BMC Plant Biology

RESEARCH

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The rhizobacterial *Priestia megaterium* strain SH-19 mitigates the hazardous effects of heat stress via an endogenous secondary metabolite elucidation network and molecular regulation signalling

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Abstract

Global warming is a leading environmental stress that reduces plant productivity worldwide. Several beneficial microorganisms reduce stress; however, the mechanism by which plant–microbe interactions occur and reduce stress remains to be fully elucidated. The aim of the present study was to elucidate the mutualistic interaction between the plant growth-promoting rhizobacterial strain SH-19 and soybeans of the Pungsannamul variety. The results showed that SH-19 possessed several plant growth-promoting traits, such as the production of indole-3-acetic acid, siderophore, and exopolysaccharide, and had the capacity for phosphate solubilisation. The heat tolerance assay showed that SH-19 could withstand temperatures up to 45 °C. The strain SH-19 was identified as *P. megaterium* using the 16S ribosomal DNA gene sequence technique. Inoculation of soybeans with SH-19 improved seedling characteristics under high-temperature stress. This may be due to an increase in the endogenous salicylic acid level and a decrease in the abscisic acid level compared with the negative control group. The strain of SH-19 increased the activity of the endogenous antioxidant defense system, resulting in the upregulation of GSH (44.8%), SOD (23.1%), APX (11%), and CAT (52.6%). Furthermore, this study involved the transcription factors *GmHSP*, *GmbZIP1*, and *GmNCED3*. The findings showed upregulation of the two transcription factors *GmbZIP1* (17%), *GmNCED3* (15%) involved in ABA biosynthesis and induced stomatal regulation, similarly, a downregulation of the expression pattern of *GmHSP* by 25% was observed. Overall, the results of this study indicate that the strain SH-19 promotes plant growth, reduces high-temperature stress, and improves physiological parameters by regulating endogenous phytohormones, the antioxidant defense system, and genetic expression. The isolated strain (SH-19) could be commercialized as a biofertilizer.

Keywords SH-19, Temperature stress, Phytohormones, ROS

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Introduction

Higher plants are constantly exposed to abiotic stresses such as heat, heavy metals, salinity, drought, cold, and UV radiation. Climate change and global warming have further intensified the occurrence of abiotic stress. Heat stress is a global issue that adversely impacts the agricultural economy [1, 2]. As sessile organisms, plants cannot escape environmental stresses and must adapt to survive. Optimal temperatures are crucial for plant growth and development; temperatures above this range can disrupt biological and biochemical processes, leading to cellular damage and even plant death [3, 4]. Heat stress, defined as a temperature above 24 °C (75°F) is the most deleterious abiotic stress frequently affecting plants [5, 6].

Heat stress triggers a rapid accumulation of free radicals such as reactive oxygen species (ROS) in plants, leading to increased membrane fluidity, and disruption of cellular structure [7, 8]. Furthermore, endogenous phytohormones, such as abscisic acid (ABA) and salicylic acid (SA), are also modulated. However, plants have evolved and become sufficiently sophisticated to mitigate heat stress [9]. During heat stress, plants adapt their cellular activities by releasing heat shock proteins, which aid in homeostatic adjustments by relocating subcellular chloroplast ribosomes. Furthermore, plants make physiological and morphological adjustments in response to heat stress by realigning leaves relative to the light angle to avoid exposure, rolling leaves to conserve water, xylem enlargement due to closed stomata, evaporative cooling, modification of lipid membranes, production of waxy cuticles, and development of trichomes [10]. Other sophisticated mechanisms to mitigate heat stress in plants include modulation of phytohormones such as ABA that regulate stomatal closure, enhancement of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) enzymatic antioxidant activities, as well as accumulation of non-enzymatic antioxidants like glutathione (GSH), and overexpression of specific genes such as chaperones [11, 12]. On the other hand, plant growth-promoting rhizobacteria (PGPR) have been widely reported to play crucial roles in enhancing plant adaptation and growth under abiotic stresses such as drought and heat. The mechanisms employed by these microbes to mitigate stress involve the stimulation of phytohormones such as indole-3-acetic acid (IAA), abscisic acid (ABA), sequestering minerals such as iron that are in fixed form in the soil and making them available to plants, solubilization of insoluble calcium phosphates and other forms of phosphates for plant absorption, and the production of multifunctional exopolysaccharides (EPS) important for moisture absorption. Additionally, PGPR are known to produce organic acids, sugars, and amino acids that plays a significant role in plant metabolism, and delay of senescence. It is widely known that the phytohormone-induced stress signalling activities mediated by PGPR regulates metabolic processes such as antioxidant activities, reactive oxygen species (ROS) scavenging and the regulation of ABA biosynthesis gene responsive to dehydration thereby regulating stomatal behaviour during heat stress while regulating ethylene levels [13, 14]. While these mechanisms were reported to provide some protection to plants under heat stress, prolonged exposure to heat stress can still be fatal to plants. Temperatures beyond 36 °C have been reported to inhibit pollen germination, fertilization, coleoptile elongation, and affects key enzymes involved in nitrate (NO_3^-) and ammonium (NH_4^+) absorption, as well as uptake. This elevated heat levels reduce relative water content, resulting in plant cell organelle damage and a decrease in photosynthetic efficiency [15]. Various strategies are being implemented to alleviate the antagonistic effects of heat stress. These include breeding, tissue culture, agrochemicals, plant growth regulators, etc., but they have limitations. The most recent trend in abiotic stress mitigation is the use of biostimulants, i.e., growth-promoting microorganisms that are eco-friendly and non-opportunistic [16, 17].

Exploiting beneficial microbes for growth promotion and stress alleviation is a promising sustainable agricultural approach. Plant growth-promoting rhizobacteria (PGPR) are free-living bacteria that play a crucial role in plant growth either directly or indirectly by fixing nitrogen, solubilizing insoluble nutrients, and secreting phytohormones [18]. Previously, several studies have been conducted on stress-tolerant microbes in various essential crop species, such as wheat, barley, rice, maize, and soybeans [19, 20]. Moreover, beneficial microbes can establish an adhesive aptitude to increase the mutualistic interaction between the host plant and microbe to reduce stress (ABA) and chaperones [21–23].

Soybean is an essential cereal crop that is cultivated globally. According to the Food and Agriculture Organization (FAO), the global production of soybeans is 176.6 million [24]. Soybean is rich in proteins and oil, and it has been used in various food product development [25]. The present study was conducted on the soybean cultivar Pungsannamul containing isoflavone, and proteins, which is an essential source of carotene [26]. The reason for selecting the model plants is that they are extremely sensitive to temperature. This study was designed to decipher plant growth-promoting rhizobacteria, analyse their antioxidant potential, elucidate their mutualistic relationship with the host plant and perform phytohormonal and transcriptomic analyses.

Methodology

Microbe collection, isolation, and growth-promoting traits

The microbes were isolated from the rhizospheric soil around *Artemisia vulgaris* plant near Pohang Beach, Republic of South Korea following a method of [27]. The soil sample at the root zone of *A. vulgaris* was collected from 15 cm above the surface, stored in a zipper bag, submerged in an icebox at 0 – 5 °C and transported to the laboratory. The samples were later eluted with 100 mL of dH_2O and was serially diluted to 10^{-9} CFU/mL and streaked on the Luria Bertani agar (LB-Agar) media consisting of LB (25 g/L), agar (16 g/L), and glucose (0.33%). The samples were then incubated at 28 °C for 3 days. The resultant colonies that emerged were isolated, and re-cultured on the same medium to obtain pure isolate. Our research team reported the adhesive potential and surface features of the microbes [28]. Furthermore, multiple plant growth-promoting characteristics, such as the production of exopolysaccharides (EPS), the production of siderophores, phosphate solubilization, and the production of indole acetic acid, were analyzed following the instructions of [29].

Briefly, to assess the EPS content, Congo red assay plates were prepared. The isolates were inoculated and incubated at 27–30 °C for three days. The colonies that grew on the Congo red medium plates were confirmed to produce EPS. Salkowski's reagent was used to detect the presence of auxin. The transformation of the reagent colour to pink is positive for indole acetic acid. Furthermore, siderophores were evaluated on chrome azurol S plates. Trypticase activity was checked for the phosphate solubilization index, and soy agar plates supplemented with tricalcium phosphate were prepared. All the procedures were performed in five replicates.

Heat tolerance assay

The ability of SH-19 to withstand heat stress was assayed using the procedure previously described by [30], with modifications. Briefly, freshly grown colonies of strain SH-19 were inoculated in triplicate on freshly prepared LB broth and incubated following a 24-h cycle using a BF-60SIR (Bio free shaking incubator, Seoul, South Korea). Un-inoculated LB broth served as the control. The temperature was controlled after inoculating the cultures in nutrient broth at 150 rpm within a range of 25 °C–50 °C, with intervals of at least 5 h. The minimum, optimum, and maximum temperatures of the isolate were determined by measuring the optical density (OD) at 600 nm over a 3-day period.

Molecular identification

The isolate was identified using PCR-amplification of the 16S rRNA with universal primers 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′-CGG CTT ACC TTG TTA CGA CTT-3′) and compared to NCBI database sequences. The PCR process included denaturation at 95 ° C for 5 min, followed by 30 cycles of denaturation at 94 ° C for 1 min, annealing at 57 ° C for 40 s, and primer extension at 72 ° C for 1.5 min. The final extension step was at 72 ° C for 10 min. The PCR products were purified by 1% agarose gel electrophoresis for sequencing and analysis (SolGent Co., Ltd., Daejeon, Republic of Korea). Sequences were aligned using the Clustal*W* program in MEGA11, and a phylogenetic tree was constructed using the neighbour-joining (NJ) technique [27] with 1000 boot-strap replications for statistical support.

Pot experiment

Soybean seeds (variety, Pungsannamul) were purchased from the seed storage centre of Kyungpook National University (Daegu, South Korea). The seeds were surface sterilized with 2.5% sodium hypochlorite and rinsed three times with sterilized water. The seeds were germinated in trays containing horticultural soil, maintaining a temperature of 28 °C–30 °C. After seven days of uniform germination (the first unifoliate leaves fully emerged), the plants were transferred to experimental pots (10 × 9 cm) filled with autoclaved soil (horticultural soil). The plants were divided into different groups: A) a control group (only water added), B) soybean plants treated with SH-19 (groups A and B were kept at 27 °C–30 °C), C) inoculated and uninoculated soybean plants subjected to heat stress at 40 °C for five days, and D) inoculated and uninoculated soybean plants subjected to heat stress at 40 °C for 10 days. Before inoculation, microbes were grown in LB media and kept in a shaking incubator for 3 days. Then 3 days old microbes were harvested and centrifuged at 4,000 × g for 10 min. The collected pellets were diluted and inoculated (50 mL) into the pots via soil drenching. The experiment was performed in five replicates. The relative humidity was maintained at 60%–70%. After 10 days of applied stress, growth attributes such as chlorophyll content, seedling length, and weight were measured. The plants were immediately harvested to preserve the secondary metabolites and stored at – 80 °C. Chlorophyll was measured using a chlorophyll fluorometer (FIM 1500, ADC Bioscientific, Ltd., UK).

Assays for endogenous phytohormones

Quantitative measurement of ABA

ABA quantification was carried out according to earlier guidelines provided by [31]. In brief, a freeze-dried plant sample was mixed with isopropanol. The solution was combined and filtered using vacuum filtration. An internal standard (0.5 ng) was added. Solvent–solvent

extraction was performed using dichloromethane and ethyl acetate. This mixture was dried and injected into a gas chromatography-mass spectrometry (GC/MS) system for ABA quantification.

SA profile assay

A freeze-dried plant sample was ground using a homogenizer. For endogenous quantification of SA, the plant sample (100 mg) was mixed in 90% methanol and centrifuged at 10,000 × g for 7 min. This procedure was repeated twice. The obtained supernatant was dried using a rotary evaporator, rinsed with 5% trichloroacetic acid buffer, sonicated, and vortexed. An SA internal standard (isopropanol: cyclopeptone: isopropanol; 49.5:1:49.5) was added, and solvent–solvent extraction was performed. The upper layer was obtained and dried using nitrogen in an N drier. Diazomethane was added as a methylating agent and injected into a high-performance liquid chromatography system for SA quantification [32].

Analysis of antioxidant profiles

The method of antioxidant analysis was followed according to a previously described method [33]. Catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and GSH were measured using a spectrophotometer. The OD of the different antioxidants was measured at absorbance levels of 240, 420, 290, and 290 nm.

RNA extraction and genetic expression

Total RNA was extracted from the leaves using QIAzol Lysis Reagent. cDNA was synthesized using a reverse transcription kit, and the genomic expression of three different genes (*GmHSP*, *GmbZIP1*, and *GmNCED3*) was determined. The procedure was followed according to that previously described by [27]. Actin was used as a control.

Statistical analysis

The experiments were performed two times with five replications. MEGA11 software was used to construct the phylogenetic tree. The statistical analysis was conducted using one-way ANOVA with the help of SAS on demand for Academics (Version 3.1.0, SAS Institute Inc., Cary, NC, USA) with significance evaluated at $p < 0.05$ using Duncan's multiple range test (DMRT). GraphPad Prism (version 5.0, San Diego, CA, USA) presented the graphical visualization.

Results

Multiple plant growth-promoting traits

From the soil of *Artemisia* plants, 73 microbes were isolated and screened for various plant growth-promoting traits [30]. Among all the isolates, five exhibited considerable growth-promoting characteristics, which was previously reported by our research team, as well as their biochemical potential. This study also revealed that the selected SH-19 strain can form biofilms even at 45 °C [34]. Therefore, in the present study, we extended the analysis to a larger scale. The results showed that SH-19 showed multiple growth-promoting features such as ability to produce EPS, indole acetic acid, siderophores, and solubilize phosphate (Table 1) and supplementary Figure (S1).

Thermotolerance assay results

The selected microbes were screened for thermotolerance at various temperatures ranging from 25–50 °C. The results suggested that the growth of microbes was optimal at 25–40 °C. Surprisingly, even at 40 °C, the microbes were stable. However, after 40 °C, a reduction in the number of cells was observed. These results suggest that the survival of SH-19 plants tended to decrease at 45 °C, but at 50 °C, the microbes could not tolerate much heat stress. There was a zero reading at 55 °C when measured via spectrophotometry, as shown in Fig. 1.

Molecular identification

The results of molecular analyses show that strain SH-19 is closely related to *Priestia megaterium*. Strain SH-19 sequence with accession number ON935600 deposited at NCBI Blast:gb|ON935600| (nih.gov) shows that this strain is uniquely placed as a member of the *Bacillaceae* family. Based on the neighbour-joining (NJ) approach, a phylogenetic tree was built, as shown in Fig. 2.

Results of the pot experiments

A comparison with the standard and normal controls revealed that SH-19 enhanced heat tolerance, as shown by the seedling characteristics (biomass and root shoot length). After five days of heat stress, the shoot length (39.5%), root length (13.45%), fresh weight, and dry weight (22%–37%) decreased. However, inoculation with SH-19 significantly increased shoot length (17.8%), root length (16.73%), fresh weight (29.32%), and dry weight (14%–40%). After five days, similar patterns of growth attributes were observed, and there were no significant

Table 1 Multiple plant growth-promoting features of SH-19

Bacterial strain	Plant growth-promoting traits			
SH-19	IAA	PSI	Siderophore Production	EPS
	+++	+++	++++	+++

N.B: *IAA* Indole acetic acid, *PSI* Phosphate solubilization index, *EPS* Exopolysaccharides are the plant growth-promoting features that exhibit moderate (+++) and high (++++) levels

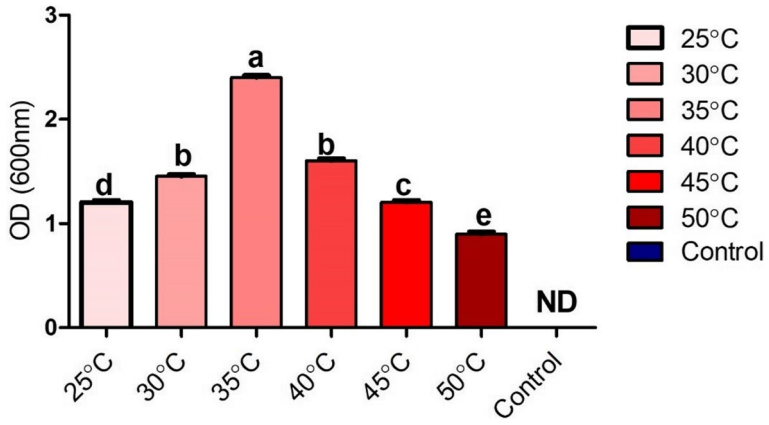

Fig. 1 Thermotolerance assay of SH-19 at different temperatures. Different lowercase letters on top of the bars indicate significant differences between the treatments at $p \leq 0.05$. The error bar represents the mean ± standard error of the mean (SEM) among the replicates, and ND represents the un-inoculated control

Fig. 2 Phylogenetic analysis of SH-19 using MEGA11. The NJ technique was used to generate the phylogenetic tree, using a 70% cut-off value and 1000 bootstrap strap replications

differences thereafter. In (Table 2), there are notable changes on soybean growth and development during the ten days of heat stress.

Chlorophyll analysis revealed that upon exposure to heat stress, the chlorophyll content decreased by 24% and 32% for chlorophyll a and chlorophyll b, respectively;

Table 2 Effects of SH-19 on the development and growth of heat-stressed soybean plants. The data are expressed as the mean ± standard deviation (SD) of analyses performed in triplicate

Days	Parameters	Control	SH-19	HS	HS + SH-19
0	SL (cm)	5.2 ± 0.2^{a}	5.00 ± 0.5^{b}	4.91 ± 0.2^{c}	5.3 ± 0.5^{a}
	RL (cm)	6.9 ± 0.3^{c}	7.98 ± 0.8^{a}	7.2 ± 0.1^{c}	7.3 ± 0.4^{b}
	SFW (g)	5.9 ± 0.4^{d}	6.31 ± 0.2^{c}	7.04 ± 0.4^{a}	6.6 ± 0.1^{b}
	SDW (g)	0.80 ± 0.4^{c}	1.02 ± 0.1^{a}	0.91 ± 0.2^{b}	0.85 ± 0.3^{c}
5	SL (cm)	21.9 ± 3.2^{b}	26.21 ± 1.12^{a}	12.9 ± 1.4^{d}	14.2 ± 1.42^{c}
	RL (cm)	27.5 ± 2.1^{b}	28.72 ± 2.4^{a}	21.3 ± 1.2^{d}	23.9 ± 4.21^{c}
	SFW (g)	9.92 ± 1.8^{b}	13.92 ± 1.45^{a}	6.91 ± 0.4^{c}	8.92 ± 3.54^{d}
	SDW (g)	1.82 ± 0.3^{c}	1.98 ± 0.6^{a}	1.3 ± 0.5^{d}	1.42 ± 0.4^{b}
10	SL (cm)	27.22 ± 2.21^{b}	30.21 ± 2.1^{a}	16.9 ± 1.5^{d}	16.4 ± 0.9^{c}
	RL (cm)	29.21 ± 3.5^{b}	32.25 ± 2.4^{a}	19.9 ± 1.7^{d}	22.2 ± 1.21^{c}
	SFW (g)	19.4 ± 1.2^{b}	24.94 ± 3.1^{a}	6.4 ± 0.4^{d}	6.9 ± 0.1^{c}
	SDW (g)	2.84 ± 0.5^{b}	3.42 ± 0.4^{a}	1.28 ± 0.3^{c}	1.8 ± 0.2^{d}

[a,b,c,d]Different alphabetic letters represent a significant difference according to Duncan's multiple range test at $p \leq 0.05$

SL Shoot length, *RL* Root length, *SFW* Shoot fresh weight, *SDW* Shoot dry weight, *HS* Heat stress

similarly, carotenoid levels decreased by 41%. After five days of heat stress and inoculation with SH-19, the chlorophyll (chlorophyll a, 13.20%; chlorophyll b, 14.90%) and carotenoid (12.99%) contents increased compared with those in the control group. A fluorescence study also revealed that heat stress significantly reduced the chlorophyll content by 10%–42%. Inoculation of the plants with SH-19 significantly increased the chlorophyll content by 6.04–26%.

Endogenous phytohormones analysis

There were no discernible differences in the endogenous ABA or SA levels between the normal and standard control plants without heat stress. However, upon exposure to heat stress for 5 and 10 days, the ABA content significantly increased twofold, by 5% and 10%, respectively, and the SA activity increased by 13.3% and 43%, respectively. However, the application of SH-19 under temperature stress for 5 and 10 days reduced the SA activity by four- and eightfold, respectively (40% and 71%). All results are provided in Fig. 3A and B.

Antioxidant analysis results

Heat stress induces the formation of ROS, especially $O_2^{\bullet}$ and H_2O_2 radicals, in the shoots of soybean plants. The antioxidant values were determined, and the results were significant in comparison with those of the control group. All the results are presented in Fig. 4.

The application of SH-19 resulted in an increase in the SOD level of 0.42% compared with that of the control; however, when heat stress was introduced, the SOD level increased up to 50% on day 10, and the application of SH-19 under heat stress increased the SOD activity by 23.1%.

Initially, the activity of CAT decreased by 10%; however, when heat stress was introduced, CAT activity increased by 33.3% on day 10. Furthermore, the application of SH-19 under heat stress increased the activity of CAT by 52.6% compared with that in the control treatment (Fig. 4B). For APX, the initial application of SH-19 decreased APX activity by 33% compared with that in the control plants, and heat stress significantly increased APX activity by 60%. The application of SH-19 under heat stress increased APX activity by 11% compared with that in the control treatments (Fig. 4C). The application of SH-19 increased the GSH activity by 10%; however, when heat stress was introduced, the GSH activity significantly increased by 52.6%. Further application of SH-19 under heat stress did not enhance the changes in the level of GSH activity on day 10 compared with that in the control plants (Fig. 4D).

Gene expression patterns

Heat stress significantly modulates gene expression

Compared with the control, heat stress markedly downregulated the expression of the SH-19 *GmHSP* gene by 29% on day 5 (46%). Compared with the control treatment, heat treatment significantly downregulated the expression of the SH-19 *GmHSP* gene by 25% (Fig. 5A). Similarly, the application of SH-19 modulated the expression of *GmbZIP1* by 8.3%, and heat treatment downregulated the expression of *GmbZIP1* by 8% compared with that of the control. The application of SH-19 during heat treatment significantly enhanced the expression of *GmbZIP1* by 17% compared with that in the control (Fig. 5B). However, the relative expression of *GmNCED3* was low (1.2%), and the application of SH-19 increased

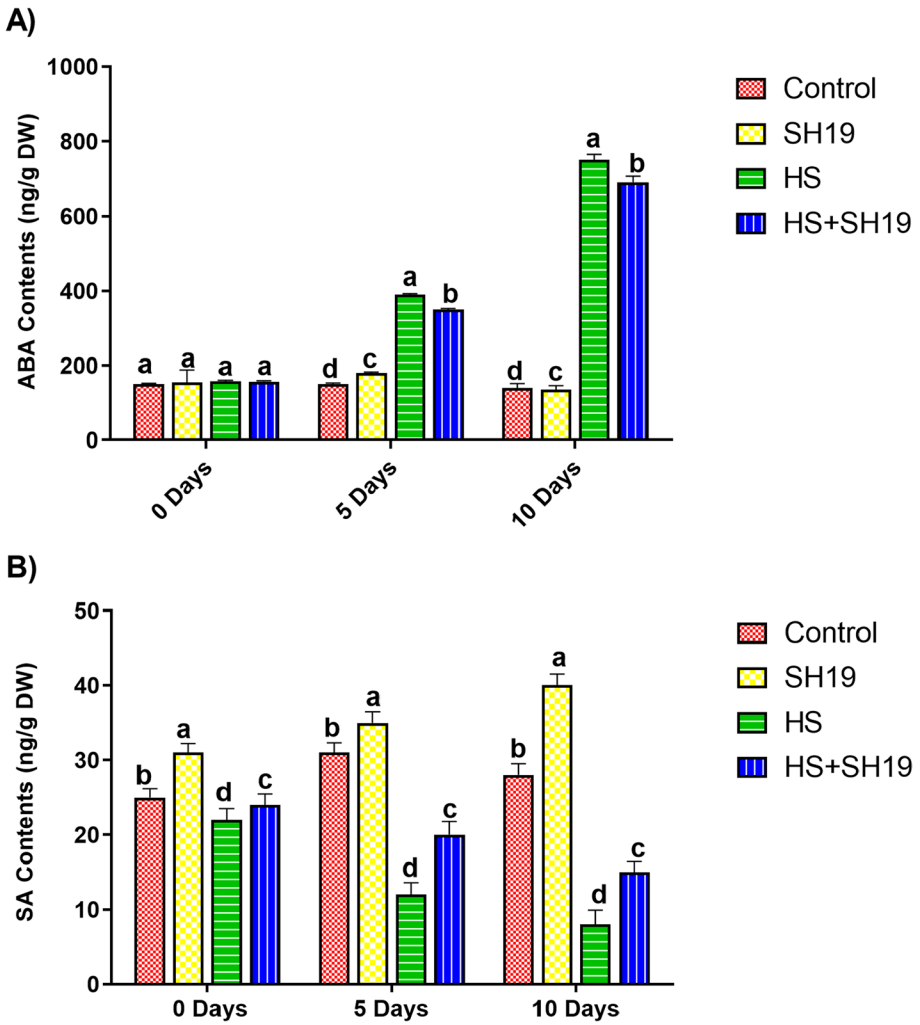

Fig. 3 Quantitative measurement of (**A**) abscisic acid (ABA) and (**B**) salicylic acid (SA) in plant samples. Different lowercase letters on top of the bars indicate significant differences between treatments at $p \leq 0.05$. The mean ± standard error of the mean (SEM) of the replicates is shown by the error bar

the expression of *GmNCED3* by 17%. Compared with the control treatment, heat stress significantly reduced the expression of *GmNCED3* by 16.7%. However, the application of SH-19 under heat stress significantly enhanced the expression of *GmNCED3* by 15% compared with that in the control (Fig. 5C).

Discussion

Environmental stress causes a substantial reduction in the worldwide yield of crops [35]. Heat stress is a major contributor to crop loss worldwide, impacting several aspects of plant growth and development [36, 37]. Heat stress intensifies the oxidation and denaturation of biomolecules such as amino acids, lipids, and carbohydrates due to the buildup of free radicals in plant cells [4, 38]. Plant defenses are strengthened by the metabolic reprogramming of cellular processes in response to stress. These include gene activation, phytohormone regulation, antioxidant defense system activation, and secondary metabolite buildup. The reprogramming does not happen when stress levels rise. [39, 40].

The present study was conducted to isolate and screen heat-tolerant plant growth-promoting rhizobacteria and determine their effectiveness at elevated temperatures. Among several previously identified isolates, SH-19 is a heat-tolerant microbe that can grow and survive at a high temperature of 45 °C, and this result is significant to that of [41], who reported that the strain was able to tolerate a maximum temperature of 30 °C. Our strain showed enhanced heat tolerance compared to other strains, thus making it a novel heat-tolerant strain evaluated on soybean plants. The plant growth-promoting traits of strain

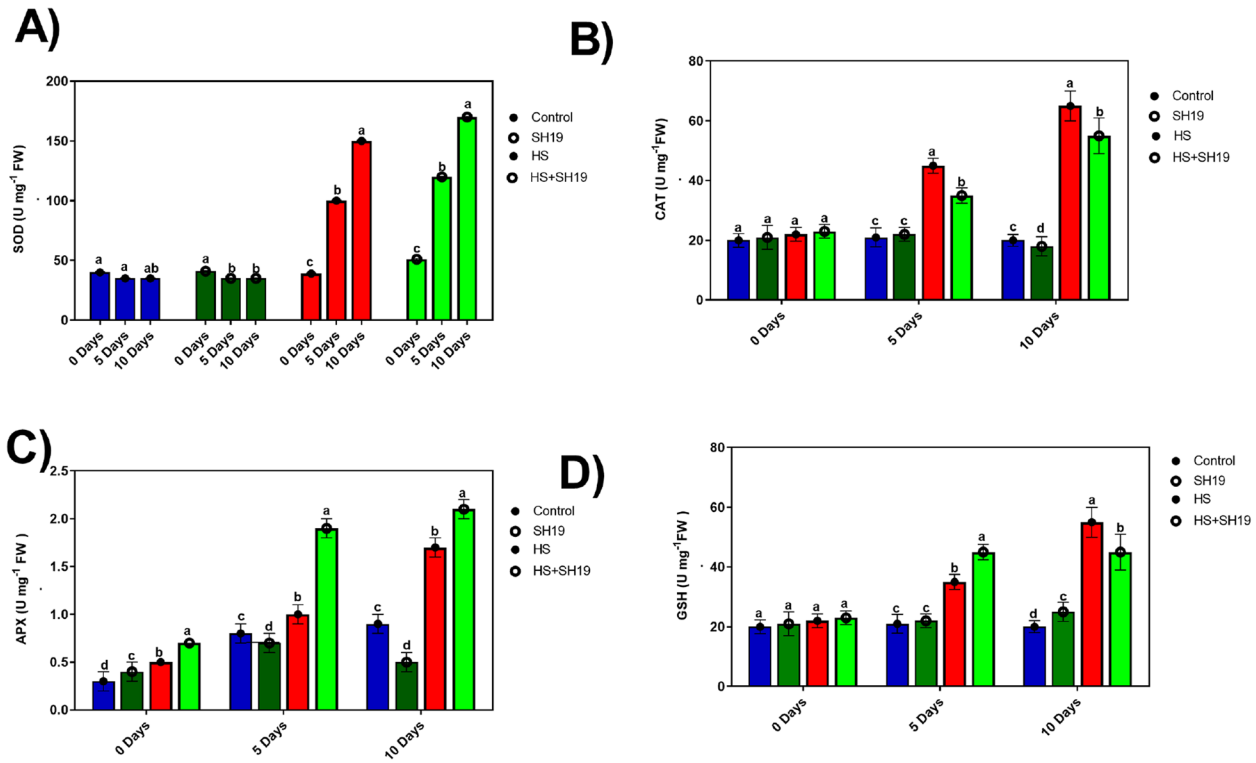

Fig. 4 Effects of SH-19 on the antioxidant activities of (**A**) superoxide dismutase (SOD), (**B**) catalase (CAT), (**C**) ascorbate peroxidase (APX), and (**D**) glutathione (GSH) in soybean plants under heat stress. The mean of five replicates is shown for each data point, and the mean ± standard error (SE) is shown as the error bars. A significant difference between the treatments at $p \leq 0.05$ is indicated by different lowercase letters above the bars

SH-19 were evaluated. The results show that it can produce EPS, which is essential for plant metabolism, to provide carbohydrates for cellular respiration. The ability of strain SH-19 to produce EPS from glucose in the medium, as shown by dark-colored deposits, indicates its potential to survive in the plant rhizosphere while providing plant cells with valuable sugars for survival during heat stress. These findings are similar to those reported for plants [27]. The demonstration of phosphate solubilization by inoculation with the SH-19 strain is also a substantial advantage. Phosphates are available in fixed forms in most soils; therefore, they cannot be absorbed by plants. The conversion of insoluble calcium phosphate in the agar medium demonstrated the ability of strain SH-19 to solubilize fixed phosphates in the soil, increase their availability for plants during heat stress, and produce indole acetic acid. These findings are in line with the previous results reported by [42]. After confirming the PGP characteristics, SH-19 was evaluated for molecular identification. The results suggest that it is a new family member of the *Bacillaceae*.

In the present study, during heat stress, significant differences in ABA and SA levels were observed. ABA is a stress hormone, and its level increases as stress increases. Heat-tolerant microbes reduce the level of ABA and enhance heat stress resistance, which is consistent with the previous findings obtained by [43]. SA is an important phytohormone in the plant defense system. In reaction to heat stress, there is a reduction in the SA level; however, the application of SH-19 improved the endogenous SA level. This result is consistent with the previous findings obtained by [44, 45]. Numerous studies have shown that microbes have the potential to modulate endogenous phytohormone levels; therefore, the results reveal that the SH-19 strain has the potential to modulate phytohormone levels.

Microbes are emerging biostimulants that have the potential to scavenge free radicals [46, 47]**.** In the present study, the levels of four antioxidant molecules, SOD, CAT, APX, and GSH, were evaluated. The results showed that heat stress enhanced the levels of free radicals such as ROS. The application of the heat-tolerant microbe SH-19 improved the antioxidant defense system. This study demonstrated that *P. megaterium* has the potential to modulate the antioxidant defense system by regulating redox balance.

The present study revealed that the levels of heat shock protein genes (*GmHSP*s) increased under heat

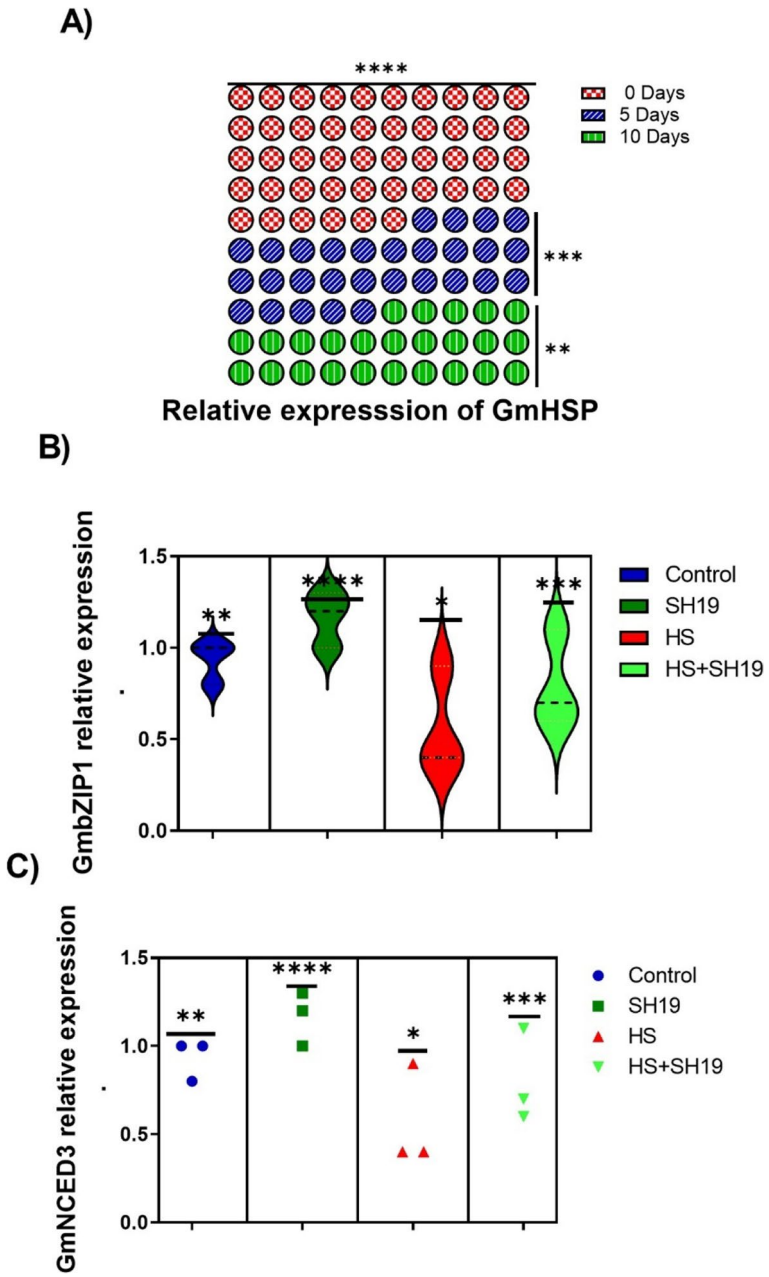

Fig. 5 Effect of strain SH-19 on the relative gene expression in soybean plants under heat stress. Each data point represents the mean of five replicate measurements, the error bars represent the standard error (SE), and each asterisk represents a significant difference at $p \leq 0.05$

stress, but with SH-19 application, there was a reduction in *GmHSP* expression. These results are in line with the previous findings obtained by [48] who reported that inoculation with PGPR strain can reduce the expression of *GmHSP*. Similarly, the genetic expression of two other genes, *GmbZIP1* and *GmNCED3*, was assessed. *GmbZIP1* is involved in the ABA signal transduction pathway; therefore, our results agree with the hypothesis that SH-19 modulates heat stress via transcriptional and phytohormonal analysis.

Conclusion

This work has identified and characterized a new strain of heat-tolerant bacterium, SH-19, which shows great promise as a PGPR to improve soybeans' ability to withstand heat stress. When exposed to heat

stress, SH-19 displayed a variety of reactions, including altered transcription of stress-responsive genes, an increased antioxidant defense system, and modifications in phytohormones (such as salicylic acid and abscisic acid). SH-19 inoculation to soybean plants produces biologically active metabolites and induces the plant-microbial interaction that promotes active plant growth and stress tolerance. It is a promising and environmentally friendly method of achieving sustainable agronomy. However, further research is needed to assess its efficacy in diverse cultivars and agroclimatic conditions.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05534-2.

Supplementary Material 1.

Acknowledgements
The present study was supported by Brain Korea 4th phase provided by ministry of Korea. The authors extend their appreciation to the Researchers Supporting Project number (RSP2024R118) of King Saud University, Riyadh, Saudi Arabia.

Author's contributions
SS conceptualised, did the experiment as part of her project, and wrote the initial draft. AAS, HOE did the critical review editing, writing and Statistical analysis. OP, MIUH and SMK, perform the formal analysis. TNIAA, BWY helped in the expression analysis of genes. IJL supervised, validated the results.

Funding
This research was supported by the Korea Basic Science Institute (National Research Facilities and Equipment Center) grant funded by the Ministry of Education (2021R1A6C101A416). We are grateful to I-JL for his kind support during supervision and to the KNU NGS Center (Daegu, South Korea) for providing laboratory equipment for quantitative PCR. Researchers supporting the project (RSP2024R118) at King Saud University.

Availability of data and materials
The data sets associated with present work are available from the corresponding authors on reasonable request.

Declarations

Ethics approval and consent to participate.
Not applicable.

Consent for publication
Not applicable.

Competing interest
The authors declare no competing interests.

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Received: 19 April 2024 Accepted: 21 August 2024
Published online: 04 September 2024

References

1. Kumar S. Abiotic stresses and their effects on plant growth, yield and nutritional quality of agricultural produce. Int J Food Sci Agric. 2020;4:367–78.
2. Farooq MS, Uzair M, Raza A, Habib M, Xu Y, Yousuf M, Yang SH, Ramzan Khan M. Uncovering the research gaps to alleviate the negative impacts of climate change on food security: a review. Front Plant Sci. 2022;13:927535.
3. Balfagón D, Zandalinas SI, Mittler R, Gómez-Cadenas A. High temperatures modify plant responses to abiotic stress conditions. Physiol Plant. 2020;170(3):335–44.
4. Medina E, Kim S-H, Yun M, Choi W-G. Recapitulation of the function and role of ros generated in response to heat stress in plants. Plants. 2021;10(2):371.
5. Kopecká R, Kameniarová M, Černý M, Brzobohatý B, Novák J. Abiotic stress in crop production. Int J Mol Sci. 2023;24(7):6603.
6. Zhang Y, Xu J, Li R, Ge Y, Li Y, Li R. Plants' Response to Abiotic Stress: Mechanisms and Strategies. Int J Mol Sci. 2023;24(13):10915.
7. Li N, Euring D, Cha JY, Lin Z, Lu M, Huang L-J, Kim WY. Plant hormone-mediated regulation of heat tolerance in response to global climate change. Front Plant Sci. 2021;11:627969.
8. Zhao J, Lu Z, Wang L, Jin B. Plant responses to heat stress: physiology, transcription, noncoding RNAs, and Epigenetics. Int J Mol Sci. 2020;22(1).
9. Jagadish SK, Way DA, Sharkey TD. Plant heat stress: Concepts directing future research. Plant, Cell Environ. 2021;44(7):1992–2005.
10. Li Y, Jiang F, Niu L, Wang G, Yin J, Song X, Ottosen CO, Rosenqvist E, Mittler R, Wu Z. Synergistic regulation at physiological, transcriptional and metabolic levels in tomato plants subjected to a combination of salt and heat stress. Plant J. 2024;117(6):1656–75.
11. Rasheed R, Ashraf MA, Iqbal M, Hussain I, Akbar A, Farooq U, Shad MI. Major constraints for global rice production: changing climate, abiotic and biotic stresses. *Rice research for quality improvement: genomics and genetic engineering*: Volume 1: breeding techniques and abiotic stress tolerance. 2020:15–45.
12. Khan MSS, Ahmed S. Ikram Au, Hannan F, Yasin MU, Wang J, Zhao B, Islam F, Chen J: Phytomelatonin: A key regulator of redox and phytohormones signaling against biotic/abiotic stresses. Redox Biol. 2023;64:102805.
13. Beattie GA, Bayliss KL, Jacobson DA, Broglie R, Burkett-Cadena M, Sessitsch A, Kankanala P, Stein J, Eversole K, Lichens-Park A. From microbes to microbiomes: Applications for plant health and sustainable agriculture. Phytopathology. 2024(ja).
14. Olanrewaju OS, Glick BR, Babalola OO. Metabolomics-guided utilization of beneficial microbes for climate-resilient crops. Curr Opin Chem Biol. 2024;79:102427.
15. Sang M, Liu Q, Li D, Dang J, Lu C, Liu C, Wu Q. Heat stress and microbial stress induced defensive phenol accumulation in medicinal plant sparganium stoloniferum. Int J Mol Sci. 2024;25(12):6379.
16. Kumar V, Wani SH. Heat stress tolerance in plants: physiological, molecular and genetic perspectives: John Wiley & Sons; 2020.
17. Rasool N. Role of Plant Hormones in Mitigating Abiotic Stress. 2023.
18. Shah A, Nazari M, Antar M, Msimbira LA, Naamala J, Lyu D, Rabileh M, Zajonc J, Smith DL. PGPR in agriculture: A sustainable approach to increasing climate change resilience. Front Sustain Food Syst. 2021;5:667546.
19. Shaffique S, Khan MA, Wani SH, Pande A, Imran M, Kang S-M, Rahim W, Khan SA, Bhatta D, Kwon E-H. A review on the role of endophytes and plant growth promoting rhizobacteria in mitigating heat stress in plants. Microorganisms. 2022;10(7):1286.
20. Maitra S, Pramanick B, Dey P, Bhadra P, Shankar T, Anand K. Thermotolerant soil microbes and their role in mitigation of heat stress in plants. Soil Microbio Sustain Agri Func Ann. 2021:203–242.
21. Wang H, Wang Y, Jiang D, Xiang Z, Wang S, Kang C, Zhang W, Ge Y, Wang T, Huang L. Soil microbe inoculation alters the bacterial communities and promotes root growth of Atractylodes lancea under heat stress. Plant Soil. 2022;478(1–2):371–89.
22. Mukhtar T, Ali F, Rafique M, Ali J, Afridi MS, Smith D, Mehmood S, Souleimanov A, Jellani G, Sultan T. Biochemical Characterization and potential of bacillus safensis Strain SCAL1 to mitigate heat stress in solanum lycopersicum L. J Plant Growth Regul. 2023;42(1):523–38.
23. Ali SZ, Sandhya V, Grover M, Linga VR, Bandi V. Effect of inoculation with a thermotolerant plant growth promoting Pseudomonas putida strain AKMP7 on growth of wheat (Triticum spp.) under heat stress. J Plant Interact. 2011;6(4):239–46.

24. Kumar P, Sharma M, Abubakar AA, Nizam bin Hayat M, Ahmed MA, Kaka U, Sazili AQ: 2.16 - Soybean: Sustainability Issues. In: *Sustainable Food Science - A Comprehensive Approach*. Edited by Ferranti P. Oxford: Elsevier; 2023: 219–231.
25. Di Giorgio L, Marcantonio MA, Salgado PR, Mauri AN. Chapter 23 - Isolation, characterization, and industrial processing of soybean proteins. In: Sreekala MS, Ravindran L, Goda K, editors. Handbook of Natural Polymers, vol. 1. Thomas S: Elsevier; 2023. p. 557–75.
26. Tran HA, Nguyen CT, Lee J-D, Seo HS, Song JT. Screening for flooding tolerance in two soybean populations (EMS-induced 'Pungsannamul'population and Korean Glycine soja core population). 한국육종학회 긍둥학술발표집. 2021:210–210.
27. Shaffique S, Imran M, Rahim W, Alomrani SO, Yun B-W, Lee I-J. A newly isolated Bacillus pumilus strain SH-9 modulates response to drought stress in soybean via endogenous phytohormones and gene expression (Daegu, South Korea). Plant Stress. 2023;10:100279.
28. Shaffique S, Imran M, Wani SH, Khan MA, Kang S-M, Adhikari A, Lee I-J. Evaluating the adhesive potential of the newly isolated bacterial strains in research exploitation of plant microbial interaction. Front Plant Sci. 2022, 13.
29. Premarathna AD, Ahmed TA, Kulshreshtha G, Humayun S, Darko CNS, Rjabovs V, Hammami R, Critchley AT, Tuvikene R, Hincke MT. Polysaccharides from red seaweeds: Effect of extraction methods on physicochemical characteristics and antioxidant activities. Food Hydrocolloids. 2024;147:109307.
30. Shaffique S, Alomrani SO, Injamum-Ul-Hoque M, Peter O, Imran M, Lee I-J. Unlocking the potential of newly isolated phytohormone-producing bacterial strains for enhanced plant growth and stress tolerance. Plant Stress. 2023;10:100260.
31. Kang S-M, Shaffique S, Imran M, Jeon S-M, Wani SH, Khan MA, Odongkara P, Kwon E-H, Kang Y, Son J-I. Inoculation of chlorella and food waste improves the physio-morphological features of red pepper by regulating activating antioxidant defense System. Phyton (0031–9457). 2023;92(9).
32. Imran M, Mpovo CL, Aaqil Khan M, Shaffique S, Ninson D, Bilal S, Khan M, Kwon E-H, Kang S-M, Yun B-W. Synergistic Effect of Melatonin and Lysinibacillus fusiformis L. (PLT16) to Mitigate Drought Stress via Regulation of Hormonal, Antioxidants System, and Physio-Molecular Responses in Soybean Plants. Int J Mol Sci. 2023;24(10):8489.
33. Jafri SAA, Khalid ZM, Khan MR, Ashraf S, Ahmad N, Karami AM, Rafique E, Ouladsmane M, Al Suliman NMS, Aslam S. Evaluation of some essential traditional medicinal plants for their potential free scavenging and antioxidant properties. J King Saud Univ Sci. 2023;35(3):102562.
34. Shaffique S, Imran M, Wani SH, Khan MA, Kang S-M, Adhikari A, Lee I-J. Evaluating the adhesive potential of the newly isolated bacterial strains in research exploitation of plant microbial interaction. Front Plant Sci. 2022;13:1004331.
35. Farhad M, Kumar U, Tomar V, Bhati PK, Krishnan J. N, Kishowar-E-Mustarin, Barek V, Brestic M, Hossain A. Heat stress in wheat: a global challenge to feed billions in the current era of the changing climate. Front Sustain Food Syst. 2023;7.
36. Hill CB, Li C. Genetic Improvement of Heat Stress Tolerance in Cereal Crops. Agronomy. 2022;12(5):1205.
37. Ashraf MA, Iqbal M, Rasheed R, Hussain I, Riaz M, Arif MS. Environmental stress and secondary metabolites in plants: an overview. Plant Metabolites Reg Under Environ Stress. 2018:153–167.
38. Kan Y, Mu XR, Gao J, Lin HX, Lin Y. The molecular basis of heat stress responses in plants. Mol Plant. 2023;16(10):1612–34.
39. Jiang X, Wassie M, Zhou W, Wang H, You J, Ma G, Zhang M. Transcriptomic Analysis Provides Novel Insights into the Heat Stress-Induced Response in Codonopsis tangshen. Life. 2023;13(1):168.
40. Huang D, Ding L, Wang S, Ding R, Qiu X, Li J, Hua Z, Liu S, Wu R, Liang X. Metabolomics reveals how spinach plants reprogram metabolites to cope with intense stress responses induced by photoaged polystyrene nanoplastics (PSNPs). J Hazard Mat. 2024:133605.
41. Khan MA, Asaf S, Khan AL, Jan R, Kang S-M, Kim K-M, Lee I-J. Thermotolerance effect of plant growth-promoting Bacillus cereus SA1 on soybean during heat stress. BMC Microbiol. 2020;20(1):1–14.
42. Xia Y, Yuan Y, Li C, Sun Z. Phosphorus-solubilizing bacteria improve the growth of Nicotiana benthamiana on lunar regolith simulant by dissociating insoluble inorganic phosphorus. Communications Biology. 2023;6(1):1039.
43. Shaffique S, Khan MA, Imran M, Kang S-M, Park Y-S, Wani SH, Lee I-J. Research progress in the field of microbial mitigation of drought stress in plants. Front Plant Sci. 2022;13:870626.
44. Arif Y, Sami F, Siddiqui H, Bajguz A, Hayat S. Salicylic acid in relation to other phytohormones in plant: A study towards physiology and signal transduction under challenging environment. Environ Exp Bot. 2020;175:104040.
45. Monte I. Jasmonates and salicylic acid: Evolution of defense hormones in land plants. Curr Opin Plant Biol. 2023;76:102470.
46. Egamberdieva D, Wirth SJ, Alqarawi AA. Abd_Allah EF, Hashem A: Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. Front Microbiol. 2017;8:2104.
47. Porter SS, Bantay R, Friel CA, Garoutte A, Gdanetz K, Ibarreta K, Moore BM, Shetty P, Siler E, Friesen ML. Beneficial microbes ameliorate abiotic and biotic sources of stress on plants. Funct Ecol. 2020;34(10):2075–86.
48. Khan MA, Asaf S, Khan AL, Jan R, Kang S-M, Kim K-M, Lee I-J. Thermotolerance effect of plant growth-promoting Bacillus cereus SA1 on soybean during heat stress. BMC Microbiol. 2020;20(1):175.

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