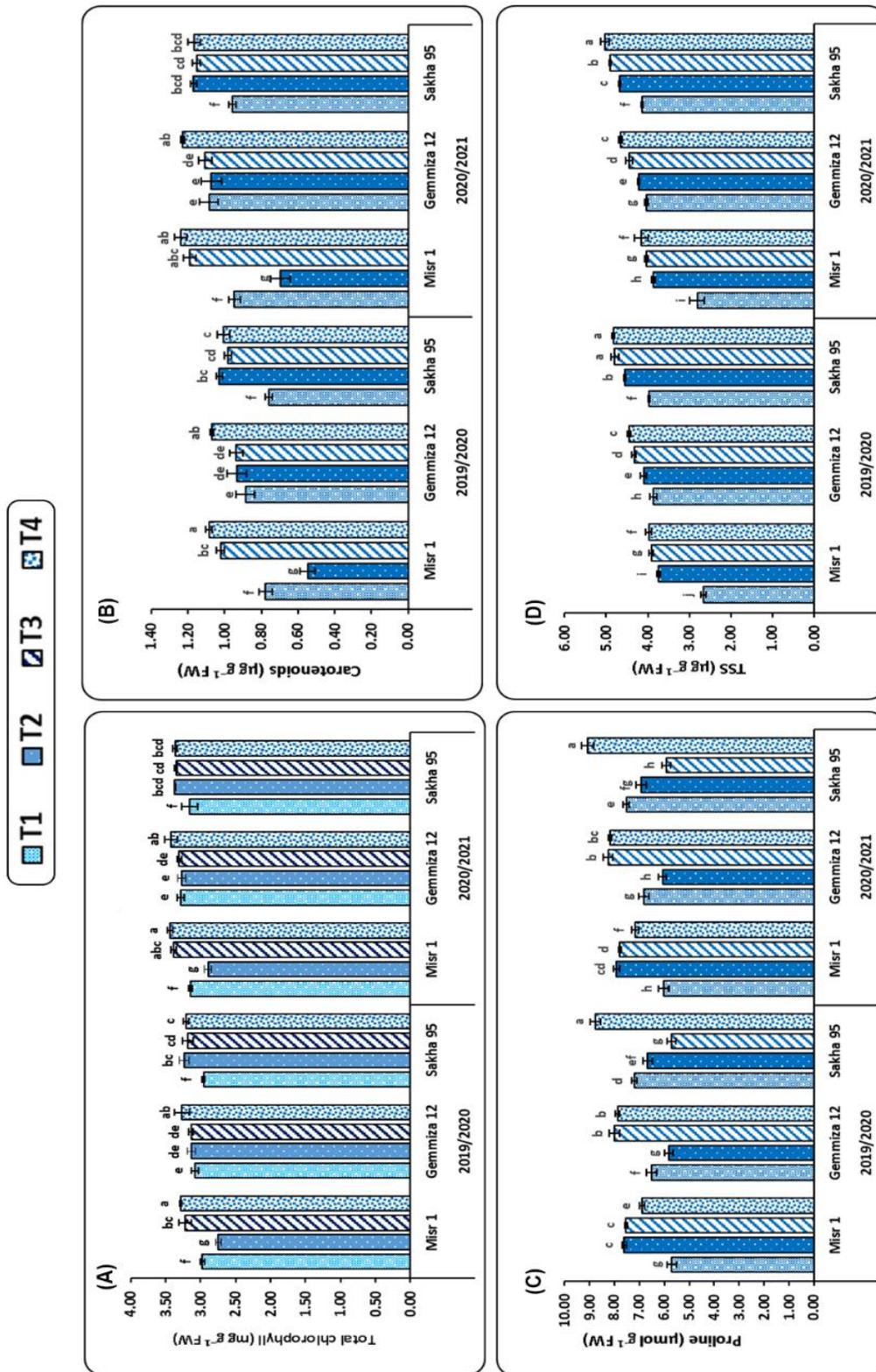


Fig. 2. Effect of PGPR soil application, PS foliar application, and their combination on total chlorophyll (A), carotenoids (B), proline (C), and total soluble sugars, TSS (D) in three wheat cultivars after 70 days from seed sowing during two growing seasons 2019/2020 and 2020/2021. Means of the same growing season with different letters indicate significant differences among treatments at $P < 0.05$. Values are means \pm standard deviation (SD) from 3 replicates. PGPR, plant growth-promoting rhizobacteria; PS, potassium silicate; T1: control (no PGPR or PS); T2, PGPR; T3, PS, T4, PGPR+PS. TSS, total soluble sugar.

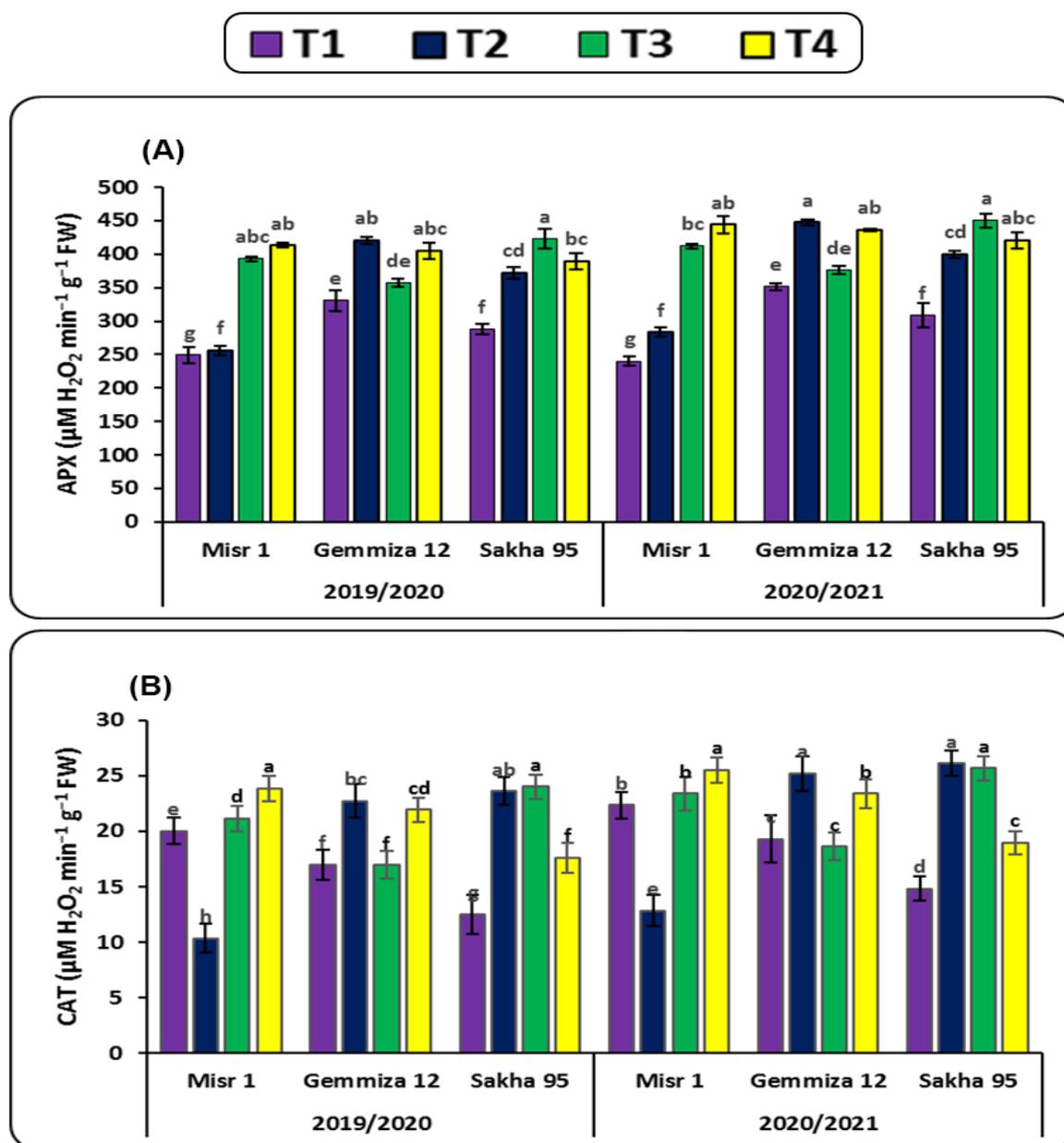


Fig. 3. Effect of PGPR soil application, PS foliar application, and their combination on APX (A) and CAT activity (B) in different cultivars of leaves wheat plants at 70 days from sowing during two growing seasons 2019/2020 and 2020/2021. Means of the same growing season designated with different letters indicate significant differences among treatments according to the Duncan's test ($P < 0.05$). Values are means \pm standard deviation (SD) from 3 replicates (Means \pm SD). PGPR, plant growth-promoting rhizobacteria; PS, potassium silicate; T1, control; T2, PGPR; T3, PS; T4, PGPR+PS; APX, ascorbate peroxidase; CAT, catalase.

Cultivation of wheat plants in salt-affected soil is associated with an increase in the salt concentration in the zone of the root, especially Na^+ , weakening the plant cells and growth. The high soil Na^+ and Cl^- interfere with K^+ and Ca^{2+} absorption, leading to nutritional disturbance (Manchanda and Garg 2008 and Nadeem *et al.* 2019). Our results attained that the combined treatment of PGPR+PS increased N, K^+ %, and K^+/Na^+ ratio while decreasing Na^+ % in the leaves of the three different tested cultivars of wheat (Table 4). In line with that, Khalifa *et al.* (2021) showed that soil inoculation with appropriate PGPR strains (*Azospirillum lipoferum*+*Bacillus circulance*) and phosphogypsum (9 ton ha^{-1}) increased the uptake of N in maize plants grown in saline soils. In addition, application of PGPR and PS enhanced ion balance in soil solution through the reduction of Na^+ in the root zone by the production of indole and exopolysaccharide, that can bind to Na^+ and mitigate its absorption and increased the absorption of K^+ in

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wheat plants (Ali et al. 2014; Shrivastava and Kumar, 2015). It therefore seems likely that the combined effect of PGPR and PS has a greater ability to maximize the absorption of K⁺ and reduce the content of Na⁺, compared to the individual applications of these treatments in salt-stressed wheat plants.

To further understand the inhibition caused by salinity on plant growth, photosynthetic pigments, proline and TSS were measured in wheat plants (Figure 2). Wheat plants are known to be sensitive to the negative effects of different stresses i.e. salinity, which led to a decrease in photosynthesis pigments and physiological characters (Singh et al., 2020; Datir et al., 2020; Sheteiwy et al., 2021b). It has been demonstrated that the synergistic effect of microbial inoculation and foliar application of PS improves water use efficiency, increases available soil water and reduces osmotic stress in salt-affected soils; thus, improving the photosynthetic system of the plant and increasing the leaf properties (Salem et al. 2017) and enhancing ATPase, DNA and RNA synthesis in wheat leaves (Ali et al. 2020). In

addition, co-application of PGPR+PS improved the photosynthetic pigments and the accumulated sugar content, which, in turn, improved the growth and yield of plant under saline stress compared to control plants. Proline accumulation also increased in the combined treatment (PGPR+PS) under saline stress compared to untreated plants (Figure 2). Several studies have reported high proline accumulation in response to various abiotic stresses (Alotaibi et al. 2021; Sheteiwy et al. 2021c). Ma et al. (2008) have reported that salinity stress induces *P5CR* gene, which is involved in proline metabolism, control proline levels and maintain lower levels of proline degradation under stress in wheat, soybean, *Arabidopsis* and pea plants. Under abiotic stress, the application of PGPR and/or mycorrhiza can also increase the transcript levels of *P5CS*, *P5CR*, *PDH* and *P5CDH* genes that are associated with proline metabolism (Sheteiwy et al. 2021c). Together, these results reveal that the combined treatment acquired wheat plants higher osmotic adjustment under saline stress to enhance salinity tolerance.

Table 5. Effect of soil application by PGPR, foliar application by PS and their combination on grain yield (ton ha⁻¹), straw yield (ton ha⁻¹), biological yield and harvest index (%) of three wheat cultivars during two growing seasons 2019/2020 and 2020/2021.

Year	cultivars (C)	Treatment (T)	Grain Yield (ton ha ⁻¹)	Straw Yield (ton ha ⁻¹)	Biological Yield	Harvest Index (%)
2019/2020	Misr 1	T1	5.10 ± 0.55 g	9.23 ± 0.15 i	14.33 ± 0.18 i	35.60 ± 0.17 e
		T2	6.67 ± 0.20 f	9.55 ± 0.95 g	16.22 ± 0.75 g	41.12 ± 0.62 b
		T3	7.11 ± 0.74 e	9.90 ± 0.31 e	17.02 ± 0.34 f	41.80 ± 0.18 a
		T4	7.75 ± 0.13 b	11.90 ± 0.46 b	19.65 ± 0.13 c	39.42 ± 0.19 d
	Gemmiza 12	T1	5.16 ± 0.61 g	9.42 ± 0.25 h	14.58 ± 0.24 h	35.40 ± 0.16 f
		T2	6.74 ± 0.83 f	9.68 ± 0.46 f	16.43 ± 0.89 g	41.05 ± 0.33 b
		T3	7.33 ± 0.56 d	10.18 ± 0.56 d	17.51 ± 0.25 e	41.85 ± 0.30 a
		T4	7.86 ± 0.67 b	12.14 ± 0.23 a	20.00 ± 0.99 b	39.31 ± 0.18 d
	Sakha 95	T1	5.14 ± 0.74 g	9.59 ± 0.44 fg	14.73 ± 0.55 h	34.92 ± 0.21 c
		T2	7.15 ± 0.94 e	9.85 ± 0.67 e	17.01 ± 0.69 f	42.06 ± 0.14 a
		T3	7.57 ± 0.33 c	10.47 ± 0.90 c	18.05 ± 0.43 d	41.97 ± 0.14 a
		T4	8.10 ± 0.77 a	12.17 ± 0.12 a	20.28 ± 0.29 a	39.96 ± 0.16 e
2020/2021	Misr 1	T1	5.21 ± 0.15 g	9.45 ± 0.61 i	14.66 ± 0.18j	35.55 ± 0.16 e
		T2	6.81 ± 0.19 f	9.73 ± 0.71 gh	16.55 ± 0.28 h	41.17 ± 0.50 b
		T3	7.26 ± 0.13 e	10.07 ± 0.34 e	17.33 ± 0.29 f	41.89 ± 0.22 a
		T4	7.88 ± 0.21 b	12.07 ± 0.38 b	19.95 ± 0.61 c	39.49 ± 0.17 cd
	Gemmiza 12	T1	5.27 ± 0.11 g	9.64 ± 0.55 h	14.91 ± 0.16 i	35.35 ± 0.16 e
		T2	6.90 ± 0.13 f	9.85 ± 0.62 f	16.76 ± 0.19 g	41.20 ± 0.32 b
		T3	7.47 ± 0.16 d	10.35 ± 0.29 d	17.82 ± 0.36 e	41.91 ± 0.29 a
		T4	7.98 ± 0.27 b	12.35 ± 0.31 a	20.33 ± 0.78 b	39.26 ± 0.18 d
	Sakha 95	T1	5.25 ± 0.14 g	9.81 ± 0.49 fg	15.06 ± 0.21 i	34.88 ± 0.21 f
		T2	7.31 ± 0.14 e	10.02 ± 0.70 e	17.34 ± 0.31 f	42.18 ± 0.14 a
		T3	7.71 ± 0.23 c	10.64 ± 0.41 c	18.36 ± 0.32 d	42.02 ± 0.15 a
		T4	8.22 ± 0.37 a	12.38 ± 0.51 a	20.61 ± 0.69 a	39.91 ± 0.19 c
F-test						
Cultivar (C)			***	***	***	***
Treatment (T)			***	***	***	***
Interaction (C X T)			***	***	***	***

Means of the same growing season designated with different letters indicate significant differences among treatments according to the Duncan's test (*P*<0.05). Values are means ± standard deviation (SD) from 3 replicates. PGPR, plant growth-promoting rhizobacteria; PS, potassium silicate; T1, control; T2, PGPR; T3, PS; T4, PGPR+PS.

Plants produce many antioxidant compounds to alleviate the damaging effects of ROS (Osman and

Salim 2016; Salim et al. 2019; Khalifa et al. 2021 and Hafez et al. 2021). Thus, improving the antioxidant in plants lead to increase tolerance to various stress factors. In addition, antioxidant enzymes decrease lipid peroxidation that improves cell membrane permeability; thus, mitigating the negative effect of salinity. Our results showed a significant ($P < 0.05$) increase in APX and CAT activities when PGPR and PS were combined compared to the individual applications or control treatment (Figure 3). The transcriptional activation of antioxidant activities could scavenge ROS in plant cells under the stress condition. In agreement with our results, Naveed et al. (2014) have reported that the application of *Burkholderia phytofirmans* PsJN on wheat plants subjected to biotic stress enhanced enzymatic antioxidant activities and increased growth. In addition, wheat plants inoculated with *A. lipoferum* can be protected from the negative effects of abiotic stresses by the changes in the antioxidant defense system (Agami et al. 2016).

Microbial activity plays a positive impact in the decomposition of organic matters in soil, by supporting the soil fertility through increasing the different levels and availability of nutrients and increasing soluble organic matter (Machulla et al. 2005; Nayak et al. 2011; Gao et al. 2020b; El-Sawah et al. 2021). The decomposed organic matter increases the soil water-holding capacity, improves soil chemistry and regulation of osmosis, and roots secretions that improve microbial respiration rate and normal microbial flora (Kaya et al. 2006; Gao et al. 2020b). In addition, soil key enzymes, such as dehydrogenase and urease, were significantly activated upon the application of both PGPR and PS together compared to the control (neither PGPR nor PS) treatment under salinity stress (Table 5). This could be attributed to the ability of PGPR to improve physicochemical properties particularly soil structure, and boost microbial activity in the rhizosphere (El-Sawah et al. 2021). These findings are in agreement with other researches. For instance, Nehela et al. (2021) showed that sodic-saline soils amended with PGPR and biochar improved microbial activity and soil enzyme activities (dehydrogenase and urease) compared to non-treated control. Soil application of PGPR and foliar spraying of silica nanoparticles improved soil enzymatic (e.g., dehydrogenase and alkaline phosphatase) and physicochemical characteristics of soil; thus, this enhanced growth of plant (maize) under different treatments of irrigation water (Hafez et al. 2021).

5. Conclusion

Our study used a practically applicable strategy that included soil application of PGPR, and foliar

spraying of potassium silicate was used to reduce the negative effects of salinity stress on three wheat cultivars (*Triticum aestivum* L.). The used applications benefited wheat plants' growth, yield, and nutrient uptake. Besides, enhancements in photosynthetic pigments, proline content, total soluble sugars content, antioxidant enzymes, and soil key enzyme activities (dehydrogenase and urease) were observed in the combined application. Therefore, delivering PGPR along with foliar spraying with K_2SiO_3 , could be used as a simple, cost-effective, and efficient method for enhancing wheat plant growth and productivity in salt-affected soil. However, more research is required to optimize beneficial plant-microbe relationships to increase crop productivity under salinity stress.

6. Conflicts of interest

The authors declare that they have no competing interests.

7. Formatting of funding sources

No funding

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