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Effect of PGPR-1 Biofertilizer on Germination and Seedling Growth of Sweet Corn under Temperature Stress Conditions

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ABSTRACT

Seed germination and seedling growth are important stages affecting crop yield. However, extreme temperatures often inhibit germination and growth, preventing crops from reaching their full yield potential. Plant Growth-Promoting Rhizobacteria (PGPR) has been used to enhance seed germination and seedling growth under stressful environmental conditions. This research aimed to study the optimal methods and application rates of PGPR on the germination and seedling growth of corn under temperature stress conditions. The experiment was designed using a Split Plot in Completely Randomized Design, with the main factor being seed germination temperatures at three levels: 1) room temperature (25°C), 2) 15°C and 3) 45°C and sub-factor being method and rate of PGPR application at three levels: 1) no seed coating with PGPR-1 biofertilizer, 2) seed coated with PGPR-1 biofertilizer at 25 grams per 1 kg of seeds, left for 2 days before being sown, and 3) seed coated with PGPR-1 biofertilizer at 50 grams per 1 kg of seeds, sown immediately. The results showed that seed germination at room temperature resulted in the highest average germination rate, germination index, seedling height, and root length, with statistically significant differences compared to germination at 15°C and 45°C. For PGPR-1 biofertilizer application, seeds coated with 50 grams of PGPR-1 and sown immediately, as well as seeds coated with 25 grams of PGPR-1 and left for 2 days, tended to have the highest seedling height and root length, but these differences were not statistically significant from no seed coating with PGPR-1 biofertilizer. Additionally, no interaction effects were found between the germination temperature and the rate of PGPR-1 biofertilizer application on all traits studied.

Keywords: PGPR- biofertilizer, seed germination, sweet corn, temperature stress.

INTRODUCTION

Seed germination and seedling growth are important stages that directly affect crop yield. However, stress factors such as extreme temperatures often inhibit seed germination and crop growth, preventing crops from reaching their full yield potential. In recent years, the use of Plant Growth-Promoting Rhizobacteria (PGPR) has gained increasing attention due to its ability to enhance seed germination and seedling growth under stressful environmental conditions. PGPR provides a variety of ecological services and can play an essential role as crop yield enhancers and biological control agents. They can promote root development in plants, increase their capacity to absorb water and nutrients from the soil, increase stress tolerance, reduce disease and promote root development (Agbodjato and Babalola, 2024). Using plant growth-promoting rhizobacteria (PGPR) can be advantageous to enhance plant productivity and safeguard against environmental stresses (Rafique *et al.* 2024). Application of microbial fertilizer can help crops grow normally under water stress and improve leaf gas exchange rates and relative chlorophyll content; promote tomato root growth, plant water consumption, dry matter and plant water use efficiency (Liu *et.al.*2024). PGPR proved to enhance plants' ability to thrive and survive unfavorable conditions (Prusty *et al.,* 2023) The PGPRs influenced positively the growth of the saffron plant by increasing the growth of the aerial part, on the multiplication, the corms biomass and finally enhanced the content of secondary metabolites (crocin, crocetin, pic-rocrocin, and safranal) which reflected the saffron quality (Chamkhi *et.al.*2023). Inoculation of efficient PGPR not only enhanced yields, but also influenced the micronutrient concentration in grains (Kumar *et.al.*2014). The use of bio-fertilizers, especially the combined application of PGPR and vermicompost (F4), showed significant improvements in oil accumulation, grain yield, and the quantity and quality of oil under drought conditions (Nasrollahzadeh *et.al.* 2023). PGPR improved the enzymatic activity of SOD, CAT, and POD in tobacco leaves and significantly enhanced the IAA, GA, and ZR content under salt stress and improved the soil nutrient status and physiological state of tobacco seedlings (Shang, *et al.*2023). PGPRs impart induced systemic tolerance (IST) to plants towards drought stress through a variety of mechanisms like improvement of antioxidant system, production of ACC-deaminase and phytohormones, nitrogen fixation, phosphate solubilization, siderophore and exopolysaccharides production, enhanced root and shoot system, amplified photosynthesis rates and carotenoid production (Ahluwalia *et.al.*2021). The PGPR effects were related to the stress temperature, and PGPR can better promote plant growth and development in the temperature range of 5◦C–14 ◦C under cold stress and 40◦C–50 ◦C under heat stress (Zhang *et.al.*2023). This research aimed to study the optimal methods and application rates of PGPR on the seed germination and seedling growth of corn under temperature stress conditions.

MATERIALS AND METHOD

1) Experimental Plan

A Split Plot in Completely Randomized Design (Split Plot in CRD) with 4 replications was undertaken. The main and sub- factors are as follows:

Main Factor: Germination temperature 3 levels

1. Seed sown at room temperature (25°C)

2. Seed sown at 15°C

3. Seed sown at 45°C

Sub-Factor: Method and rate of PGPR application at 3levels

1.No seed coated with PGPR-1 biofertilizer

2.Seed coated with PGPR-1 biofertilizer at 25 grams per 1 kg of seeds, left for 2 days before being sown.

3.Seed coated with PGPR-1 biofertilizer at 50 grams per 1 kg of seeds, sown immediately.

2) Experimental Method

Sweet corn seeds var. Chainat 2 were coated with PGPR-1 biofertilizer according to the sub-factors mentioned above. After that, 100 seeds per treatment per replication were sown in sand trays and placed at different temperatures as the main factor mentioned above for 14 days.

3) Data Recording

3.1 Root length (cm): Ten seedling samples were randomly measured from the base to the tip of the root and then averaged to be root length per plant.

3.2 Germination percentage (%): Normal seedlings were daily counted for 7 days, after that the germination percentage was calculated as follows:

Germination percentage (%)

= Number of total germinated seeds x100

Total number of seeds tested

3.3 Germination index (GI): The number of normally germinated seedlings was counted daily for 14 days, after which the germination index (GI) was calculated using the following formula:

Germination index (GI) = $\sum Gt/Dt$

Gt = is the number of germinated seeds on day t

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Dt = is the time corresponding to Gt in days.

Ten seedlings were randomly measured from the base to the tip, then averaged to be seedling height/plant.

3.4 Seedling dry weight (g): Ten seedling shoots without their roots were placed in hot air oven at 70 °C Celsius for 24 hours, then weighed them and averaged to be shoot dry weight per plant

3.5 Seedling height (cm.): Ten seedling samples were randomly measured from the base of the shoot to the top then averaged to be Seedling height per plant.

4) Data Analysis

Analysis of variance was performed according to the Split plot in Completely Randomized Design and compared the differences between the means using the Least Significant Difference (LSD) method at a 95% confidence level using the Statistical Tool for Agricultural Research (STAR) software.

RESULTS AND DISCUSSION

1. Germination Percentage

Temperature had a significant effect on seed germination. Seeds placed at room temperature had the highest average germination rate of 97.83%, followed by seeds placed at 45°C and 15°C, with average germination rates of 67.67% and 29.58%, respectively, showing statistically significant differences. For the rate of coating corn seeds with PGPR-1 biofertilizer, seeds coated with 50 grams of PGPR-1 and sown immediately had the highest average germination rate of 75.67%, followed by seeds coated with 25 grams of PGPR-1 and left for 2 days before being sown , and seeds not coated with PGPR-1, with average germination rates of 62.00% and 57.42%, respectively, but these differences were not statistically significant. It was in line of Giri *et al.* (2023) who reported that the maximum rice seed germination was recorded in the PGPR consortium inoculated pot soil. The use of bio-fertilizers, separately or in combination, increased the germination of *B. tomentellus Boiss* (Delshadi *et.al.*2017). However, there was no interaction effect between germination temperature and the rate of coating corn seeds with PGPR-1 biofertilizer on germination (Table 1).

Table 1 Effects of temperature and PGPR-1 biofertilizer coating rate on germination percentage(%)

Temperature (M)	PGPR-1 Biofertilizer Coating Rate (g/kg(seed) (S)			Average (M)
	No coating	25 grams, left for 2 days	50 grams, sown immediately	
Room temperature	96.50	97.00	100.00	97.83 a[1/]
15°C	13.50	23.50	51.75	29.58 c
45°C	62.25	65.50	75.25	67.67 b
Average (S)	57.42	62.00	75.67	
LSD.05 (M)	19.21			
LSD.05 (S)	ns			
LSD.05 (M)x(S)	ns			
% CV. (M)	29.58			
% CV. (S)	28.27			

ns =Not significantly different,[1/]Different letters in the same column indicate statistically

significant differences.

2. Germination Index

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Seeds were sown at room temperature gave the highest average germination index of 41.13 which significantly differed from the seeds sown at 45°C and at 15°C with average germination index of22.57 and 9.50 respectively. For the rate of PGPR-1 biofertilizer, seeds coated with 25 grams of PGPR-1 and left for 2 days had the highest average germination index of 25.73, followed by seeds coated with 50 grams of PGPR-1 and sown immediately, and seeds not coated with PGPR-1, with germination index of 24.97 and 22.49, respectively however, these differences were not statistically significant. The rhizobacteria KBEndo3P1 increased the germination index of cucumber seedlings by 50% (Pérez-García *et.al.* 2023), Divyanshu *et.al.* (2022) reported that barley plants treated by PGPR through pot trial showed remarkable increase in seed germination percentage and vigor index. Additionally, there was no interaction effect between germination temperature and the rate of PGPR-1 biofertilizer on the germination index (Table 2).

Table 2: Effects of temperature and PGPR-1 biofertilizer coating rate on germination index.

Temperature (M)	PGPR-1 Biofertilizer Coating Rate (g/kg(seed) (S)			Average (M)
	No Coating	25 grams, left for 2 days	50 grams, sown immediately	
Room temperature	39.47	43.95	39.98	41.13 a[1/]
15°C	9.25	9.23	10.02	9.50 c
45°C	18.77	24.01	24.92	22.57 b
Average (S)	22.49	25.73	24.97	
LSD.05 (M)	4.96			
LSD.05 (S)	ns			
LSD.05 (M)x(S)	ns			
% CV. (M)	20.36			
% CV. (S)	21.40			

ns= Not significantly different[1/], Different letters in the same column indicate statistically significant differences.

3. Seedling Height

Seedling height was significantly influenced by temperature. Seeds placed at room temperature obtained the highest average seedling height of 9.99 cm and significantly differed from the seeds placed at 15°C and 45°C, which showed seedling heights of 2.44 cm and 1.51 cm, respectively. In term of rate of PGPR-1 biofertilizer, seeds coated with 50 grams of PGPR-1 and sown immediately had the highest average seedling height of 4.98 cm ,and did not show any significant differences from the seeds coated with 25 grams of PGPR-1 , left for 2 days before being sown and seeds not coated with PGPR-1, with average seedling heights of 4.75 cm and 4.21 cm, respectively. Application of PGPR strains significantly increased plant height of wheat (*Triticum aestivum* L.) by combined and single inoculated strain of Enterobacter (Kumar *et.al.*2014). Shahzad *et.al.* (2013) reported that rhizobacterial strain significantly increased plant height of maize. There was no interaction effect between temperature and the rate of PGPR-1 biofertilizer used for seed coating on seedling height (Table 3).

Table 3 Effects of temperature and PGPR-1 biofertilizer coating rate on seedling height (cm.).

Temperature (M)	PGPR-1 Biofertilizer Coating Rate (g/kg(seed) (S)			Average (M)
	No coating	25 grams, left for 2days	50 grams, sown immediately	
Room temperature	9.57	9.93	10.45	9.99 a[1/]
15°C	1.77	2.74	2.81	2.44 b

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Temperature (M)	PGPR-1 Biofertilizer Coating Rate (g/kg(seed) (S)			Average (M)
	No coating	25 grams, left for 2days	50 grams, sown immediately	
45°C	1.29	1.55	1.67	1.51 c
Average (S)	4.21 B2/	4.75 AB	4.98 A	
LSD.05 (M)	0.67			
LSD.05 (S)	0.60			
LSD.05 (M)x(S)	ns			
% CV. (M)	14.57			
% CV. (S)	15.06			

ns =Not significantly different, 1/Different letters in the same column indicate statistically significant differences., 2/Different letters in the same row indicate statistically significant differences.

4. Root Length

Temperature significantly affected the root length of seedlings. Seeds placed at room temperature had the highest average root length of 17.99 cm, followed by seeds placed at 15°C and 45°C, with average root lengths of 7.27 cm and 1.39 cm, respectively, showing statistically significant differences. Considering the rate of PGPR-1 biofertilizer, seeds coated with 50 grams of PGPR-1 and sown immediately had the highest average root length of 9.58 cm, followed by seeds coated with 25 grams of PGPR-1 and left for 2 days, and seeds not coated with PGPR-1, with average root lengths of 9.33 cm and 7.73 cm, respectively. These differences were statistically significant. PGPRs impart induced systemic tolerance (IST) to plants towards drought stress through a variety of mechanisms like enhancement of root system (Ahluwalia *et.al.* 2021). The HPGPR significantly improved root length of rice seedlings under salinity stress (Ashik Mahmud *et.al.*2023). However, there was no interaction between temperature and the rate of PGPR-1 biofertilizer used as seed coating on root length (Table 4).

Table 4 Effects of temperature and PGPR-1 biofertilizer coating rate on root length(cm.).

Temperature (M)	PGPR-1 Biofertilizer Coating Rate (g/kg(seed) (S)			Average (M)
	No coating	25 grams, left for 2 days	50 grams, Sown immediately	
Room temperature	17.43	18.08	18.43	17.99 a1/
15°C	4.48	8.60	8.71	7.27 b
45°C	1.27	1.31	1.58	1.39 c
Average (S)	7.73 B2/	9.33 A	9.58 A	
LSD.05 (M)	1.92			
LSD.05 (S)	1.53			
LSD.05 (M)x(S)	ns			
% CV. (M)	21.68			
% CV. (S)	20.12			

ns =Not significantly different,1/Different letters in the same column indicate statistically significant differences., 2/Different letters in the same row indicate statistically significant differences.

5. Seedling Dry Weight

Seedling dry weights were sown at room temperature and had the highest of 1.24 grams, , showing statistically significant differences from the seeds placed at 45 and 15 °C with average dry weights of 0.76 grams and 0.66 grams, respectively. For the rate of PGPR-1 biofertilizer, seeds coated with 50 grams of PGPR-1 and sown immediately tended to obtain the highest average dry weight of 0.98 grams, followed by seeds coated with 25 grams of PGPR-1 and left for 2 days, and seeds not coated with PGPR-1, with average dry weights of 0.83 grams and 0.82 grams, respectively, however no statistically significant differences were found. Wang *et. al.* (2021) found that the biochar combined with PGPR significantly increased dry matter accumulation of tomato. Using the PGPR isolates as inoculum affects positively the corms biomass of saffron (Crocus sativus L.) (Chamkhi *et.al.*2023). Shahzad *et.al.* (2013) reported that rhizobacterial strain significantly increased total biomass of maize. However, there was no interaction effect between temperature and the rate of PGPR-1 biofertilizer on dry weight (Table 5).

Table 5 Effects of temperature and PGPR-1 biofertilizer coating rate on seedling dry weight(g.).

Temperature (M)	PGPR-1 Biofertilizer Coating Rate (g/kg(seed) (S)			Average (M)
	No coating	25 grams, left for 2 days	50 grams, sown immediately	
Room temperature	1.17	1.18	1.34	1.24 a
15°C	0.69	0.70	0.87	0.76 b1/
45°C	0.61	0.62	0.74	0.66 c
Average (S)	0.82 B2/	0.83 B	0.98 A	
LSD.05 (M)	0.08			
LSD.05 (S)	0.08			
LSD.05 (M)x(S)	ns			
% CV. (M)	9.37			
% CV. (S)	10.86			

ns =Not significantly different,1/Different letters in the same column indicate statistically

significant differences., 2/Different letters in the same row indicate statistically significant differences.

CONCLUSION

From this study, we can conclude that seed germination at room temperature resulted in the highest average germination rate, germination index, seedling height, and root length, with statistically significant differences compared to germination at 15°C and 45°C. For PGPR-1 biofertilizer application, seeds coated with 50 grams of PGPR-1 and sown immediately, as well as seeds coated with 25 grams of PGPR-1 and left for 2 days, tended to have the highest seedling height and root length, but these differences were not statistically significant from no seed coating with PGPR-1 biofertilizer. Additionally, no interaction effects were found between the germination temperature and the rate of PGPR-1 biofertilizer application on all traits studied**.**

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