International Journal of Horticultural Science and Technology
Journal homepage: https://ijhst.ut.ac.ir

Enhanced Cold Tolerance in Tomato Seedlings: Exploring the Synergy of *Streptomyces* Bacteria and Ghatti Gum

Iraj Khazaei[1], Naser Alemzadeh Ansari[1]*, Rouhollah Karimi[2]*

1 Department of Horticultural Sciences, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran
2 Department of Horticulture and Landscape Engineering, Faculty of Agriculture, Malayer University, Malayer, Iran

*Corresponding author's email: ansari_n@scu.ac.ir, r.karimi@malayeru.ac.ir

ARTICLE INFO

Article history:

Received: 9 November 2024,
Received in revised form: 28 December 2024,
Accepted: 21 April 2025,

Article type:

Research paper

Keywords:

Antioxidant enzymes,
Biofertilizer,
Cold stresssugars,
Nutrition

ABSTRACT

This study aimed to investigate the effects of *Streptomyces rimosus* (*Sr*) bacteria and ghatti gum (Gg) on the cold tolerance of tomato seedlings (cv. Izmir). The experiment was conducted as a split-plot design in a randomized complete block design with three replications. The main plot consisted of two temperature levels (24 and 4 °C), and the sub-plot included four fertilizer treatments (control, *Sr*, Gg, and *Sr* + Gg combination). The results showed that most traits were significantly influenced by temperature, treatment, and their interaction. At 4 °C, the highest levels of catalase, ascorbate peroxidase, glutathione peroxidase, malondialdehyde, hydrogen peroxide, phenol, flavonoids, and proline were observed. The combined treatment of *Sr* + Gg increased the activity of antioxidant enzymes and reduced free radicals. Moreover, the highest amounts of iron, phosphorus, and potassium were observed in the *Sr* + Gg treatment at greenhouse temperature. In conclusion, the combined application of *Sr* + Gg under cold stress conditions improved the cold tolerance of tomato seedlings by increasing the activity of antioxidant enzymes, reducing electrolyte leakage, and increasing protein and proline content. Therefore, the use of *Sr* + Gg combination is recommended in areas prone to cold damage.

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most popular and widely consumed vegetable fruit around the world (Sadashiva et al., 2013). Farm tomato products are widely used for fresh and industrial consumption worldwide, with greenhouse types being particularly popular for fresh consumption. Tomatoes are cultivated globally due to their adaptability and local demand. Iran ranks seventh among the world's leading tomato producers, producing nearly 6.4 million t of tomatoes annually between 2015 and 2020 (FAO, 2021). Low temperatures, starting below 15 ºC, are an abiotic stress that negatively impacts tomato growth and yield by reducing photosynthesis, respiration, cell membrane function (Gerszberg et al., 2017; Shomali et al., 2022), and increasing reactive oxygen species (ROS) and protein degradation (Gerszberg et al., 2017; Fayezizadeh et al., 2023). Cold stress leads to oxidative stress, causing electrolyte leakage (EL) and the formation of ROS (Karimi et al., 2016). These ROS damage cell membranes, chloroplasts, proteins, and nucleic acids, disrupting normal cell metabolism and causing damage to cell membranes, chloroplasts, and proteins (Shomali et al., 2022; Ihtisham et al., 2023). During exposing to low temperature, plants undergo a series of physiological and biochemical changes, which lead to an increase in plants' resilience in stressful conditions. For example, changes in the composition and arrangement of membrane lipids, the accumulation of sucrose and other simple sugars, proline and glycine betaine, specific proteins and the activation of enzymatic and non-enzymatic antioxidant systems (Aslamarz et al., 2009, 2010; Karimi and Ershadi,

2015; Karimi et al., 2016; Karimi, 2017). Tomato growth and yield are limited by environmental stress factors (Gerszberg et al., 2017). Environmental conditions can cause abiotic stress, negatively impacting the growth, production, yield, and quality of products (Aslamarz et al., 2010). In Izmir tomatoes, abiotic stresses can lead to a 70% reduction in yield, depending on the plant's growth period and stress duration (Krishna et al., 2019).

Recently, the use of microorganisms as biofertilizers has been considered due to their high efficiency and compatibility with plants (Kaboosi et al., 2016; Mahanty et al., 2016; Bhardwaj et al., 2018). Among biofertilizers, some species of plant growth promoting rhizobacteria (PGPR) have been used to increase salinity tolerance (Karimi et al., 2022a,b; Sarlak et al., 2024). *Streptomyces* (*Sr*) species, gram-positive bacteria known for producing bioactive compounds like antibiotics and immune compounds, have been studied for their potential to improve plant growth and protection. Some species are symbiotic with plants, enhancing growth and protein content. Indole acetic acid (IAA) production by Streptomyces species has been reported to increase growth, protein content, antioxidant enzymes activity, and play a role in stress reduction tolerance (Manullang et al., 2020). According to studies, the application of *Streptomyces* in the root zone of some plants such as beans (Nassar et al., 2003), tomatoes (El-Tarabily, 2008), wheat (Sadeghi et al., 2012) and grapes (Karimi et al., 2022a) increased tolerance to environmental stresses through the production of polyamines, the accumulation of compatible solutes, the increase in phosphate solubility, the production of IAA in the root and the production of siderophores in the rhizosphere.

Natural molecules like ghatti gum (Gg) and chitosan are being used to enhance stress tolerance in plant products by stimulating defense system responses, neutralizing biotic and abiotic stresses, and improving product yield and quality (Quitadamo et al., 2021). Gg, which is generally known as Indian gum, is prepared from the *Anogeissus latifolia* tree belonging to the *Combretaceae* family (Singh et al., 2024). Gg is a non-starch polysaccharide with high molecular weight, water solubility, and excellent emulsification, stabilization, concentration, heat tolerance, pH stability, carrier, and biodegradable properties. It is widely used in food, pharmaceutical, wastewater treatment, and hydrogel formation (Singh et al., 2024). Previous studies have shown that Gg coating can reduce frost in horticultural products like grapes during cold storage (Eshghi et al., 2021). The use of Gg and chitosan in cold storage of Rishbaba grapes reduces EL percentage, malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) content, indicating their role in enhancing low temperature tolerance in horticultural products (Eshghi et al., 2021). Research on the impact of Gg on plant cold tolerance has not yet been conducted. However, studies have shown that chitosan, a natural polysaccharide, can enhance plant weight, nutrient content, total phenolic compounds, soluble carbohydrates, proline and amino acids, photosynthetic pigments, and antioxidant enzyme activity in banana plants under cold stress (Wang et al., 2021). To our knowledge, the combination effect of Gg polysaccharide and *Sr* PGPR on plants' ability to withstand cold has not been the subject of any research. Our hypothesis is that the combination of these two materials can improve cold tolerance in tomato plants by affecting the absorption of nutrients and some physiological and biochemical changes. In order to ascertain whether Sr PGPR and Gg have any mutual effects on the membrane stability indices, compatibility osmolytes, antioxidant enzyme activity, and nutritional content of Izmir tomato leaves under low temperature stress, the current study was carried out.

Materials and Methods

Plant materials and experimental treatments

In order to investigate the effect of Sr PGPR and Gg on the cold tolerance of tomato seedlings (*Solanum lycopersicum* L. CV. Izmir) in the greenhouse, an experiment was carried out in the form of split plots based on a randomized complete block design with three replicates. The main plot included two temperature levels of 24 °C (greenhouse conditions) and 4 °C (normal chilling temperature). The sub-plot includes four fertilizer treatments (chemical and biological) called controls (without *Sr* inoculation and Gg treatment, soaked with distilled water), *Sr* PGPR (colony concentration, CFU Unit) 10^6), Gg (2%) and the combination of Sr PGPR + Gg. Streptomyces strain C-2012 inoculum (*S. rimosus* (JX839830)) was purchased from the Water and Soil Research Institute of the Ministry of Jihad Agriculture.

Growth condition and cold treatments

Tomato seeds were placed in one litter of inoculum for 30 min to inoculate with *Sr* PGPR or Gg. The culture medium was sterilized in an autoclave at 121 °C for 30 min before starting the experiment. After germination, the seedlings were regularly watered three times a week using Hoagland's solution until the emergence of 5 true leaves. After this stage, the plants were divided into two groups, half of the plants treated with *Sr* PGPR, Gg and the combination of these two treatments (*Sr* PGPR + Gg). In each treatment, half of seedlings were placed in the greenhouse (first group; 24 ºC) and the other half of the plants were exposed to 4 ºC (second group; 4ºC) in a cooling chamber (Phytotron, Rad Electronics, Tehran) for two consecutive days with 6 h chilling in each day. After applying the cold treatment, the

cooling chamber temperature was gradually increased (2 ºC h^{-1}) to the room temperature and the seedlings were transferred to the greenhouse for 2 d and then mature leaves were sampled from the middle parts of the plant canopy to measure the following indices.

Photosynthetic pigments
The concentration of chlorophylls (Chl) *a*, *b* and total Chl and carotenoids were measured spectrophotometrically and expressed in terms of mg g^{-1} of fresh weight (FW) (Lichtenthaler et al., 1987).

EL assay
In order to measure EL, leaf samples were taken from each group of plants and immersed separately in 70 mL test tubes containing 40 mL of distilled water. The tubes were placed on a shaker at a speed of 120 rpm for 20 h. Then their electrical conductivity (EC_1) was measured with a conductivity meter (Atago, Japan). Then the tubes were autoclaved for 20 min (121 ºC) and the electrical conductivity (EC_2) were re-measured and EL calculated from the following relationship ($EL = (\frac{EC_1}{EC_2}) \times 100$) (Campos et al., 2003).

MDA and H_2O_2 assay
Membrane lipid peroxidation was measured based on the concentration of MDA produced as a result of damage to the membrane and its reaction with thiobarbituric acid. After extraction with trichloroacetic acid, the absorbance of the samples was read at two wavelengths of 600 and 532 nm and expressed on the basis of µmol g^{-1} FW (Health et al., 1968). H_2O_2 content was measured according the method of Loreto and Velikova (2001). The H_2O_2 concentration of the samples was calculated by comparing their absorbance at 390 nm wavelength and its standard curve in the range from 100 to 1000 µmol mL^{-1} and expressed as µmol g^{-1} FW.

Soluble protein assay
Soluble protein concentration was measured with Brilliant Blue (G-250) color reagent at a wavelength of 595 nm (Bradford, 1976). To measure proline, extraction was done with the help of sulfosalicylic acid and measurement was done with the presence of acetic acid and ninhydrin reagent. The absorbance of the samples was measured at a wavelength of 518 nm and the proline concentration of the leaf was determined in µmol g^{-1} FW (Bates et al., 1973).

Antioxidant enzymes activity assay
To measure the activity of antioxidant enzymes, fresh leaf tissue samples (100 mg) were pulverized in liquid nitrogen and kept at -80 ºC until further use. The activities of catalase (CAT; Bergmeyer, 1970), guaiacol-peroxidase (GPX; Herzog and Fahimi, 1973) and ascorbate peroxidase (APX; Nakano and Asada, 1981) enzymes were measured at wavelengths of 290, 240 and 465 nm, respectively.

Total phenol and flavonoid assay
Extraction and measurement of total phenol was done by Folin-Ciocalteu reagent method and the samples was read at a wavelength of 765 nm and expressed based on mg of gallic acid g^{-1} FW (Velioglu et al., 1998).
Total flavonoid was measured using aluminum chloride (10%) and potassium acetate (1M) according to Chang et al. (2002) method and absorption of the samples was read at 415 nm wavelength by a spectrophotometer. For each extracts, the amount of total flavonoids was calculated as mg of quercetin g^{-1} FW.

Total soluble sugar and proline assay
Total soluble sugar was measured by colorimetric method with the help of anthrone in the presence of sulfuric acid and reading at 625 nm wavelength (Irigoyen et al., 1992). The concentration of soluble sugar was determined based on the glucose standard curve and expressed as mg g^{-1} FW.
To measure proline, extraction was done with the help of sulfosalicylic acid and measurement was done in the presence of acetic acid and ninhydrin reagent. The absorbance of the samples was measured at a wavelength of 518 nm and the proline concentration of the proline was determined in terms of µmol g^{-1} FW.

Leaf nutrients analysis
Potassium (K) was measured using a flame photometer (G 405, Germany), iron (Fe) and zinc (Zn) by an atomic absorption device (model AANALYST70, USA) and leaf phosphorous (P) by a spectrophotometer at a wavelength of 470 nm according to the method of Karla (1998).

Data analysis
Statistical analysis of data was done using SAS 9.4 statistical software. The comparison of means was done using Duncan's multiple range test at the 5% probability level.

Results
Photosynthetic pigments
The simple effect of temperature and treatment on the content of leaf Chl *a* was significant at the 1% probability level, but the interaction effect of temperature × treatment on the content of these photosynthetic pigments was not significant (Table 1). As temperature decreased from 24 °C to 4 °C, the content of leaf Chl *a* decreased in all plants (Fig. 1). The content of leaf Chl *b* of tomato plants was affected by the simple effects of temperature and

treatments, and their interaction on the content of this photosynthetic pigment was not significant (Table 1). Low temperature stress (4 ºC) led to a decrease in leaf Chl *b* content compared to plants located at 24 ºC (Fig. 2).

Table 1. Analysis of Variance (ANOVA) results of the effect of temperature and different treatments on the content of photosynthetic pigments in tomato plant leaves.

S. O V.	DF	Chlorophyll *a*	Chlorophyll *b*	Total Chlorophyll	Carotenoids
Block	2	0.007	0.002	0.01	0.00
Temperature	1	0.26**	0.18**	0.88**	0.68**
First error	2	0.001	0.001	0.001	0.004
Treatments	3	0.01**	0.01**	0.19**	0.007**
Tem.× Treat.	3	0.004^{ns}	0.004^{ns}	0.012*	0.009**
Error	12	0.002	0.0017	0.003	0.0012
C.V.		4.44	7.46	3.16	3.37

^{ns}, * and ** respectively, no significant difference and significant difference at the probability level of 0.05 and 0.01.

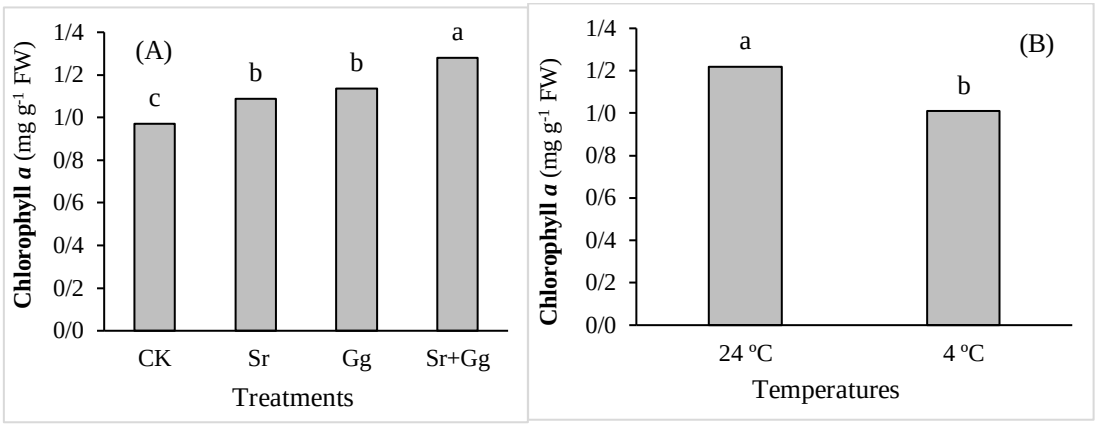

Fig. 1. (**A**) The simple effect of different treatments of *Streptomyces* rhizobacteria and ghatti gum; (**B**) and the simple effect of temperature on the content of chlorophyll *a* in tomato plant leaves. CK; control, Sr; *Streptomyces* rhizobacteria, Gg; ghatti gum, Sr + Gg; combination of *Streptomyces rhizobacteria* and ghatti gum. The means with same Duncan's multiple range test letters in each column are not statistically significant ($P \leq 0.01$).

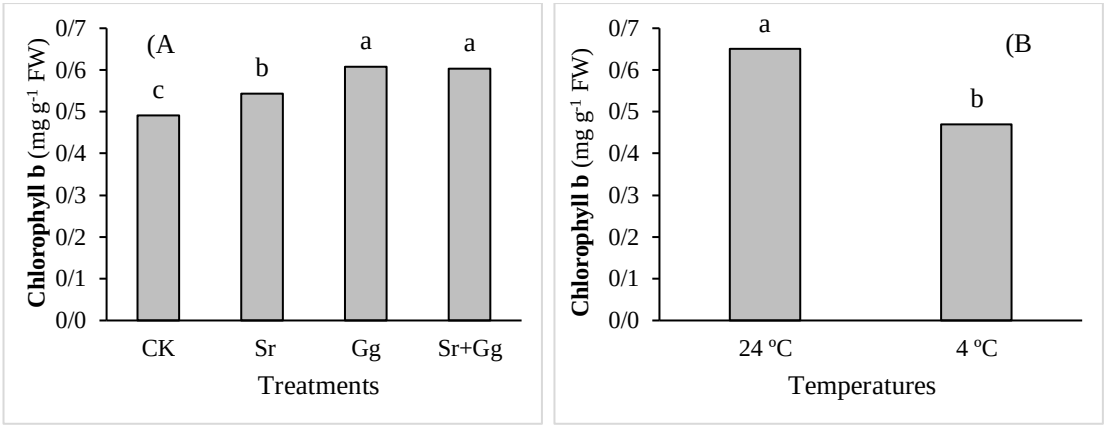

Fig. 2. (**A**) The simple effect of different treatments of *Streptomyces* rhizobacteria and ghatti gum; (**B**) and the simple effect of temperature on the chlorophyll content of tomato plant leaves. CK; control, Sr; *Streptomyces* rhizobacteria, Gg; ghatti gum, Sr + Gg; combination of *Streptomyces rhizobacteria* and ghatti gum. The means with same Duncan's multiple range test letters in each column are not statistically significant ($P \leq 0.01$).

The simple effect of temperature and treatments ($P \leq 0.01$) and their interaction ($P \leq 0.05$) on the leaf total Chl content of tomato plants were significant (Table 1). Transferring the plants to low temperature (4 ºC)

led to a decrease in leaf total Chl content in all tomato seedlings. Interestingly, the plants treated with the combination of *Sr* + Gg had higher total Chl content under low temperature stress compared to control plants. Of course, in terms of the total Chl content, no significant difference was observed with plants treated with Gg alone (Fig. 3).

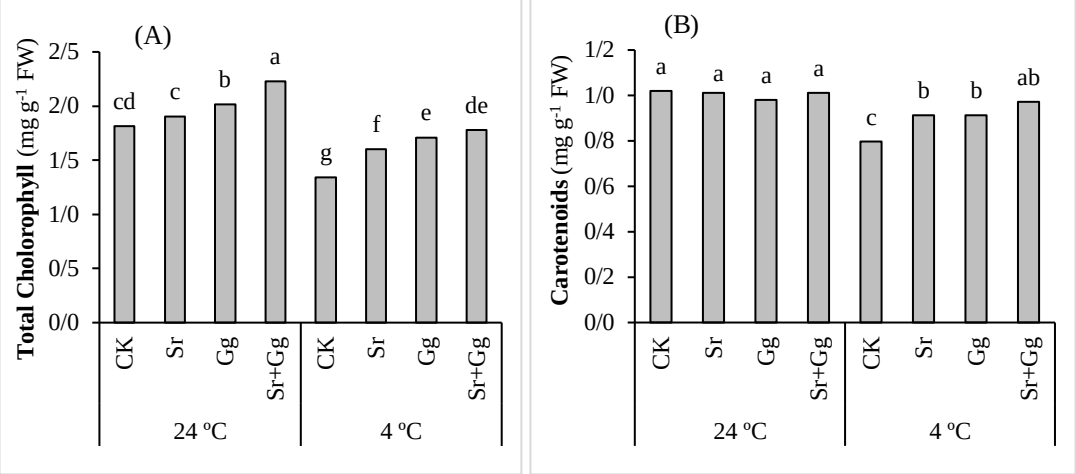

Fig. 3. (**A**) Interaction of temperature (24 and 4 °C) and different treatments of *Streptomyces* rhizobacteria and ghatti gum on total chlorophyll; (**B**) and carotenoid content of tomato plant leaves. CK; control, Sr; *Streptomyces* rhizobacteria, Gg; ghatti gum, Sr + Gg; combination of *Streptomyces rhizobacteria* and ghatti gum. The means with same Duncan's multiple range test letters in each column are not statistically significant ($P \leq 0.01$).

The effect of temperature, treatment and their interaction on leaf carotenoid content was significant at 1% probability level (Table 1). Under cold stress (4 ºC), the leaf carotenoid content decreased compared to plants under greenhouse temperature conditions (24 ºC). Under the temperature of 4 ºC, a significant difference was observed between the carotenoid content of plants treated with *Sr* + Gg and the control plants, but no statistically significant difference was observed between the carotenoid content of plants treated with Gg, *Sr* and their combination (Fig. 3B).

Membrane stability indices (EL, MDA and H_2O_2)

The effect of temperature, treatment and their interaction on the amount of leaf EL was significant at the 1% probability level (Table 2). Low temperature stress (4 ºC) led to an increase in the leaf EL compared to plants located at a temperature of 24 ºC (Fig. 4).

The effect of temperature, treatment and their interaction on leaf H_2O_2 content was significant at 1% probability level (Table 2). At the temperature of 24 °C, no significant difference was observed between the leaves H_2O_2 content measured in all plants (Fig. 5).

Table 2. Analysis of Variance (ANOVA) results of the effect of temperature and different treatments on the stability indices of the leaf membrane of tomato seedlings.

S. O V.	DF	EL	MDA	H_2O_2
Block	2	0.54	0.002	0.08
Temperature	1	8756.58**	18.89**	22.62**
First error	2	0.98	0.97	0.14
Treatments	3	211.62**	8.35**	1.56**
Tem.× Treat.	3	163.78**	2.63**	0.43**
Error	12	0.67	0.23	0.06
C.V.		2.63	15.98	13.87

^{ns}, * and ** respectively, no significant difference and significant difference at the probability level of 0.05 and 0.01.

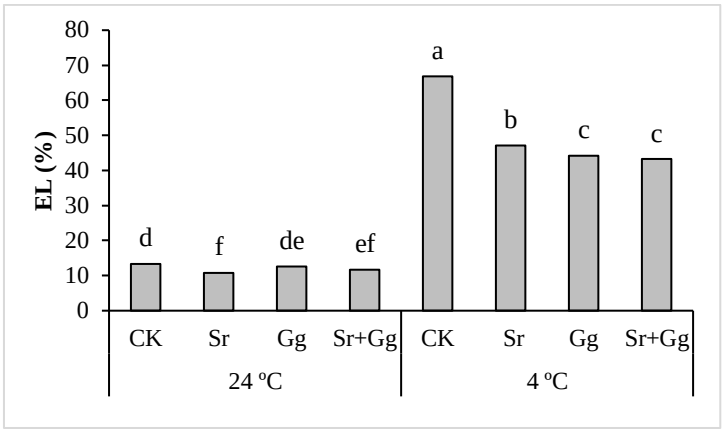

Fig. 4. The interaction of temperature (24 and 4 ºC) and different treatments of *Streptomyces* rhizobacteria and ghatti gum on the electrolyte leakage (EL) of from tomato plant leaves. CK; control, Sr; *Streptomyces* rhizobacteria, Gg; ghatti gum, Sr + Gg; combination of *Streptomyces rhizobacteria* and ghatti gum. The means with same Duncan's multiple range test letters in each column are not statistically significant ($P \leq 0.01$).

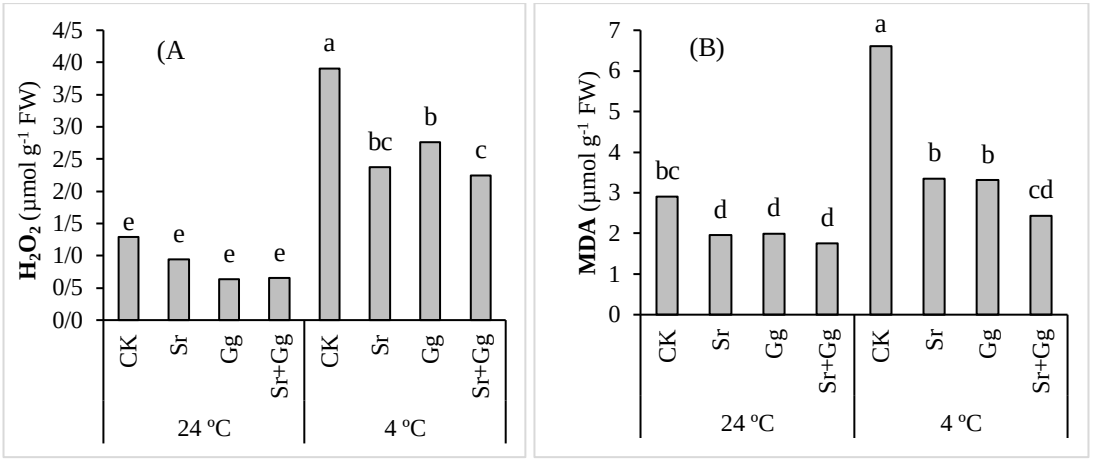

Fig. 5. (**A**) The interaction of temperature (24 and 4°C) and different treatments of *Streptomyces* rhizobacteria and ghatti gum on the content of hydrogen peroxide (H_2O_2); (**B**) and malondialdehyde (MDA) of tomato leaves. CK; control, Sr; *Streptomyces* rhizobacteria, Gg; ghatti gum, Sr + Gg; combination of *Streptomyces rhizobacteria* and ghatti gum. The means with same Duncan's multiple range test letters in each column are not statistically significant ($P \leq 0.01$).

The content of MDA in the leaves of tomato plants was significantly (0.01) affected by temperature, treatment and their interaction. In plants under 24 °C, there was no significant difference in terms of MDA content between plants treated with *Sr* and Gg, but there was a significant difference between these treatments and the control plants in terms of MDA content (Fig. 5).

Soluble protein

The leaf soluble protein content of tomato seedlings was affected by the simple effects of temperature and treatment, but the interaction effect of temperature × treatment was not significant (Table 3). Low temperature stress (4 °C) led to an increase in leaf soluble protein content compared to plants grown at 24 °C (Fig. 6).

Table 3. Analysis of Variance (ANOVA) results of the effect of temperature and different treatments on the activity of antioxidant enzymes and soluble protein content of leaves of tomato seeds.

S. O V.	DF	Protein	CAT	GPX	APX
Block	2	0.04	2.35	6.53	0.23
Temperature	1	23.39**	277.12**	124.8**	431.06**
First error	2	0.06	2.98	1.25	0.08
Treatments	3	4.81**	5.91**	9.21	19.66**
Tem.× Treat.	3	0.13^{ns}	7.13**	3.68 ^{ns}	8.14**
Error	12	0.10	0.34	2.13	0.36
C.V.		8.40	9.66	4.96	8.67

^{ns}, * and ** respectively, no significant difference and significant difference at the probability level of 0.05 and 0.01.

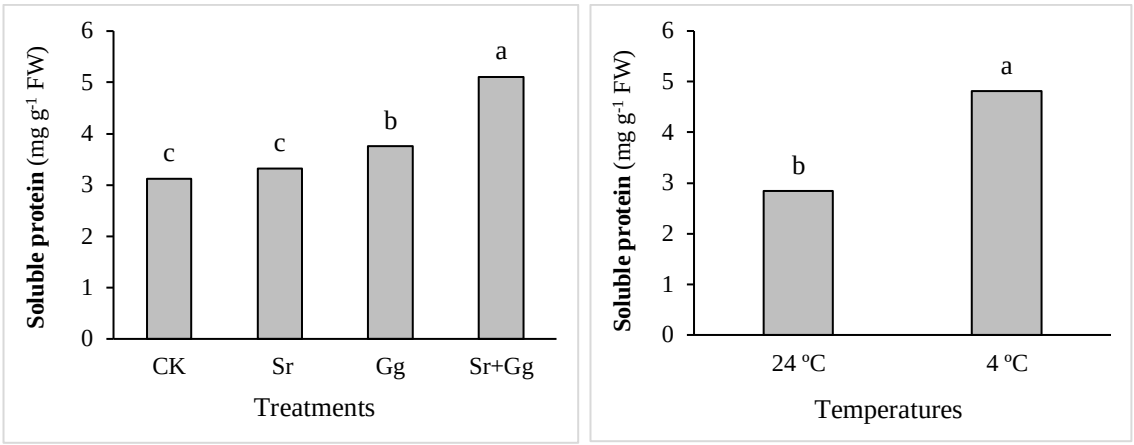

Fig. 6. (**A**) The simple effect of different treatments of *Streptomyces* rhizobacteria and ghatti gum; (**B**) and the simple effect of temperature on the soluble protein content of tomato leaves. CK; control, Sr; *Streptomyces* rhizobacteria, Gg; ghatti gum, Sr + Gg; combination of *Streptomyces rhizobacteria* and ghatti gum. The means with same Duncan's multiple range test letters in each column are not statistically significant ($P \leq 0.01$).

Antioxidant enzymes

The activity of CAT enzyme in the leaves of tomato seedlings was significantly ($P \leq 0.01$) affected by temperature, treatment and the interaction (Table 3). In the plants grown at 24 °C, no significant difference was observed between the plants treated with *Sr* and Gg in terms of leaf CAT activity (Fig. 7).

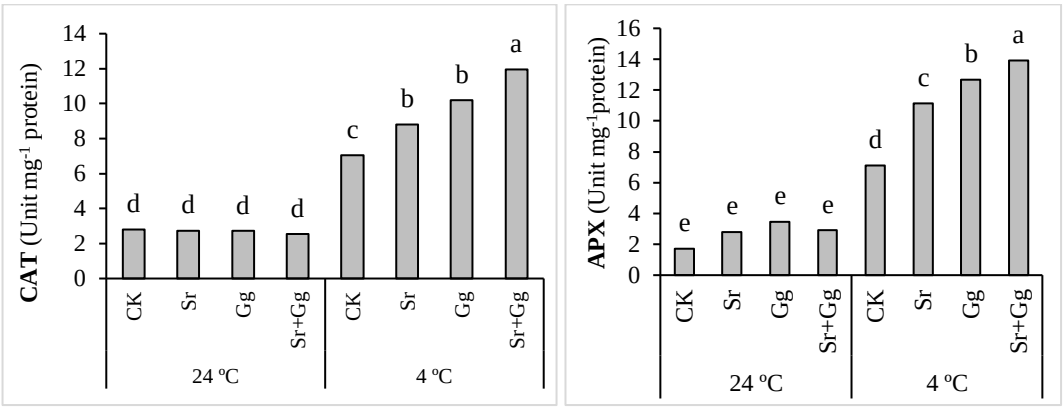

Fig. 7. (**A**) The interaction of temperature (24 and 4ºC) and different treatments of *Streptomyces* rhizobacteria and ghatti gum on the activity of catalase enzyme (CAT); (**B**) and ascorbate peroxidase (APX) of tomato plant leaves. CK; control, Sr; *Streptomyces* rhizobacteria, Gg; ghatti gum, Sr + Gg; combination of *Streptomyces rhizobacteria* and ghatti gum. The means with same Duncan's multiple range test letters in each column are not statistically significant ($P \leq 0.01$).

The effect of temperature, treatment and their interaction on APX activity of tomato plant was significant at the 1% probability level (Table 3). In the plants remained at 24 ºC, there was no significant difference in the activity of this enzyme between the plants treated with Sr, Gg or their combination (Fig. 7). The leaves of tomato seedlings was significantly ($P \leq 0.01$) affected by the simple effects of temperature and treatment, but the interaction effect of temperature × treatment was not significant (Table 3).

Cold stress (4 ºC) led to an increase in the activity of GPX enzyme compared to plants located at a temperature of 24 ºC (Fig. 8).

Total phenol

The simple effect of temperature and treatments at the level of 1% and their interaction at the level of 5% were significant on the leaf total phenolic content of tomato seedlings (Table 4). At 24 ºC, a significant difference was observed between the leaf total phenol content measured in all plants. In this temperature treatment, the application of Gg alone and in combination with *Sr* led to a significant increase in the phenol content compared to the control (Fig. 9).

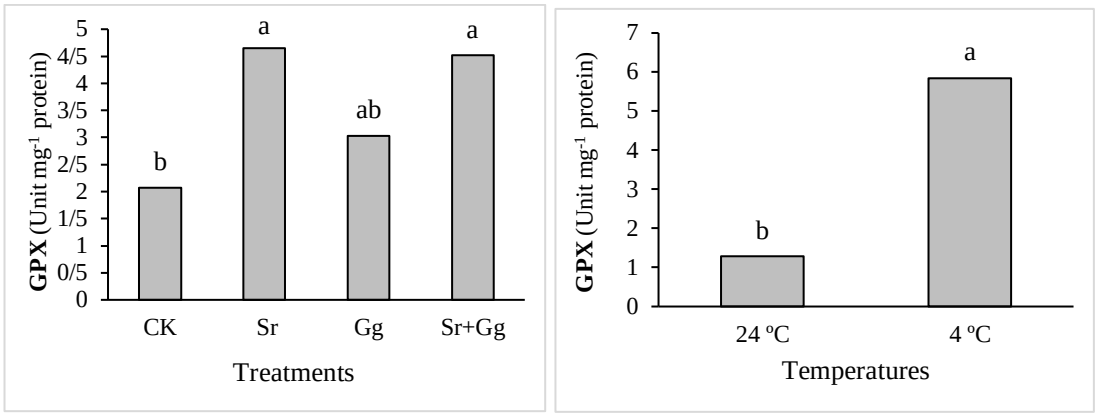

Fig. 8. (**A**) The simple effect of different treatments of *Streptomyces* rhizobacteria and ghatti gum; (**B**) and the simple effect of temperature on the guaiacol peroxidase of tomato plant leaves. CK; control, Sr; *Streptomyces* rhizobacteria, Gg; ghatti gum, Sr + Gg; combination of *Streptomyces rhizobacteria* and ghatti gum. The means with same Duncan's multiple range test letters in each column are not statistically significant ($P \leq 0.01$).

Table 4. Analysis of Variance (ANOVA) results of the effect of temperature and different treatments on the content of some biochemical indicators of tomato plant leaves.

S. O V.	DF	Phenol	Flavonoids	Sugar	Proline
Block	2	0.177	0.30	1.93	0.18
Temperature	1	6.62**	6.10**	361.46**	37.29**
First error	2	0.05	0.04	1.59	0.62
Treatments	3	5.72**	3.11**	37.52**	4.84**
Tem.× Treat.	3	0.82*	0.33**	2.87^{ns}	1.77*
Error	12	0.22	0.02	1.28	0.52
C.V.		11.33	8.01	7.49	26.81

^{ns}, * and ** respectively, no significant difference and significant difference at the probability level of 0.05 and 0.01.

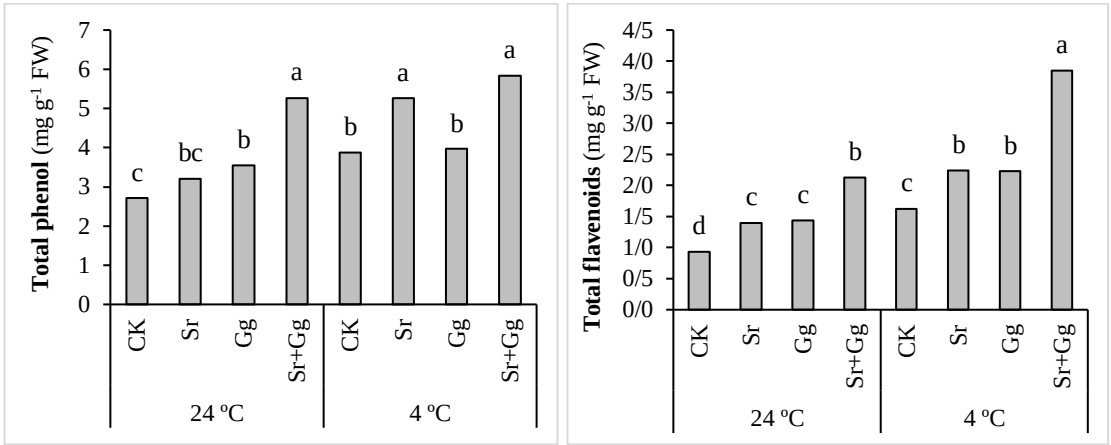

Fig. 9. (**A**) The interaction of temperature (24 and 4 °C) and different treatments of *Streptomyces* rhizobacteria and ghatti gum on the content of total phenol; (**B**) and total flavonoid of tomato plant leaves. CK; control, Sr; *Streptomyces* rhizobacteria, Gg; ghatti gum, Sr + Gg; combination of *Streptomyces rhizobacteria* and ghatti gum. The means with same Duncan's multiple range test letters in each column are not statistically significant ($P \leq 0.01$).

Total flavonoids

The effect of temperature, treatment and their interaction on leaf flavonoid content was significant at 1% probability level (Table 4). Low temperature stress (4 ºC) led to an increase in the flavonoid content of leaves compared to plants located at 24 ºC (Fig. 4). Among both groups of plants (greenhouse temperature and cold stress), the highest flavonoid content was related to those plant treated with the combination of *Sr* and Gg (Fig. 9).

Total soluble sugar

Total soluble sugar content of tomato seedlings was significantly (0.01) affected by the simple effects of

temperature and treatment, but the interaction effect of temperature × treatment was not significant (Table 4). Low temperature stress (4 ºC) led to an increase in the total leaf soluble sugar content compared to plants located at 24 ºC (Fig. 10).

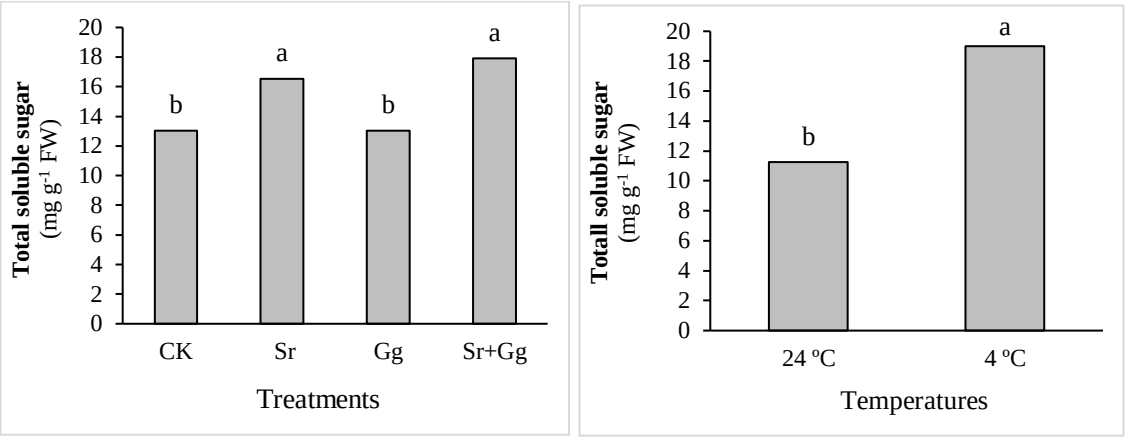

Fig. 10. (**A**) The simple effect of different treatments of *Streptomyces* rhizobacteria and ghatti gum; (**B**) and the simple effect of temperature on the soluble sugar content of tomato plants leaves. CK; control, Sr; *Streptomyces* rhizobacteria, Gg; ghatti gum, Sr + Gg; combination of *Streptomyces rhizobacteria* and ghatti gum. The means with same Duncan's multiple range test letters in each column are not statistically significant ($P \leq 0.01$).

Proline

The simple effect of temperature and treatments was significant at the 1% probability level and their interaction at the 5% probability level on the leaf proline content of tomato plants (Table 4). At the temperature of 24°C, no significant difference was observed between the measured leaf proline content in all plants (Fig. 11).

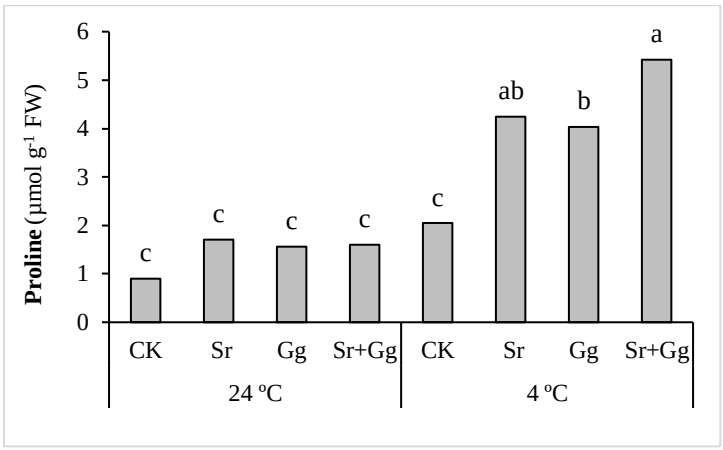

Fig. 11. The interaction of temperature (24 and 4 ºC) and different treatments of *Streptomyces* rhizobacteria and ghatti gum on the proline content of tomato plant leaves. CK; control, Sr; *Streptomyces* rhizobacteria, Gg; ghatti gum, Sr + Gg; combination of *Streptomyces rhizobacteria* and ghatti gum. The means with same Duncan's multiple range test letters in each column are not statistically significant ($P \leq 0.01$).

Leaf nutrients

The effect of temperature, treatment and their interaction on leaf K content was significant at 1% probability level (Table 5). Among the studied treatments, the highest leaf K content was related to the plants treated with the combination of *Sr* and Gg under 24 ºC condition (Fig. 12).

The simple effect of temperature and treatments at the level of 1% and their interaction at the level of 5% on the P content of the leaves of tomato seedlings were significant (Table 5). Among the studied treatments, the highest P content at 24 ºC was observed in the treatment of *Sr* + Gg, which did not significantly differed from the treatment of *Sr* alone (Fig. 12).

The effect of temperature, treatment and their interaction on leaf Fe content was significant at 1% probability level (Table 5). Among the studied treatments, the highest leaf Fe content was related to the plants treated with the combination of *Sr* and Gg under the 24 °C (Fig. 13).

Table 5. Analysis of Variance (ANOVA) results of the effect of temperature and different treatments on the content of some nutrients in the leaves of tomato plants.

S. O V.	DF	K	P	Fe	Zn
Block	2	0.00	0.00	2.74	2.76
Temperature	1	0.153**	0.001*	929.76**	1.15^{ns}
First error	2	0.005	0.001	23.78	1.82
Treatments	3	0.78**	0.006**	486.15**	543.73**
Tem.× Treat.	3	0.083**	0.001*	66.67**	14.45^{ns}
Error	12	0.011	0.0003	6.94	9.29
C.V.		6.29	7.35	2.12	3.79

^{ns}, * and ** respectively, no significant difference and significant difference at the probability level of 0.05 and 0.01.

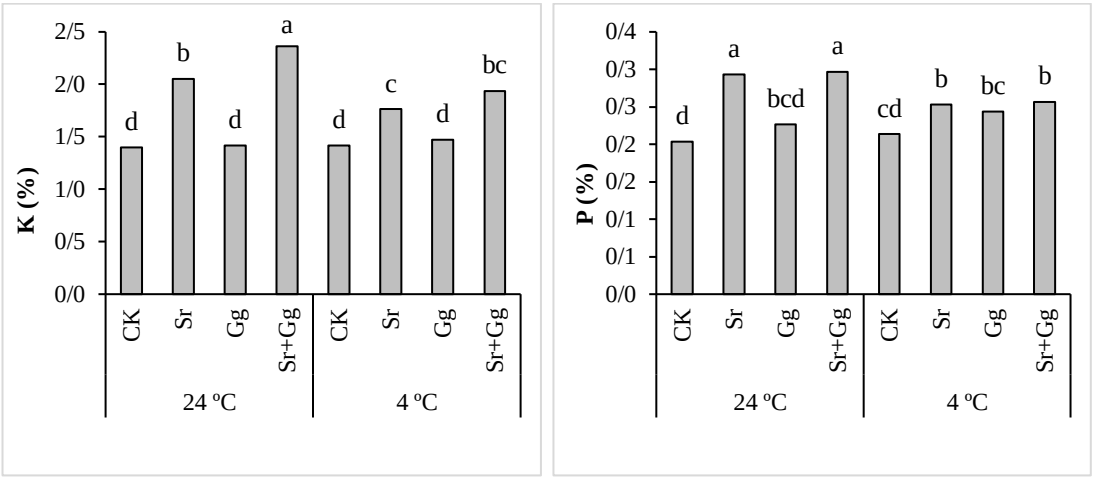

Fig. 12. (**A**) Interaction of temperature (24 and 4°C) and different treatments of *Streptomyces* rhizobacteria and ghatti gum on potassium; (**B**) and phosphorus content of tomato plant leaves**.** CK; control, Sr; *Streptomyces* rhizobacteria, Gg; ghatti gum, Sr + Gg; combination of *Streptomyces rhizobacteria* and ghatti gum. The means with same Duncan's multiple range test letters in each column are not statistically significant ($P \leq 0.01$).

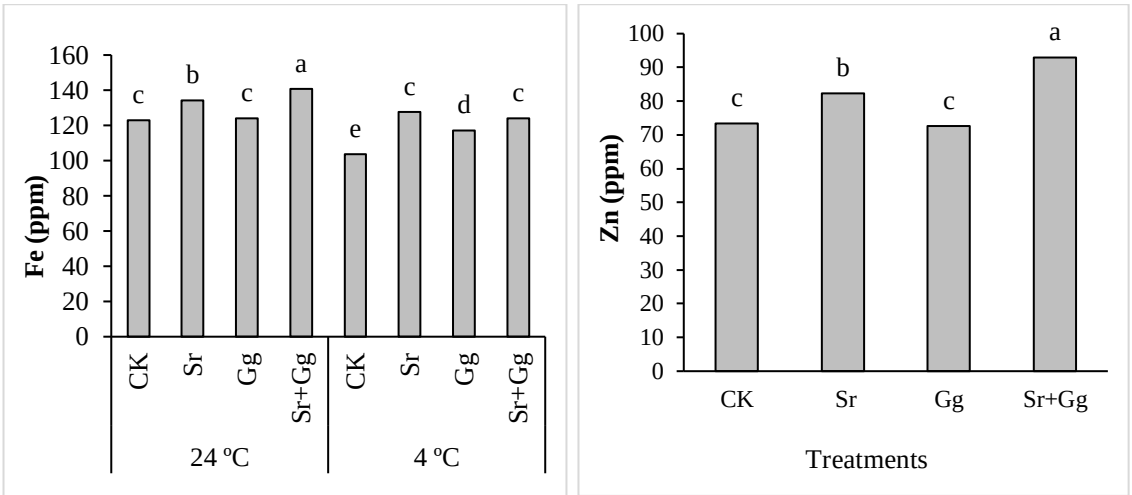

Fig. 13. (**A**) Interaction of temperature (24 and 4°C) and different treatments of *Streptomyces* rhizobacteria and ghatti gum on potassium; (**B**) and phosphorus (Panel B) content of tomato plant leaves**.** CK; control, Sr; *Streptomyces* rhizobacteria, Gg; ghatti gum, Sr + Gg; combination of *Streptomyces rhizobacteria* and ghatti gum. The means with same Duncan's multiple range test letters in each column are not statistically significant ($P \leq 0.01$).

The content of leaf Zn of tomato plants was significantly ($P \leq 0.01$) affected by the treatment, but the effect of temperature and the interaction effect of temperature × treatment on the content of this element were not significant. Among the studied treatments, the highest content of Zn was related to the plants that were treated with the combination of *Sr* + Gg (Fig. 13).

Discussion

Low temperatures can impact germination, nutrient absorption, flowering, and growth of tomato plants, with most cultivars and genotypes experiencing cold stress damage at 0-12 °C (Fayezizadeh et al., 2023; Sadashiva et al., 2013). Tomato seedlings' chilling sensitivity delays planting, so using resistant cultivars or treatments can reduce low temperature damage risk and enable earlier planting in fields and greenhouses (Fayezizadeh et al., 2021; Sadashiva et al., 2013). Photosynthetic pigments, crucial internal factors, can limit photosynthesis rate. Cold-exposed plants showed a significant decrease in Chl content without *Sr* and Gg treatment, possibly due to oxidative damage to chloroplast membranes or increased chlorophyllase enzyme activity (Karimi et al., 2016). In the present study, the stability of Chl under cold stress was higher in tomato seedlings treated with *Sr* + Gg combination compared to the control. No change or even increase in total Chl content has been reported in plants treated with *Sr* in grapes (Karimi et al., 2022a) and *Calendula officinalis* under salt stress (Sarlak et al., 2024) and treated with Gg in grapes under cold stress (Paveh and Karimi, 2024). The ability of *Sr* + Gg treatment to reduce the lipids peroxidation of membrane in chloroplasts, especially thylakoid membrane structures, through increasing the activity of free radical neutralizing enzymes is one of the possible mechanisms to reduce leaf Chl degradation under cold stress.

Tomato seed priming with *Sr* and Gg positively reduced EL, MDA, and H_2O_2 content in plants under low temperature stress, as the cell membrane is a key target for damage. An increase in the level of MDA is one of the indices that show the extent of damage to the membrane, and in other words, it reflects the oxidative damage to membrane lipids (Gulen et al., 2008; Karimi et al., 2016). Cold stress leads to increased production of ROS due to changes in phospholipid composition and arrangement in the cell membrane, causing membrane integrity disruption and ion leakage (Karimi et al., 2016; Juurakko and Walker, 2021). One of the reasons for the significant reduction in EL, MDA and H_2O_2 content in plants treated with *Sr* PGPR is the increase in intracellular K^+ content and the activity of antioxidant enzymes, which leads to a reduction in oxidative degradation caused by cold stress in plants. In a study on grapes (Karimi et al., 2022a) and *Calendula officinalis* (Sarlak et al., 2024), inoculation of plants with *Sr* PGPR significantly decreased the leaf EL, MDA and H_2O_2 content compared to untreated plants under salinity stress. The ability of *Sr* PGPR to increase drought stress in corn (Chukwuneme et al., 2020), salt stress in wheat (Akbari et al., 2020) has been reported. Also, in a study on grapes, it was found that the vines treated with Gg have more cold tolerance (Paveh and Karimi, 2024). These findings confirm the positive effect of the combined application of *Sr* and Gg on the stability of the cell membrane of tomato seedling under stress, which can be used as an agronomic operation to increase cold tolerance especially at early season in tomato farms.

Pretreatment of tomato seeds with *Sr* and Gg increased total soluble sugar and proline content in leaves under cold stress, reflecting the sugar's main role in osmotic regulation in plants, as confirmed by several studies (Paveh and Karimi, 2024; Sarlak et al., 2024; Fayezizadeh et al., 2024). Accumulation of osmotic regulators is one of the mechanisms of tolerance of plants to low temperature stress (Juurakko and Walker, 2021), which seed priming enhances nutrient absorption in tomato seedlings, potentially influencing their tolerance to cold temperatures, particularly during early growth stages (Karimi et al., 2017). A study found that foliar application of Gg increased proline and leaf sugar content during early spring budbreak stages and improved cold tolerance in grapevine plants (Paveh and Karimi, 2024). Also, in a study on tomato seedlings, the combined use of trehalose as a disaccharide and *Serendipita indica* fungus led to an increase in sugar and proline, membrane stability and cold tolerance of plants (Kaboosi et al., 2023). Proline is one of the compatible solutions that produced in plant tissues during exposure to low temperature stress (Chukwuneme et al., 2020).

Soluble proteins, which regulate osmotic pressure and protect plants against low temperatures, significantly enhance their cold tolerance (Chukwuneme et al., 2020; Sadashiva et al., 2013). *Sr* and Gg may enhance soluble protein biosynthesis, a crucial mechanism for intracellular osmotic regulation, thereby reducing damage to leaf cells from cold stress (Karimi et al., 2022a; Narsing et al., 2022). Inoculation of vine roots with *Sr* PGPR increased the sugar, protein and proline content of leaves compared to uninoculated grapes under salt stress (Karimi et al., 2022a). Also, in wheat seedlings inoculated with *Sr* PGPR, the amount of leaf proline and soluble sugars increased in plants under salt stress compared to control (Akbari et al., 2020). Tomato seedling treated with *Sr* and Gg may exhibit improved cell membrane lipid integrity and function under cold stress due to increased accumulation of these compounds.

Gg can enhance the antioxidant system, boost phenylalanine amonialyse enzyme activity, increase phenol and flavonoid content in fruits under cold stress conditions (Eshghi et al., 2021). The use of Gg in increasing cold tolerance in tomato plants has not been extensively studied, with most studies focusing on post-harvest issues. Ghatti gum, a polysaccharide with high viscosity, has been used as an edible coating alone or in combination with chitosan and ascorbic acid to extend grapes postharvest life (Eshghi et al., 2021). Phenols and flavonoids are part of the non-enzymatic antioxidant system that play an important role in neutralizing ROS produced in plants under cold stress (Fayezizadeh et al., 2023; Karimi et al., 2015). Therefore, any treatment that leads to the increase of these secondary metabolites can indirectly increase cold tolerance (Chukwuneme et al., 2020). The increase in phenol and flavonoids in plants treated with *Sr* + Gg aligns with the enhancement of the antioxidant system and cold tolerance in Izmir tomato plants, which can effectively absorb, neutralize, and remove ROS or peroxidases (Hu et al., 2020).

By activating both enzymatic (*i.e.*, CAT, GPX and APX) and non-enzymatic (*i.e.*, phenolic and flavonoid compounds) antioxidant systems, the application of Gg plays an important role in cell membrane stability (Eshghi et al., 2021; Paveh and Karimi, 2024). Also, the ability of *Sr* PGPR to stimulate the antioxidant system in inoculated plants has led to the removal of ROS and greater membrane stability (Karimi et al., 2022a; Narsing et al., 2022). The application of *Sr* + Gg increased the activity of ROS inhibiting enzymes in wheat cultivars under cold stress, and increased their tolerance to salt stress through higher antioxidant enzyme activities (Akbari et al., 2020). Also, changes in CAT and GPX activity have been detected in grape roots inoculated with *Sr* PGPR (Karimi et al., 2022a), which confirms our results. Tomato seedlings inoculated with Sr and Gg showed higher antioxidant activity, reduced leaf H_2O_2, MDA and EL, confirming the effectiveness of these treatment in reducing cold stress and strengthening the antioxidant system (Karimi et al., 2016), leads to increased cold tolerance and continued growth under cold stress in plants treated with *Sr* + Gg (Narsing et al., 2022). This result shows that the combination of *Sr* + Gg has a synergistic effect on the stability of leaf cell membrane in tomato seedlings under cold stress.

Tomato seedlings treated with *Sr* + Gg had higher content of K, P, Fe and Zn compared to control plants. The increased accumulation of certain nutrients in treated plants can impact osmotic regulation, energy supply for basic cell metabolism, and antioxidant enzyme activity in tomato seedlings under cold stress (Chukwuneme et al., 2020). In line with our results, root inoculation of rooted grape cuttings with *Sr* PGPR under salt stress led to an improvement in the leaf nutrient status (Karimi et al., 2022a). The inoculation of mycorrhizal fungi and zinc treatment in greenhouse grown grapevine saplings increased cold tolerance, indicating their impact on nutrient accumulation and membrane stability after exposure to cold stress (Karimi et al., 2022b). The increase in nutrients content in *Sr*-inoculated plants may be related to the growth of hairy roots induced by auxin (Sadeghi et al., 2012) or the osmotic regulation induced by Gg as a polysaccharide, which led to more absorption of nutrients by plant roots (Paveh and Karimi, 2024). The potential of *Sr* PGPR to produce siderophores as a high-affinity Fe chelating compound (Sadeghi et al., 2012) and changes in the microbial community structure of the root rhizosphere (Hu et al., 2020) is another possible option that increases nutrient absorption and cold tolerance in tomato seedlings treated with this microorganism (Karimi et al., 2022).

Conclusion

This study showed that priming Izmir tomato seeds with *Sr* + Gg improved their physiological and nutritional indices under 24 and 4 ºC conditions. Inoculation with *Sr* PGPR increased both leaf Chl and nutrients contents through P solubility and affecting nutrients adsorption by soil pH regulation (*i.e.* higher Zn and Fe absorption). On the other hand, the *Sr* PGPR + Gg application increased compatible osmolytes, stimulated the enzymatic antioxidant system, and preserved cell membrane integrity of tomato seedling under cold stress. This suggests that priming with *Sr* + Gg can increase seedlings cold tolerance in early establishment stages. In the field of the application of this microorganism, no limitations have been reported so far, and it even has the ability that in salty soils where the absorption of elements by the plant roots is limited, inoculation with this microorganism leads to the improvement of the absorption of elements and strengthening the growth and stability of the plant in the conditions environmental stresses.

Acknowledgments

We are thankful to the Research Council of Shahid Chamran University of Ahvaz.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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