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Thermotolerant PGPR consortium B3P modulates physio-biochemical and molecular machinery for enhanced heat tolerance in maize during early vegetative growth

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Abstract

Background Global warming and irregular changes in temperature are a serious threat to plant growth with a significant negative impact on yield. Global maize productivity has decreased significantly due to sudden temperature fluctuations and heat waves especially in the regions severely hit by climate change.

Results The current study demonstrates the potential of beneficial bacteria for inducing heat tolerance in maize during early growth. Three *Bacillus* spp. AH-08, AH-67, SH-16, and one *Pseudomonas* spp. SH-29 showed the ability to grow and exhibited multiple plant-beneficial traits up to 45 ± 2 °C. At temperatures of 45 and 50 °C, *Bacillus* sp. SH-16 exhibited upregulation of two small heat shock proteins (HSP) of 15 and 30 kDa, while SH-16 and AH-67 showed upregulation of two large HSP of 65 and 100 kDa. Plant-inoculation with the consortium B3P (3 *Bacillus* + 1 *Pseudomonas* spp.) was carried out on six hybrid maize varieties pre-grown at 25 ± 2 °C. Heat shock was applied to 10-day-old seedlings as: 3 h at 38°C, 48 h recovery period, and then 48 h at 42°C. The B3P treatment showed significant improvement in the overall plant growth (plant height, root & shoot fresh & dry weight, root and leaf area) with a higher level of CAT, POD, total chlorophyll, and carotenoids, while low concentration of MDA. A non-significant difference was observed in case of total cell protein and amino acids after B3P-treatment under stress. The expression of *HSP1* and *HSP18* in Malka and YH-5427 while *HSP70* and *HSP101* were higher in FH-1046 and Gohar as compared to non-inoculated treatment.

Conclusions These findings indicate that heat-tolerant plant-growth promoting rhizobacteria (Ht-PGPR) exert versatile, multiphasic and differential response to improve plant growth and heat-tolerance in different maize varieties during seedling/ early vegetative growth. Subsequent research will be focused on the field evaluation of these PGPR to see the field and yield response of this consortium under natural temperature fluctuations in field.

Keywords Global warming, Climate change, Heat stress, Plant Growth Promoting Rhizobacteria (PGPR), Maize, Heat shock proteins

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Background

In natural and agricultural habitats, plants are exposed to continually varying or sudden fluctuations in temperature. Plant growth is severely affected at high temperatures due to stress-induced cell damage, generation and accumulation of reactive oxygen species, protein misfolding (Tariq and Iqbal 2010), (Mathur et al. 2019), and disruption of the photosynthetic machinery (Kajla et al. 2015). Although, the reproductive (anthesis and grain filling) stage is more sensitive in cereals (Schauberger et al. 2017) but during early seedling development, temperature of 42 °C could inhibit both shoot and root growth (Iloh et al. 2014). Maize (*Zea mays*) is among the crops significantly affected due to temperature fluctuations, where each degree rise in temperature may reduce its production by up to 13% (Izaurralde et al. 2011). The crop is suffering from serious heat stress events as a result of climate change which will be more intense, frequent, and longer lasting in the 2nd half of this century causing even larger yield reductions in the future (El-Sappah and Rather 2022).In addition, the climate variability is large enough to offset the impact of the increase in production due to technological improvements in both advanced (China, Brazil, Russia, USA) as well as developing countries (South Asia and Southern Africa) increasing the risks and uncertainties on global food security.

Plants exhibit two types of thermotolerance responses; basal and acquired. These responses are implemented by the production of molecular chaperones such as heat shock proteins (HSPs), antioxidants, accumulation, and adjustment of compatible solutes (Wahid et al. 2007). Based on these innate plant response mechanisms, various traditional and contemporary breeding strategies have been employed to increase plant thermotolerance or to develop transgenic plants but the success remains limited mainly because of the complexity of the trait, limitation of available genetic resources, phenotypic flexibility, and variability of assimilate partitioning under heat stress (Wahid 2007).

Beneficial microbes are a major constituent of the rhizosphere microbiome having a direct growth-stimulating impact on plants. These plant growth-promoting rhizobacteria (PGPR) directly assist plants by improving their vital nutrients (e.g., nitrogen, phosphorous, iron or zinc, etc.) and water intake by their inherent potential of nutrient fixation/solubilization or root proliferation due to the production of phytohormones (e.g., indole acetic acid (IAA), cytokinin, and gibberellin). Likewise, their antagonistic potential, exopolysaccharides, and ACC-Deaminase activities enable plants to tolerate a range of biotic and abiotic stresses (García et al. 2017), (Ahmad et al. 2022). The heat-resilient microbes exhibit above-mentioned plant-beneficial traits at elevated temperatures enabling plants to withstand heat stress with simultaneous increase in their growth (Kaushal and Wani 2016), (Elshafie et al. 2017). At present, microbial consortia based on beneficial bacteria are available in the market and applied widely in farmer fields (Imran et al. 2022). By contrast, climate-resilient especially heat-resilient formulations are very little known.

The present study aimed to design a heat-resilient microbial inoculum for inducing microbe-mediated heat stress tolerance primarily in maize and then other crops. We hypothesized that multi-stress tolerant PGPR will act smartly to protect maize plant against heat stress. The bacteria were isolated from the desert and tested in vitro and in vivo to evaluate their impact on early vegetative growth of maize under high temperatures. The current study indicates that a bacterial consortium B3P based on heat-resilient bacteria have the potential to stimulate early vegetative growth of maize and can be tested further as heat-resilient microbial inoculum in maize and other crops under field condition.

Results

Isolation of heat-tolerant bacteria

A large collection of different bacteria/morphotypes ($n=135$) were obtained from different soil and root samples during this study on both LB and NFM at different temperatures. Of a total of 135, four bacteria were selected that contained PGP traits (P, Zn solubilization, calcite degradation, IAA production, nitrogen fixation, and EPS production) as well as high-temperature tolerance. These four bacterial isolated *AH-08, AH-67, SH-16,* and *SH-29* were able to grow up to 50 ± 2 °C and showed plant growth-promoting traits up to 45 ± 2 °C.

Morphological and functional characters of bacteria

The morphological, biochemical, and physiological data of these strains and functional characterization i.e., IAA production, nitrogen fixation, phosphorus, zinc, and calcium solubilization are mentioned in Table 1 and Fig. 1. The data revealed that *B. subtilis* AH-08 showed a P-solubilization potential of 331.07 µg/L after 14dpi, while calcium-solubilization up to 45 °C, produced 50 ppm IAA, and fix nitrogen at 50 °C. The *B. cereus* AH-67 solubilized phosphorus 203.0 µg/L after 14dpi, while solubilized zinc up to 45 °C and produced 100 ppm IAA. The *B. badius* strain *SH-16* solubilized 331.0 µg/L phosphorus at 14dpi, and produced 75 ppm IAA up to 45 °C. The *Pseudomonas koreensis* SH-29 solubilized 140.8 µg/L P, after 14dpi, and fix nitrogen up to 45 °C.

Determination of total cell proteins under heat stress

The analysis of whole cell proteins under heat stress indicates that in bacterial strains many proteins were

Table 1 Detailed morphological, biochemical, and physiological characteristics of bacterial isolates obtained from Cholistan desert used in the present study

Characteristics	*B. subtilis* AH-08	*B. cereus* AH-67	*B. badius* SH-16	*P. koreensis* SH-29	Characteristics	*B. subtilis* AH-08	*B. cereus* AH-67	*B. badius* SH-16	*P. koreensis* SH-29
Isolation from/ medium	Soil/ L. B	Soil/ L. B	Soil/L. B	Soil/L. B	**KOH test**	-	-	-	-
Colony color / edges/elevation	Off-white/ undulate/flat elevation	Cottony White/ entire/flat	Pale-Yellow/ filamentous/flat	White/lobate/flat	**Catalase**	+	+	+	+
Colony morphology/size	Gummy, Wrinkled/ medium	Filamentous/ large	Round/ Large	Wrinkled/ medium	**Biofilm**	Strong	Intermediate	intermediate	Week
Cell shape/motility	Rod/ highly motile	Rod/ highly motile	Rod/ motile	Rod/ motile	**Temperature tolerance**	50 °C	50 °C	50 °C	50 °C
16S *rRNA* accession	MZ779051	MZ779053	ON340744	MZ779057	**Salt tolerance**	7.5%	2.5%	7.5%	7.5%
Gram staining	Positive	Positive	Positive	Negative	**Siderophore Production**	+	-	-	+
P-Solubilization (zone diameter mm)	+(5)	+(4)	+(3)	+(1.83)	**Zinc Solubilization**	-	+(2.21)	+	+(1.5)
Ca- Solubilization (zone diameter mm)	+	+(4.5)	+	-	**IAA Production (ppm)**	++(50)	++(100)	++(75)	+
Antibiotic Resistance									
Gentamycin (10 mg)	S	S	S	S	**Streptomycin (10 mg)**	R	R	R	R
Rifampicin (5 mg)	R	R	R	R	**Ciprofloxacin (5 mg)**	S	S	S	S
Nalidixic acid (30 mg)	MS	MS	MS	MS	**Kanamycin (30 mg)**	R	R	R	R
Ceftriaxone (30 mg)	S	S	S	S	**Neomycin (10 mg)**	S	S	S	S
Amikacin (30 mg)	R	R	R	R					
Carbon source utilization and enzymatic reaction									
ONPG	+	+	+	+	**SODIUM CITRATE**	+	-	-	+
SODIUM MALONATE	-	-	-	+	**LYSINE DECORBOXYLASE**	+	+	+	+
ARGININE DIHYDROLASE	-	-	-	-	**ORNITHINE DECORBOXYLASE**	-	-	-	-
H2S PRODUCTION	-	-	-	-	**UREA HYDROLYSIS**	+	-	+	+
TRYPTOPHANE DEAMINASE	-	-	-	+	**INDOLE**	-	-	-	-
ACETION	+	+	+	+	**GELATIN HYDROLYSIS**	+	+	+	+
ACID FROM GLUCOSE	+	+	+	+	**NO3/N2 PRODUCTION**	+	+	+	-/+
ACID FROM MALTOSE	+	+	+	+	**ACID FROM SUCROSE**	+	+	+	+
ACID FROM MANNITOL	+	-	+	+	**ACID FROM ARABINOSE**	+	+	+	+
ACID FROM RHAMNOSE	+	+	+	+	**ACID FROM SORBITOL**	+	-	+	+
ACID FROM INOSITOL	-	-	+	+	**ACID FROM ADONITOL**	-	-	-	-
ACID FROM MELIBIOSE	+	+	-	+	**ACID FROM RAFFINOSE**	-	-	+	-

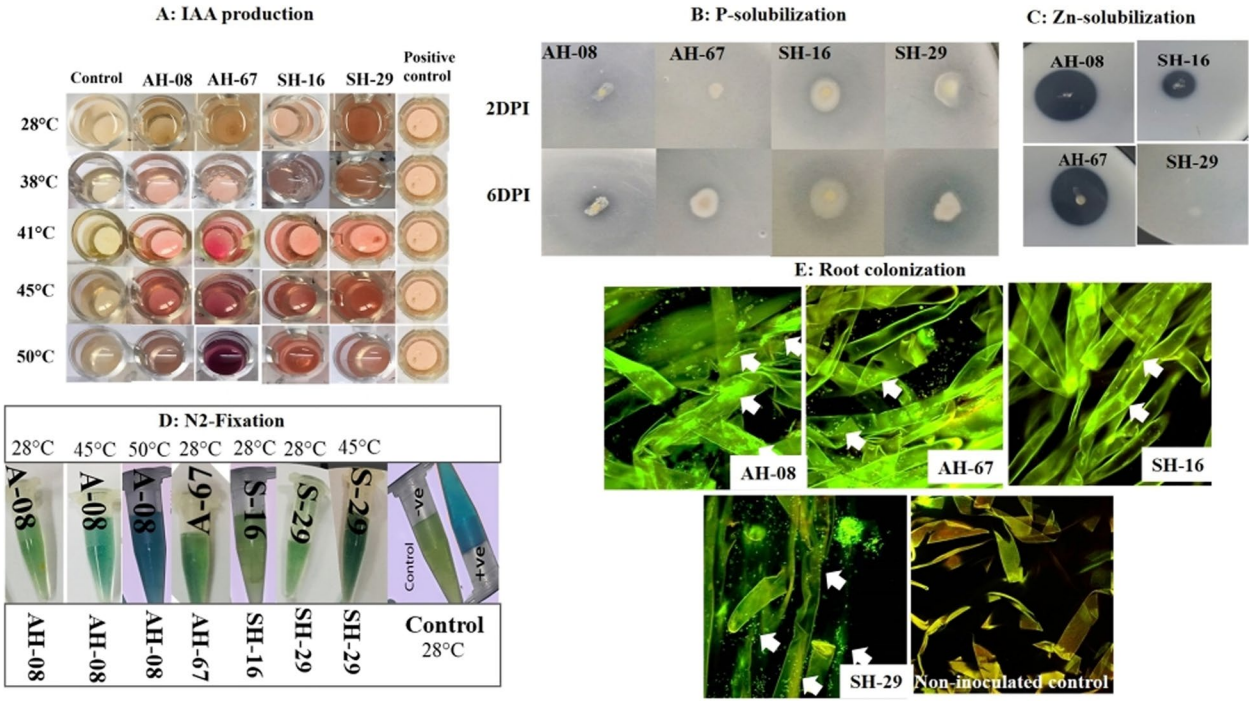

Fig. 1 In vitro PGP-activity assays for production of IAA (**A**), P-solubilization (**B**), Zn-solubilization (**C**), Nitrogen fixation (**D**), and root colonization (**E**) by heat tolerant bacteria obtained from Cholistan desert

unregulated at higher temperatures (Fig. 2). The heat shock protein upregulated includes 100, 90, 75, 70, 40, 45, and 18 kDa. The proteins from strain SH-29 could not be resolved using the same method used for others, hence needs further optimization of the extraction procedure which will be done in later experiments.

Inoculation effect on early growth of maize under induced heat stress

The data from the pot experiment collected for different plant-growth traits showed significant differences with B3P-treated under heat stress compared to non-treated plants both in heat-tolerant and heat-sensitive maize varieties.

Shoot and root length

Data shows that the shoot length of B3P-treated plants was generally higher in all maize varieties compared to their respective non-inoculated plants under heat stress (Fig. 3 A). Plant height was maximum under stress in the B3P-treated Malka variety which is a heat-sensitive variety. A maximum percent increase in shoot length was observed in FH-1046 (63%) followed by Malka (25.66%). Data regarding root length also showed a strong positive impact of B3P treatment on all maize varieties under heat stress (Fig. 3B). A maximum percent increase in root length was observed in treated plants of varieties Gohar and FH-1046 (89%) followed by Sahiwal Gold (51%).

Shoot and root weight

Plant fresh weight (both shoot and root; Fig. 3 C, D) was generally higher in all B3P-treated varieties with the highest fresh weight in Malka showing a 73% increase over respective non-inoculated control. While maximum percentage increase (treatment effectiveness) was observed in variety FH-1046 which exhibited an 84% increase in shoot fresh weight after inoculation. For root fresh weight, B3P treatment was most effective in the Sahiwal Gold variety showing an increase of 88% over control. Both shoot and root dry weights (Fig. 3E, F) followed almost the similar trend as mentioned for fresh weight. The maximum percentage increase (over control) in shoot dry weight was 92% in Sahiwal Gold while in root dry weight was 109% in YH-1745.

Leaf and root area

The data show that B3P inoculation significantly increased leaf length, leaf area, and root area in all

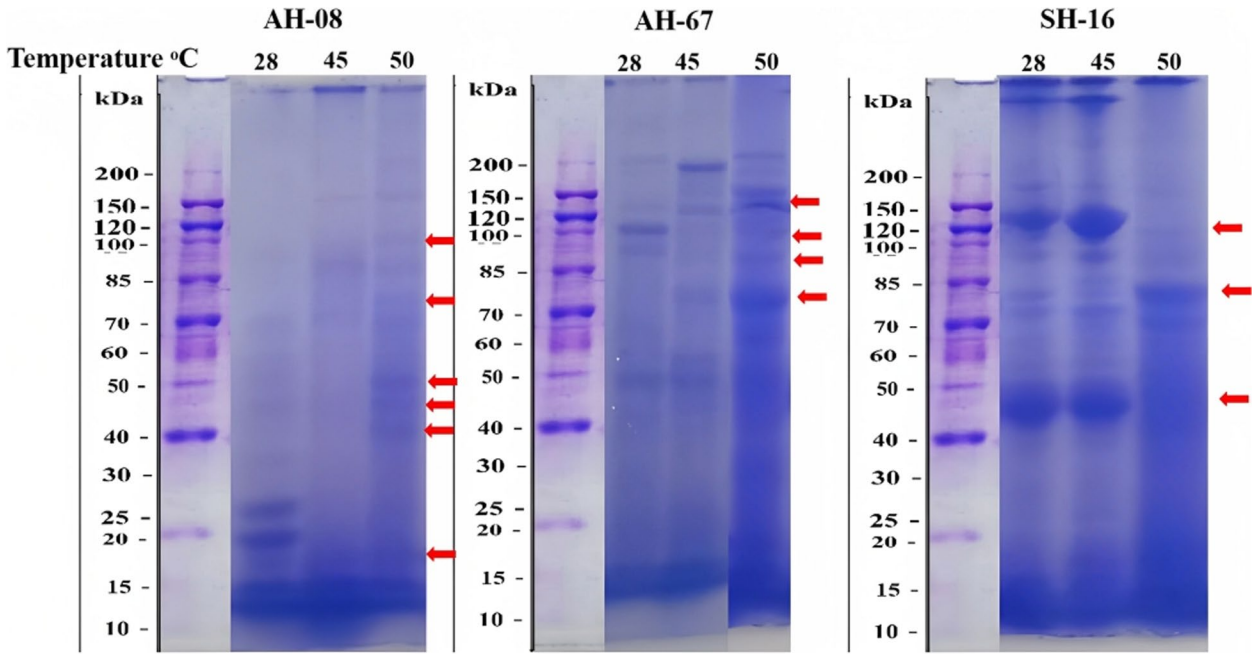

Fig. 2 Whole-cell protein profiling on SDS-PAGE of heat tolerant microbes grown at different temperature stress levels

varieties (Fig. 4). Leaf area and length were maximum in the Gohar variety showing an increase of 95% and 62% over respective non-inoculated control (Fig. 4A). The leaf area of B3P-treated varieties of Sahiwal Gold showed a non-significant response compared to the non-treated control (Fig. 4A). The minimum increase in flag leaf length was observed in Sahiwal Gold after inoculation with B3P under heat stress (Fig. 4B). Leaf chlorophyll contents (measured through SPAD) and chlorophyll a/b were generally higher in B3P-treated leaves compared to non-treated leaves in all varieties (Fig. 4C, D). A significant increase in chlorophyll contents (especially chl b) was observed for B3P-treated Gohar and Sahiwal Gold varieties under stress conditions (Fig. 4D). The data further revealed that B3P-treated plants had significantly well-developed roots (Fig. 4E) compared to non-treated control plants. A maximum increase of 100% was observed in Gohar followed by FH-1046 (42%). Maize variety Malka, however, exhibited a non-significant response of inoculation on root area parameters.

Analysis of gene expression under heat shock

Quantitative PCR analysis was performed on RNA extracted from B3P-treated and non-treated plants after 48 h of heat stress induction to see the expression of genes related to heat shock proteins. The qPCR data related to four genes (*HSP1, HSP18, HSP70*, *HSP101*) show a differential response of varieties between B3P-treated and non-treated plants. In the case of *HSP1*, all B3P-treated plants showed higher expression levels as compared to non-treated ones, but Gohar showed the highest expression of *HSP1* whereas, FH-10446 showed a maximum percentage increase over non-treated control (Fig. 5A). Similarly, B3P treatment increased the expression of *HSP18* in all varieties after heat stress compared with non-treated plants, where maximum expression was observed in variety Malka followed by YH-5427 (Fig. 5B). A similar expression response was observed for *HSP70*, where mostly B3P-treated plants showed increased expression compared to non-treated plants (Fig. 5C). Maximum expression was observed in Malka, followed by YH-5427 and FH-1046. In contrast, the highest expression of HSP101 was observed in FH-1046 treated with B3P, followed by Gohar and Sahiwal Gold, whereas other varieties exhibited comparatively lower levels of expression (Fig. 5D).

Antioxidant enzyme activities, total proteins, and amino acids under heat stress

Plant enzyme activities were analyzed 24 and 48 h after HS-2. In general, the activities of antioxidant enzymes CAT and POD increased while MDA increased after B3P-inoculation under stress. Malonaldehyde

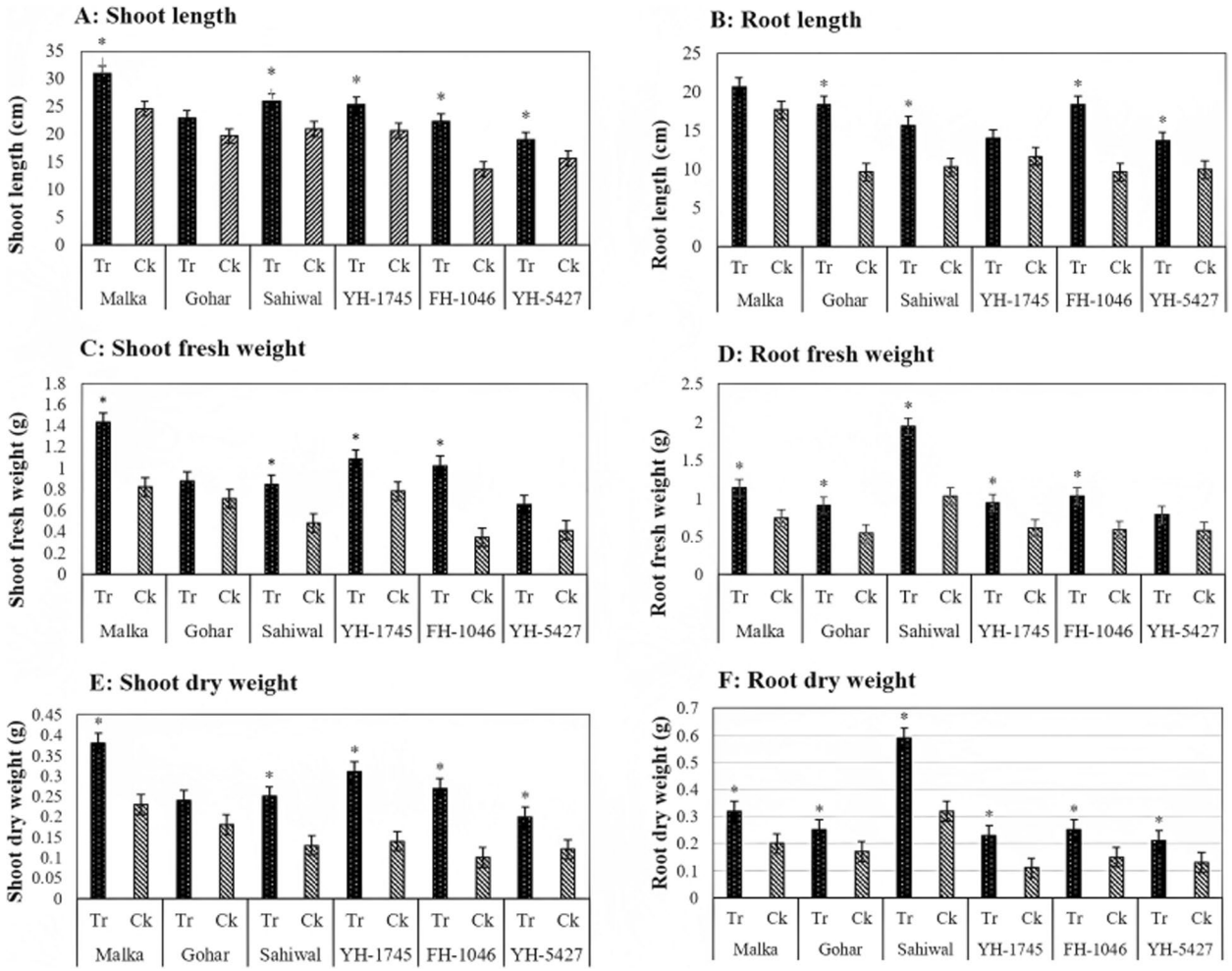

Fig. 3 Graphical representation of above ground and below ground plant parameters showing the effect of B3P inoculation on shoot length (**A**), root length (**B**), shoot fresh weight (**C**), root fresh weight (**D**), shoot dry weight (**E**), root dry weight (F) of hybrid maize varieties grown under heat stress. *The data presented is mean of 3 independent replicates. The bars represent the standard deviation. The data was subjected to ANOVA. The* ***** *on the bar indicates that treatment mean different significantly at P < 0.05 according to LSD*

activities at 24HPI (24 h post induction of HS-2) and 48HPI were significantly reduced in B3P-treated plants as compared to non-treated plants. MDA activity was reduced by up to 42% in the YH-5427 variety after B3P treatment followed by a significant reduction in varieties Gohar, Malka, and YH-1745. The highest MDA activity was observed in non-treated plants of variety YH-175 (Fig. 6A).

Peroxidase activity was lower in all varieties during early hours after stress i.e., 24HPI but increased significantly as time passed (Fig. 6 B) in most of the plants (both treated and non-treated). A maximum increase (635%) in POD activity was observed in B3P-treated plants of variety FH1046, followed by YH-5427 (96%). POD activity in varieties Malk and Gohar also increased over respective non-inoculated controls, but the increase was statistically non-significant.

Catalase activity (CAT) started during the early hours of stress in all varieties except FH-1046 (Fig. 6C). The enzyme activity increased significantly afterward (both treated and non-treated). Maximum CAT activity was observed in B3P-treated variety YH-1745 with an increase of 635%, followed by FH-1046 (96%). CAT activity in varieties Malk and Gohar also increased over respective non-inoculated controls, but the increase was statistically non-significant.

Total cell proteins at 24HPI were initially higher in all B3P-treated plants, whereas YH-1745, Sahiwal Gold, and Malka varieties showed higher total cell proteins (Fig. 6D). Protein concentration increased gradually

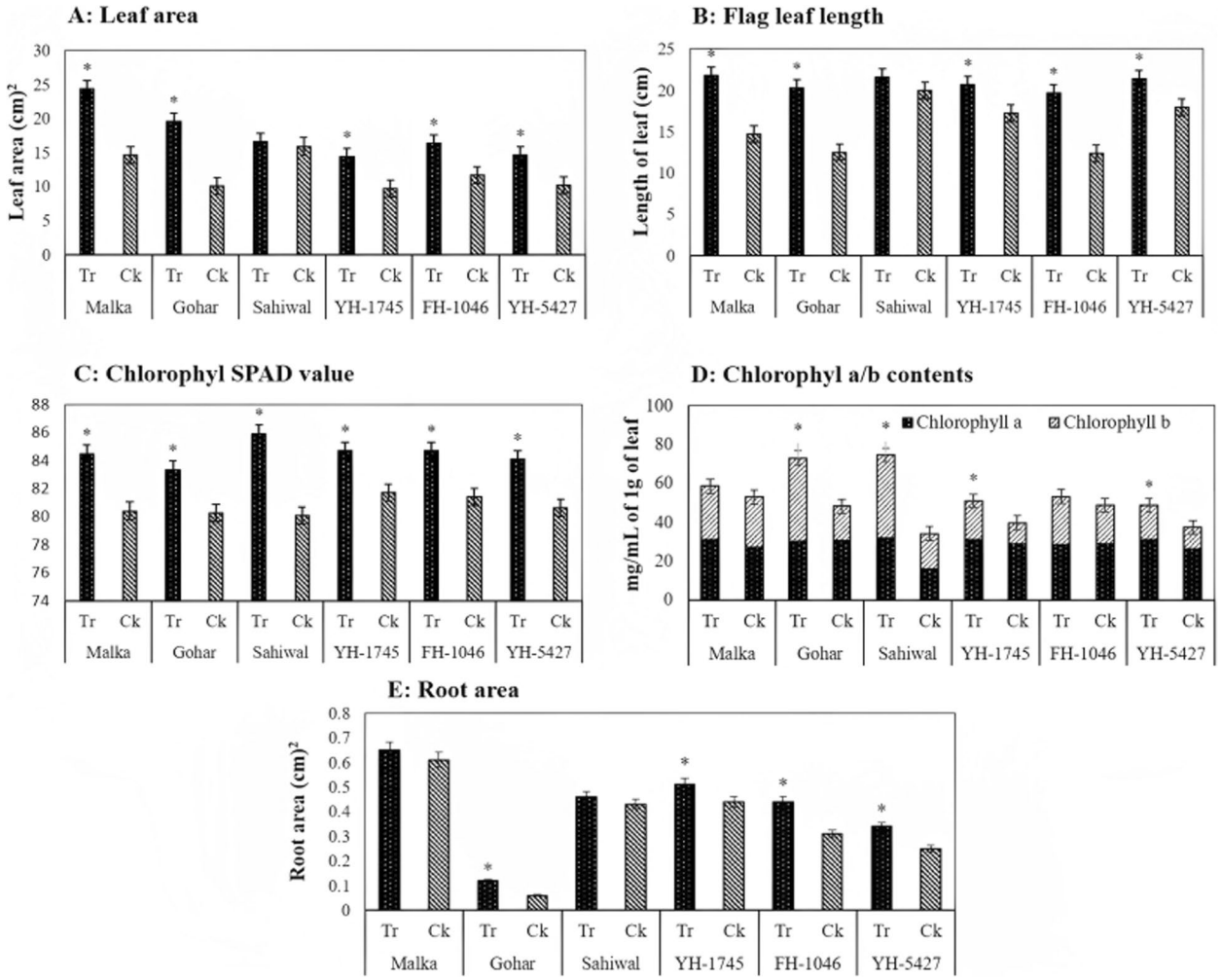

Fig. 4 Graphical representation of leaf parameters showing the effect of B3P inoculation on leaf area (**A**), flag leaf length (**B**), chlorophyl SPAD value (**C**), chlorophyl a/b contents (**D**), root area (**E**) of different maize varieties grown under heat stress. *The data presented is mean of 3 independent replicates. The bars represent the standard deviation. The data was subjected to ANOVA. The* ***** *on the bar indicates that treatment mean different significantly at P < 0.05 according to LSD*

with time after heat stress and significantly in all B3P-treated varieties compared to respective non-treated control plants. Comparatively, protein contents were higher for Gohar, Sahiwal Gold, YH-1745, FH-1046, and YH-5247.

Total amino acids of plants showed a non-significant response with or without B3P treatment after the heat stress (Fig. 6E). During initial hours (24HPI), the amino acids were maximum in YH-1745 which remained so after 48HPI.

Regression, interaction, and correlation analysis

The analysis of correlation (Supplementary Fig. 3) revealed a positive linear relationship between different growth parameters, gene expression, and enzyme activities ($r = 0.1$—0.98^{**}). A higher correlation coefficient ratio (r-value) was observed for SL: SFW ($r = 0.90$), and RFW: RDW ($r = 0.98$). Correlation analysis further revealed that enzyme and gene expression has also a significant positive correlation ($r > 0.5$) with growth parameters e.g., SL with CAT, HSP18, HSP70 ($r = 0.5$—0.6), RL with CAT, HSP18, HSP70 ($r = 0.52$—0.69), SFW/SDW with CAT, HSP18, HSP70 ($r = 0.57$—0.7), LA with HSP18, HSP70 ($r = 0.67$, 0.70), POD with HSP1 ($r = 0.75$), MDA: RA ($r = 0.52$), HSP1 with POD, CAT, HSP101 ($r = 0.64$–0.86).

Linear regression effectively modeled the positive relationship of morphological parameters with gene expression and enzyme activity, accounting for 70% of the total variance. A positive linear regression

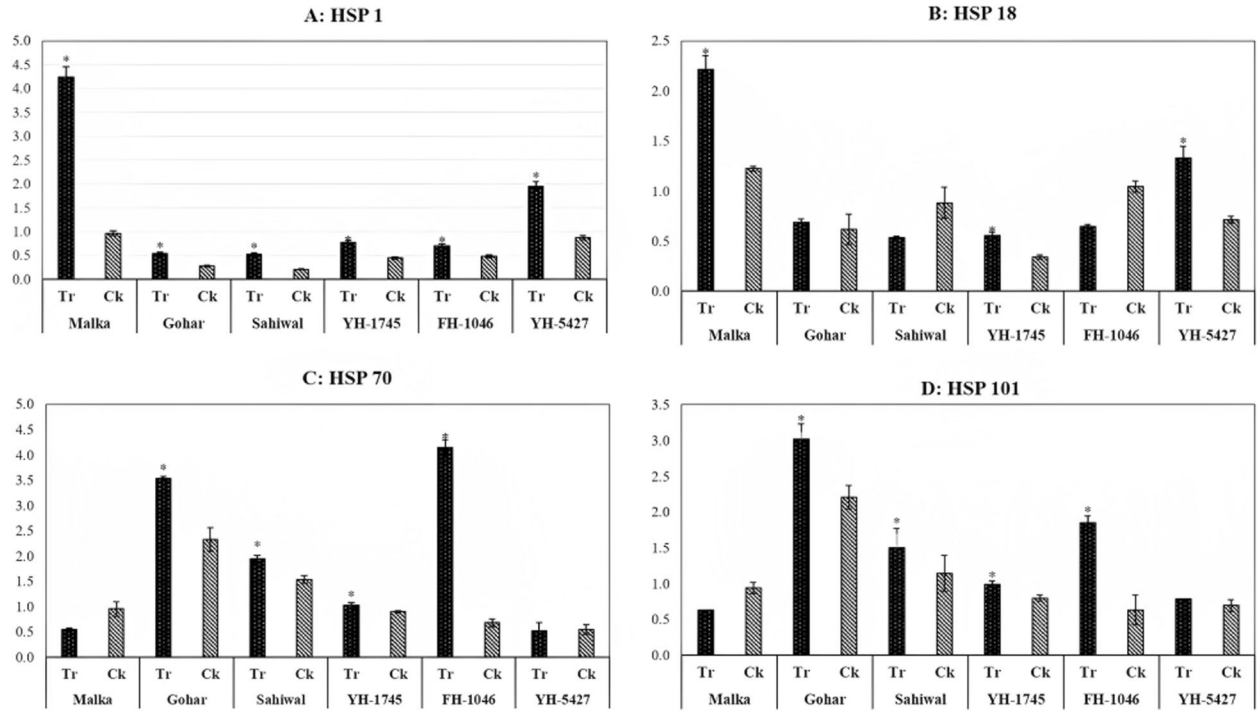

Fig. 5 Effect of B3P inoculation on expression of different heat shock proteins; HSP1 (**A**), HSP18 (**B**) HSP70 (**C**) and HSP101 (**D**) post induction of heat stress in hybrid maize varieties. *The data presented is mean of 3 independent replicates. The data was normalized with the actin gene and then subjected to ANOVA. The* ***** *on the bar indicates that treatment mean different significantly at P < 0.05 according to LSD*

was observed for dependent variables i.e., CAT ($R^2 = 0.874$), POD ($R^2 = 0.822$), MDA ($R^2 = 0.990$), total proteins ($R^2 = 1.00$), expression of HSP1 ($R^2 = 0.835$), expression of HSP18 ($R^2 = 0.762$), expression of HSP70 ($R^2 = 0.868$), expression of HSP101 ($R^2 = 0.880$) with SFW/SDW, RFW/RDW, FLL, LA, RA, and chlorophyll contents as independent variables. The PCA (Fig. 7) captured more than 89% of the variance and demonstrated the key varietal differences in the treatment response. The effect of bacterial inoculation under heat stress was more pronounced in different varieties as treated plants loaded positively on PC1 than non-treated plants.

Discussion

The current research describes the PGPR traits of bacteria native to the Desert; their heat resilience and investigates their likely role in inducing heat tolerance in maize during the early vegetative growth stage. Maize is more sensitive to heat stress than wheat and rice (Zhao et al. 2017), (Zhang et al. 2019) where for each 1°C increase in global temperature a yield penalty of 10% has been simulated (Dong et al. 2021). Each decade is becoming hotter than the last while an increase of 1.14 °C (above 1850–1900 industrial average) in global mean temperature has been recorded until 2022. Climate-smart agricultural strategies promote the adoption of high-yielding crop varieties that are resilient to climate conditions, resulting in reduced emissions and losses. Native plant-associated microbes, which naturally exist in stressed environments, assist plants in coping with stress by utilizing a diverse range of enzymes and metabolites, while also harnessing their inherent potential to promote plant growth (Niu et al. 2008). The present study lays the foundation to exploit heat-tolerant PGPR for stimulation of plant growth under heat stress with minimum impact on the yield.

Heat-tolerant PGPR were isolated from the Cholistan desert and surrounding areas (latitude: 28°46′N-29°16′N, longitude: 69°52′E- 71°29′E, altitude: 112 m). The climate of this desert is sub-tropical, arid, semi-arid, and scorching harsh, with low monsoon rainfall which increases the soil and environment temperature. The summer temperature ranges from 46 to 51 °C during the months of drought while the winter temperature varies from 0 to 1 °C. The soil is non-saline, low in organic matter with alkaline pH (Zia et al. 2021). A total of 130 distinct morphotypes (bacteria) were obtained from these samples while only 30 were able to grow at 50 ± 2 °C and contained PGP traits. The strains were

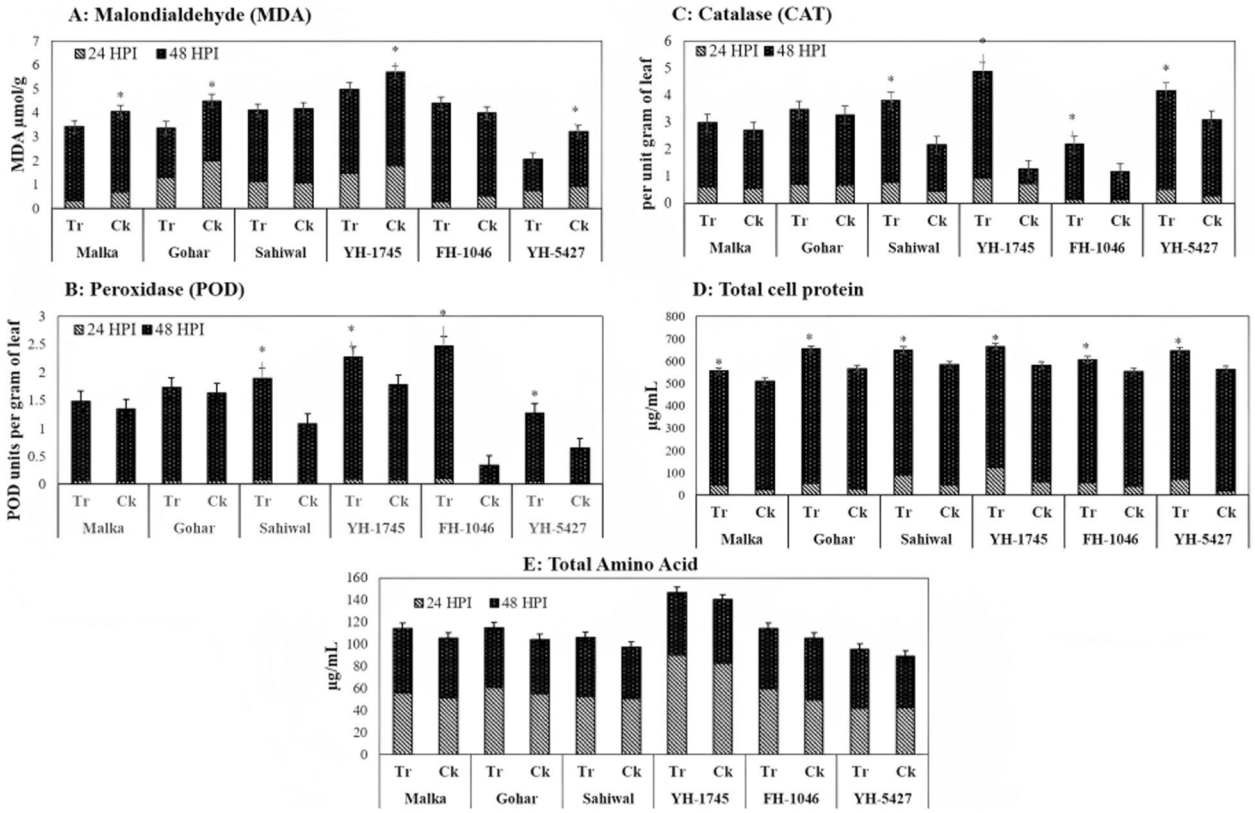

Fig. 6 Effect of B3P inoculation on enzyme activities post induction of heat stress in hybrid maize varieties; Malondialdehyde (**A**), peroxidase (**B**) catalase (**C**), total cell proteins (**D**) and amino acids (**E**). *The data presented is mean of 3 independent replicates. The* ***** *on the bar indicates that treatment mean different significantly at P < 0.05 according to LSD*

mostly *Bacillus* and *Pseudomonas* spp. These bacteria also showed differential responses to tolerate different levels of NaCl salt (2.5, 5, 7.5%) at a range of different temperatures ranging from 28 ± 2 °C to 50 ± 2 °C. Rhizospheric bacteria from high-temperature soil exhibiting PGP traits and desert PGPR (mostly *Bacillus* spp.) have been reported for PEG tolerance of up to 13%, NaCl tolerance of up to 15%, and temperature tolerance up to 70 °C (Shekhawat et al. 2021).

The isolates have shown catalase activity which shows their ability to tolerate ROS produced in response to stress. Catalase enzyme facilitates cellular detoxification and plays a key role in the defense against oxidative stress in bacteria by catalyzing the decomposition of H_2O_2. Extracellular catalase (microbes on the roots) in the vicinity of the roots may indirectly benefit the plants under stress by detoxifying ROS species produced in root cells. (Mhamdi et al. 2010) has comprhensively reviewed the role of catalases under stress and non-stressed conditions and shown that several catalases are expressed in different plant compartments e.g., leaves, roots, seeds, or pollens suggesting an important role in plant physiology. Bacterial colonization and biofilm formation are main traits for developing interaction with plants. Confocal laser scanning microscopy results showed that AH-08 had a good root colonization ability as compared to AH-67 and SH-16. This root colonization helps to interact with the host as described in earlier studies for *Bacillus subtilis* sp. under stress conditions (Beauregard et al. 2013). Along with the colonization, biofilm formation and utilization of diverse carbon sources enable them competent in the rhizosphere environment. Under heat stress, plants experience significant alterations in carbon and sugar contents, leading to cellular damage. However, these bacterial strains possess the capability to utilize a wide range of carbon sources, enabling their survival in diverse forms of soil carbon during heat-stress conditions as reported earlier in a previous study by Eida et al. (Eida et al. 2018).

Several plant hormones are involved in signaling metabolic regulation during heat stress. Bacteria also produce phytohormones such as auxins, cytokinins, gibberellins, and abscisic acid in the vicinity of plant roots that

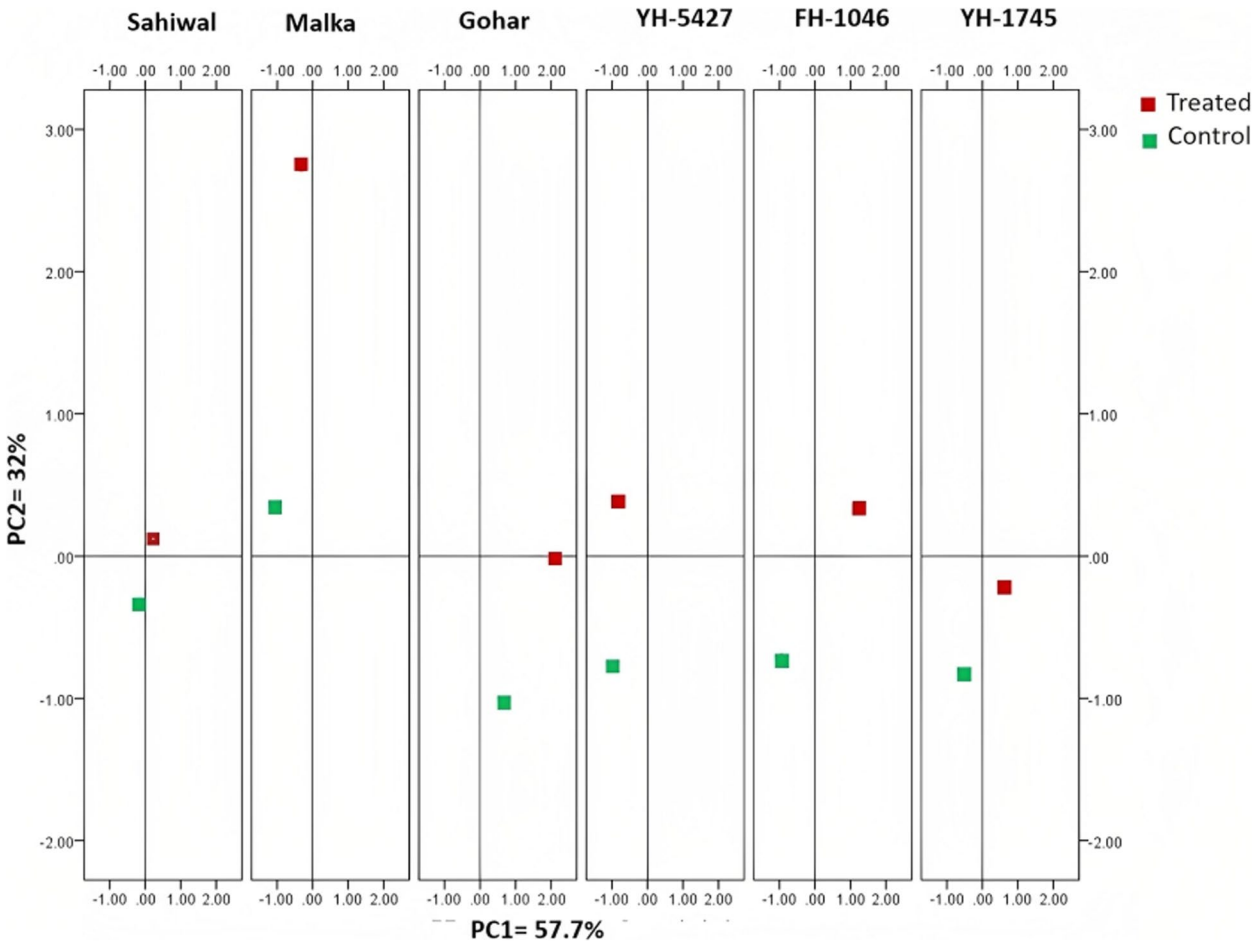

Fig. 7 Principal component analysis (PCA) sowing the response of hybrid maize varieties after heat stress towards B3P-inoculation (Total variance explained = 89.7%)

directly regulate plant growth similar to the plant's hormones. The AH-08, SH-16, and SH-29 produced IAA up to 45 °C while AH-67 produced IAA at 50 °C. IAA-producing rhizospheric bacteria play an important role in plant growth, especially under heat stress. Shekhawat et al. (Shekhawat et al. 2021) reported that bacteria produce IAA at 28 ± 2 °C and 50 ± 2 °C but Kachhap et al. (Kachhap et al. 2015) reported a reduction of 50% in IAA production by *Pseudomonas* and 16% reduction by *Enterobacter* spp. at high temperatures. It is noteworthy that the strains used in the current study did not exhibit such reductions in IAA production.

Nitrogen (N), Phosphorous (P), Calcium (Ca), and Zinc (Zn) are the essential macro and micronutrients required for plant growth and play important role in improving soil fertility. The bacteria are able to fix nitrogen and solubilize P, Zn, or Ca at higher temperatures i.e., 45 and 50 ± 2 °C thus increase nutrient availability and stimulate nutrient uptake under stressed conditions. It has been reported earlier that PGPR solubilize phosphorus (Kachhap et al. 2015) and fix nitrogen (Hungria and Franco 1993) at higher temperatures i.e., 45 °C.

Heat shock proteins (HSPs) are molecular chaperones that regulate the folding, localization, accumulation and degradation of protein molecules in the plant species and impart a generalized role in the tolerance to various stresses. Correspondingly, bacterial HSPs act to counteract and maintain metabolic activity/cellular processes under different environmental conditions and stresses. It is also reported that PGPR inoculation may modulate the induction of the HSPs in plant and protect the integrity and normal functioning of the cellular proteins. The present study demonstrates a differential expression of small and large heat shock protein and genes in heat-tolerant PGPR at different temperatures. The expression of 60–65 kDa and 10–30 kDa proteins was observed in the PGPR at 45 and 50 °C which were not expressed in control cultures grown at 28 °C. These are small heat shock proteins which are expressed in different *Bacillus* spp. and *Pseudomonas* spp. exposed to high temperatures

(Richter and Hecker 1986), (Reischl et al. 2001). qPCR data showed that B3P inoculation enhanced the expression of *HSP1* and *HSP18* in Malka and YH-5427, while the expression of *HSP70* and *HSP101* was higher in FH-1046 and Gohar, suggesting that heat resilient PGPR induced expression of different proteins in host plants which help plants to survive under heat stress. This data further suggests that heat-tolerance mechanism of different hybrid varieties varies with same inoculation. In some, it is carried out by the upregulation of *HSP70* and *HSP101* while in others, it is exerted by the upregulation of *HSP1* and *HSP18* genes. Although, all HSPs play a key role in anti-stress processes in plants.

The present study showed significant improvement in the morphological and biochemical parameters of maize under stress. The plant height, root & shoot fresh & dry weight, root, and leaf area, root length, and plant biomass of treated plants under both normal and high-temperature condition was higher generally compared to non-treated plants. Further, B3P-treated plants showed a higher level of CAT, POD, and high levels of chlorophyll a, b, proteins, and amino acids while the concentration of MDA was low in B3P-treated plants as compared to the control. The higher expression of ROS-scavenging systems i.e., CAT (Catalase), POD (peroxidase), and SOD (Superoxidase dismutase) are known to reduce the impact of ROS on plants under stress (Chen et al. 2008). Co-inoculation of lettuce with the PGPR strain *Pseudomonas* sp. and an arbuscular mycorrhiza significantly enhanced the POD, and CAT in the leaves under moderate and severe abiotic stress (Kohler et al. 2008), where the stress decreased total chlorophyll, cell protein, and amino acid and increases the MDA concentration which is mainly released after cell rupturing. Thermotolerant *Pseudomonas putida* strain *AKMP7* inoculated to wheat plants have shown an increase in plant biomass and dry weight, root and shoot length, number of tillers and spikelet, and grain formation. The inoculated bacteria also prevented the plant from cellular injury, enhanced the antioxidant enzymatic activities (SOD, APX, CAT), and increased the level of protein, proline contents, cellular sugar, amino acid, and starch, and the efficiency of chlorophyll under heat stress condition. *AKMP7* formed biofilm on plant roots which helps plants to combat adverse heat stress conditions (Ali et al. 2011). *Bacillus* spp. and *Pseudomonas* spp. from roots of desert cacti were reported to significantly enhance leaf surface area, stalk length, and fresh and dry biomass in *Z. mays* under heat stress (Kavamura et al. 2013). It has also been reported that heat-tolerant endophyte SA187 increases heat tolerance in *Arabidopsis* and wheat under control and field condition and enhance their morphological parameter (Shekhawat et al. 2021). Heat-resilient *Bacillus cereus* inoculated tomatoes have previously shown increased chlorophyll a and b, relative water contents in plants under heat stress as compared with control (Mukhtar et al. 2020). A previous study reported that PGPR enhanced the level of *CAPx*, *rbcL*, and *rbcS* in wheat plants under water deficit conditions (Zia et al. 2021). The current study also shows that photosynthetic pigments are higher in B3P-treated plants as compared to control after heat stress.

Conclusions

This study provides an insight into heat-resilient bacteria with distinctive plant-growth-promoting traits. Increasing temperature is the main climate stress, the plants are facing today due to climate change, and it is expected to increase further in the coming decades. The present study describes the bacteria that have multiple mechanisms to cope with the heat and induce plant-heat tolerance using a combination of these mechanisms e.g., expression of heat shock genes/proteins, and modulation of enzymatic activity of stress-related enzymes. The bacteria described in this study are mostly bacilli strains that are more tolerant and resistant to multiple stresses e.g., salt, water, etc. We anticipate that amelioration of heat-stress tolerance will improve plant growth not only during the early vegetative stage but plant growth and yield during later stages as well. The same may be validated in future experiments on the same or other crops.

Methods

Site description, sampling, bacterial isolation, and screening for heat tolerance

The rhizosphere soil and plant roots samples were collected from the Cholistan desert (28.5°N 70°E) and surrounding areas of Ahmadpur (29.14' °N 71.25°E) and Bahawalpur 29.35 °N 71.69 °E (Supplementary Fig. 1). The average soil and environmental temperature of the Cholistan desert are high (32 to 50 °C) with low rainfall (100 mm), alkaline sandy soil with high soil pH (7.5–8.6), and high electric conductivity(EC) supplemented with low organic matters and low soil moisture (Zia et al. 2021).

Bacteria were isolated by enrichment technique from rhizospheric roots of different weeds (tumbleweed, desert broom) and plants (cotton, maize, sorghum, pearl millet). One gram root (rinsed in distilled water) was macerated in 1 mL saline (0.8% NaCl) and 100 mL of macerate was inoculated separately to test tubes containing 5 mL LB broth and Nitrogen free malate (NFM) media (Okon and Kapulnik 1986), respectively, in four replicates per root sample. One set of replicate tubes was incubated at 28 °C while 2nd set was incubated at 38 °C, 3rd set at 45 °C and 4th set at 50 ± 2 °C. From soil samples, 1 g each

was serially diluted in saline as described (Somasegaran and Hoben 1994), and 10^{-4}—10^{-6} dilution was spread on 4-replicate LB agar plates and incubated at different temperatures as was done for roots. The tubes and plates were incubated until the appearance of visible growth in the tubes and colonies on the plates. The cultures from tubes were streaked onto LB/NMF plates for obtaining the pure colonies. Different morphotypes from each root/soil sample were selected and purified by repeated sub-culturing at respective media and temperatures. The bacteria were validated for tolerance by growing them in LB broth with continuous shaking for 3 to 4 days at 28 °C, 38 °C, 41 °C, 43 °C, 45 °C, 48 °C, and 50 ± 2 °C. The temperature tolerance/survival rate was analyzed by taking optical density at 600 nm on a spectrophotometer.

Identification of bacteria and functional characterization

The growth characteristics and colony morphologies were determined as per standard microbial procedure along with the cell size, shape, and mobility. Gram staining was performed as described (Vincent and Humphrey 1970). The catalase activity was observed by putting one drop of 5% H_2O_2 on a glass slide containing a bacterial colony and observing bubble formation within 10–15 s which indicated catalase-positive for catalase enzyme. A potassium hydroxide (KOH) test was used to identify the bacteria following (Halebian et al. 1981). A drop of KOH (3%) was dropped on a glass slide and with the help of a 2 mm loop, the bacterial colony was agitated on the slide in a circular motion, the bacterial colony became mucoid and viscous within 20 s and stuck to the loop and move upward which was an indication of gram-negative bacteria. The DNA was extracted from bacterial isolates by using CTAB methods (Gomes et al. 2000). The genomic DNA was used to amplify the 1500 bp 16S rRNA gene for taxonomic identification by using universal primers P1/P6. The sequencing was done commercially from Macrogen and analysis was done by using Mega 10X and the trimmed sequence was submitted to NCBI. The different carbon source utilization and enzymatic reaction were performed through the QTS-24 kit (DESTO, Karachi) according to the manufacturer protocol as described (Zia et al. 2021).

The production of indole-3-acetic acid (IAA) was checked using colorimetric methods with some in LB broth containing tryptophan (0.1%) (Ehmann 1977b) for 3 to 5 days incubated at 28 ± 2 °C, 38 ± 2 °C, 45 ± 2 °C, and 50 ± 2 °C. After incubation, the culture was centrifuged at 10,000 rpm and the supernatant was mixed with Salkowski reagent (1:1) as described by (Gordon and Weber 1951). The 100 ppm IAA standard was used as positive control while water was as a negative control. The plate was incubated for 30 min at 28 ± 2 °C. The results were observed by the development of color from pink to purple and purple to purplish-pink which was an indicator of IAA presence.

The nutrient solubilization was checked at different temperatures. Zinc and Phosphorus solubilization were checked by inoculation of a single bacterial colony on LGI medium containing zinc oxide (Bunt and Rovira 1955), and Pikoviskaya's media containing tri-calcium phosphate (Pikovskaya 1948) and calcite media contained calcium carbonate ($CaCo_3$) as an insoluble calcium source, respectively (Nautiyal et al. 2000). The plates were incubated at 28 ± 2 °C, 38 ± 2 °C, 45 ± 2 °C, and 50 ± 2 °C for 4 to 6 days. The development of the halo zone was considered positive for respective nutrient solubilization and the solubilization index was calculated (Pathak et al. 2017).

For salt tolerance, the bacteria were grown on LB media supplemented with 2.5%, 5%, 7.5%, and 10% NaCl and were incubated at 28 ± 2 °C, 38 ± 2 °C, 45 ± 2 °C, and 50 ± 2 °C. The ability of bacteria to fix atmospheric nitrogen was screened on a semi-solid Nitrogen free malate medium (NFM) as described (Baldani et al. 2014). The bacterial strains were grown on L.B media for 24 h at 30 °C. After incubation, 20 µL culture was transferred into an Eppendorf tube containing 1 mL NFM medium and was incubated at 28 ± 2 °C, 38 ± 2 °C, 45 ± 2 °C, and 50 ± 2 °C for 5 to 10 days. The change of color from green to blue and the development of a pellicle-like structure were considered an indication of the fixation of the nitrogen potential of the bacterium (Baldani et al. 2014).

Biofilm formation ability was tested by the crystal violet method at different temperatures (28–45 °C) (Pratt and Kolter 1999). The bacteria were grown in 3 mL L.B broth for 4 days until biofilm developed. After the formation of the biofilm, the culture was decanted carefully. The attached biofilm was washed with 4 ml distilled water and stained with 0.1% crystal violet and incubated at room temperature for 15 min. After incubation, the crystal violet solution was drained out and the biofilm was washed with 4 ml of 95% ethanol. The biofilm dissolved in distilled water. The final optical density was checked through a spectrophotometer at OD_{590nm}. Biofilm quantification was done by using the Stepanovic method (Stepanović et al. 2000). Three standard deviations above the mean OD_{590nm} of negative control were defined as the cut-off value (ODC).

The bacteria were cultured in a flask containing L.B broth for 2–4 days with continuous shaking at 38 ± 2°C. One mL of bacterial culture was spread onto solid ASS agar (Antibiotic sensitivity sulphonamide agar; Merck, Germany) plates [40 g/L, pH 7.4] with the help of a sterilized cotton swab until the culture is completely absorbed into the agar surface. The intrinsic antibiotic resistance

pattern was determined by the disc diffusion method as described by (Sarker et al. 2014) using ready-to-use antibiotic discs (Bioanalyse®, Turkey). Antibiotic discs used were ampicillin AM (10 µg), chloramphenicol C (30 µg), aztreonam ATM (30 µg), gentamycin CN (10 µg), rifampicin RA (5 µg), cefixime CFM (5 µg), amikacin AK (30 µg), ciprofloxacin CIP (5 µg), tetracycline TE (30 µg), nalidixic acid NA (30 µg), ofloxacin OFX (5 µg) and erythromycin E (15 µg). Antibiogram was observed after 24–48 h of incubation at 38 ± 2°C and analyzed using the standard chart (Sarker et al. 2014).

Determination of bacterial total cell protein

Total bacterial soluble protein was determined by using Laemmli buffer (He 2011). The bacterial culture was incubated at 28,45, and 50 °C for 15 days. The bacterial culture was centrifuged and dissolved in 200 µL Laemmli buffer and 1 mL autoclaved distilled water and then incubated at 95 °C for five minutes. The suspension was cooled down to ice and centrifuged at 10,000 × g for 10 min at 4 °C on centurion K2015R refrigerated microcentrifuge. The supernatant containing cell protein was mixed with SDS loading dye and loaded on SDS PAGE gel with a 100 kDa ladder to analyze the total cell proteins.

Plant inoculation experiment

Based on the temperature tolerance and PGP trait (P, Zn solubilization, calcite degradation, nitrogen fixation, and EPS production) at high temperatures, and elsewhere: 4 bacteria were selected for plant inoculation. Potential best strains from the collection of heat-resilient PGPR were selected for inoculation response on maize (Malka-16, Sahiwal Gold, Gohar-16, FH-1046, YH-5427, FH-1745) under high temperature at the controlled condition.

Effect of B3P treatment on plant growth under induced heat stress

A total of six maize varieties including two moderately heat-tolerant (YH-5427, FH-1046) and four heat-sensitive (Malka, Sahiwal Gold, Gohar, FH-1745) obtained from Ayyub Agriculture Research Institute (AARI), Faisalabad, Punjab, Pakistan was used in these experiments. These varieties were selected based on their performance in the AARI field under natural heat stress during previous years (Supplementary Fig. 2). Seeds were washed in 2% (v/v) sodium hypochlorite with shaking for 2 min, then it was washed with autoclaved distilled water 3–4 times. After surface sterilization seeds were coated/pelleted with filter mud containing the inoculum as described by Imran et al. (2015).

Pots were filled with 100 g autoclaved peat moss and properly labeled. Coated seeds were sown in the pot (3 seeds/pot). The seeds were germinated at 25–28 ± 2 °C at 50—60% humidity. After nine days of seed germination, the pots were placed at 38 ± 2 °C for 3 h for induction of heat stress (HS-1) to seedlings. After 3 h incubation, the pots were replaced at 25–28 ± 2 °C at 50—60% humidity for the recovery period of two days. After two days, the pots were incubated at 44 ± 2 °C for 48 h for 2nd stress (HS-2). And then shifted back to 25–28 ± 2 °C. On day 14 of seed germination, the flag leaves were sampled, placed on white paper, and photographed with a digital camera. Photographs of leaves were further processed in the ImageJ software to measure the leaf area as described by (Abràmoff et al. 2004). The leaves were collected for enzyme and gene expression analysis. Plants were uprooted from peat moss and washed with distilled water. Roots were processed for colonization studies and fresh and dry weight analysis. The effectiveness of bacterial inoculation (B3P) was determined via the following formula:

$$\text{Treatment Effectiveness}(\%) = 100\times\big(\big(\text{Dry weight } of\ B3\text{P} - \text{treated plant} - \text{Dry weight } of\ \text{Controlplant}\big)/\big(\text{Dry weight of Control plant}\big)\big)$$

Dry weight of B3P-Treated plants (inoculated with the bacterial consortium)

Dry weight of control plants is the non-inoculated plants of respective hybrid variety

Development of inoculum-B3P

For development of B3P bacterial consortium, bacteria were individually grown in 100 mL L.B broth for two days with continuous shaking at 45 °C, then the preculture was further inoculated to one liter L.B broth. After substantial growth (10^9 cells/mL), the cell pellet was obtained by centrifugation and suspended in 0.85% sterilized saline, centrifuged, and again resuspended in 100 mL dH_2O. These four inoculum were mixed in equal proportion (1:1:1:1) and the final OD of consortium was adjusted as 10^8 mL^{-1}. The inoculum was mixed at 30% v/w in the sterilized carrier material (i.e., Filter mud).

Root colonization and plant enzymes analysis

The root colonization was checked under a confocal laser scanning microscope (CLSM) using 20 µL methyl acridine orange dye (0.1 mg/mL) for 10 min in the dark by following the protocol of (Harrison et al. 2006). The samples were observed under a confocal laser scanning

microscope (CLSM) (Olympus FV 1000, Japan) using an acridine orange specific filter and imaged at 100X magnification. The bacterial population was quantified by intensity estimation using image J software.

For analysis of plant enzyme expression under heat stress, 0.1 g fresh leaf of B3P-treated and non-treated plants were homogenized in 2 mL of sodium phosphate buffer. Three biological replications were processed for enzyme analysis. Leaf samples were taken 24 and 48 h after HS-2. The leaf samples were crushed in a mortar pestle and centrifuged at 12,000 rpm for 15 min at 4 °C and stored at -20 °C till enzyme analysis (Moore and Stein 1954).

The total chlorophyll contents were measured as described (Wellburn 1994). The fresh leaves were homogenized in 80% acetone and centrifuged at 10,000 rpm for 15 min, the supernatant was directly transferred into the cuvette, and absorbance was taken at λ663nm for chlorophyll a, λ645nm for chlorophyll b, and λ480nm for carotenoids.

$$Chlorophyll\ a\left({}^{mg}/_{mL}\right) = 12.7A_{663} - 2.69A_{645}$$

$$Chlorophyll\ b\left({}^{mg}/_{mL}\right) = 22.9A_{645} - 4.68A_{663}$$

$$Chlorophyll\left({}^{mg}/_{mL}\right) = Chlorophyll\ a + Chlotophyll\ b$$

For the peroxidase (POD) enzyme, 0.1 mL supernatant of fresh leaves was taken and mixed with the reaction mixture containing 2.7 mL PBS (pH 7.0), 0.1 mL Hydrogen peroxide (H_2O_2), and 0.1 mL guaiacol. The solution was mixed gently, and absorbance was measured at λ_{470nm} for 2 min at the interval of 20 s (Zia et al. 2021). The solution of PBS, H_2O_2, and guaiacol was used blank. Enzyme activity was determined by the equation below.

$$POD\ enzymer\ activity(\mu/mL) = (\Delta Vr \times Vt) \times \frac{1000}{\varepsilon} \times 1 \times \Delta t \times 0.100$$

where Δ Vr = Absorbance of sample-absorbance of blank; Vt = Total volume of the assay; Δt = Time; ε = extinction coefficient of substance (conc. Of a substance dissolved in a given solute and measures at a given wavelength).

Catalase (CAT) enzyme activity was determined by using the protocol of (Aebi 1984). The substrate was prepared by mixing 0.15 mL of H_2O_2 with 25 mL of 0.1 M sodium phosphate buffer. Wavelength was set at 240 nm, 1 mL of the substrate, 1.9 mL of distilled water, and 0.1 mL of sample was mixed, and absorbance was recorded at 0 s and 1 min after thoroughly mixing the solution. The mixture of H_2O_2 and sodium phosphate buffer was used as blank. CAT activity was determined by the equation below.

$$CAT = \frac{change\ in\ absorbance}{2\ min} \times \frac{100}{43.6} g \times weight\ of\ sample\ per\ mL\ of\ reaction$$

Total amino acids were measured by following the protocol of (Moore and Stein 1954). The 0.5 mL supernatant was added into test tubes containing the 0.5 ml 2% Ninhydrin and 0.5 mL 10% pyridine. Test tubes were incubated at 100 °C for 30 min and absorbance was taken at λ_{570nm}. The concentration of amino acid was measured through a standard curve of Ninhydrin.

The activity of the Malonaldehyde (MDA) enzyme was calculated by following the protocol of (Gaweł et al. 2004). The 0.25 g fresh leaf was ground into 7.5% trichloroacetic acid TCA and centrifuged for 12 min at 10,000 rpm. The 0.1 ml supernatant was mixed with 1.5 ml thiobarbituric acid TBA (0.9 g TBA in 150 ml of 10% TCA) and mixed gently. The reaction mixture was incubated at 100 °C for 30 min. Absorbance was measured at λ_{600nm}, λ_{532nm}, and λ_{450nm} on a spectrophotometer.

$$MDA\left({}^{\mu mol}/_{g}\right) = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{645}$$

Total soluble protein was quantified by using the Bradford method (Bradford 1976). The fresh leaf sample (0.25 g) was homogenized in 5 mL chilled sodium phosphate buffer and centrifuged at 12,000 rpm for 10 min. The 0.3 mL supernatant was mixed with 3 mL of Bradford reagent and mixed gently. The reaction mixture was incubated at room temperature for 10 min and absorbance was measured at λ_{595nm} by using a spectrophotometer. Protein concentration was measured through a standard curve by using bovine serum albumin (BSA).

RNA extraction and gene expression analysis

Total RNA was extracted from maize leaves using TRIzol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). Three biological replicates from each treated/non-treated sample were used for extraction. The quality and quantity of RNA were assessed by electrophoresis on 2% agarose gels and by a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, USA). The cDNA was synthesized using the Revert Aid First Strand cDNA Synthesis Kit following the manufacturer's protocol. The SYBR™ Green PCR Master Mix and CFX Maestro Software for Bio-Rad CFX Real-Time PCR Systems were used to measure the gene expression of heat shock proteins. Maize-specific primers for heat shock proteins were designed (Table 2) and synthesized commercially. *Actin* was the housekeeping gene (HSG) used as an endogenous reference. The *HSP1, HSP18, HSP70,* and *HSP101* were the gene of interest (GOI). The qPCR condition was initial denaturation at 94 °C for 2 min, denaturation at 94 °C for 20 s,

Table 2 Sequence of primer pairs and their respective product size designed in this study for qPCR analysis of heat induced genes in maize

Gene	Primers Sequence (5` to 3`)	Product Size (bp)
HSP1	Forward: *ATTGCGACCACACCTCACAA*	190
	Reverse: *GAAGATGTACCAGGGCGAGG*	
HSP18	Forward:*CGATCCGACATCCGAGAGAT*	340
	Reverse:*GGTACTTGGCGTCGTCCTC*	
HSP70	Forward: *CAACGACACACGACAAGCAG*	284
	Reverse: *TTTGCTAGAGCTTGCCCACA*	
HSP101	Forward: *CTGAGACGGGGATGAAGTCG*	224
	Reverse: *TCACGGGCTTATCTACACGC*	
Actin	Forward: *ATGGCTGACGGTGAGGACATCCAGCC*	205
	Reverse: *AGGTGAGGATACCCCTCTTGGATTGG*	

annealing at 54 for 30 s, first extension for 30 s at 72 °C, 35 cycles, final extension for 5 min at 72 °C, Hold at 8 °C. The gene expression analysis was measured by using the following formula (Naqvi et al. 2017).

$$Gene\ expression\ analysis = (\%age\ efficiency\ of\ GOI)^{\Delta CT\ of\ GOI} / (\%age\ efficiency\ of\ HSG)^{\Delta CT\ of\ HSG}$$

Statistical analysis

The correlation analysis and plant inoculation data were statistically analyzed by one-way and/or two-way ANOVA using the software Statistix 10. The comparison among treatment means was done at a 5% probability level using Tukey's HSD or Fischer's LSD.

Abbreviations

PGPR Plant growth promoting rhizobacteria
HS Heat stress
HSP Heat shock proteins
IAA Indole 3-acetic acid
DPI Day post incubation
GOI Gene of interest, HSG; Housekeeping gene

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13213-023-01736-5.

Additional file 1: Supplementary Figure 1. Geographical location of the sites on Pakistan map from where the samples were collected during the present study.

Additional file 2: Supplementary Figure 2. The cobs of hybrid maize varieties showing the impact of heat on during the year 2021-22.

Additional file 3: Supplementary Figure 3. Correlation matrix showing the relationship of parameters for hybrid maize plants with and without B3P-inoculation.

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Authors' contributions

MA: Data curation; Formal analysis, Investigation; Methodology, Writing—original draft, MI: Investigation; Supervision, MSN: Validation; Visualization, FM: Investigation; Methodology, YS: Methodology, MH: Methodology, Data curation, MA: Investigation; Methodology, RZN: Methodology, Software, MA: Project administration, AI: Conceptualization; Funding acquisition; Investigation; Project administration; Resources; Software; Supervision; Writing—review & editing.

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Availability of data and materials

The strains and data generated/analyzed in this study are available in this manuscript and NCBI.

Declarations

Ethics approval and consent to participate

The study does not involve any human and animal objects so the approvals from appropriate ethics committee was not applicable.

Consent for publication

All persons gave their informed consent prior to their inclusion in the study.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Abràmoff MD, Magalhães PJ, Ram SJ (2004) Image processing with ImageJ. J Biophotonics 11:36–42

Aebi, H. (1984). "[13] Catalase in vitro," in Methods in enzymology. Elsevier), 121–126

Ahmad, M., Imtiaz, M., Shoib Nawaz, M., Mubeen, F., and Imran, A. (2022). What Did We Learn From Current Progress in Heat Stress Tolerance in Plants? Can Microbes Be a Solution. Front. Plant Sci. 13. https://doi.org/10.3389/fpls. 794782

Ali SZ, Sandhya V, Grover M, Linga VR, Bandi V (2011) 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 6:239–246. https://doi.org/10.1080/17429145.2010.545147

Baldani JI, Reis VM, Videira SS, Boddey LH, Baldani VLD (2014) The art of isolating nitrogen-fixing bacteria from non-leguminous plants using N-free semi-solid media: a practical guide for microbiologists. Plant Soil 384:413–431. https://doi.org/10.1007/s11104-014-2186-6

Beauregard PB, Chai Y, Vlamakis H, Losick R, Kolter R (2013) Bacillus subtilis biofilm induction by plant polysaccharides. Proc Natl Acad Sci 110:E1621–E1630. https://doi.org/10.1073/pnas.1218984110

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye

binding. Anal Biochem 72:248–254. https://doi.org/10.1006/abio.1976.9999
Bunt J, Rovira A (1955) Microbiological studies of some subantarctic soils. J Soil Sci 6:119–128. https://doi.org/10.1111/j.1365-2389.1955.tb00836.x
Chen XD, Wang WW, Fu B, Xiao LM, Guo Y (2008) Growth promoting effect of hydrogen-oxidizing bacteria in soybean rhizosphere. Acta Bot Sin 28:136
Dong Q, Wang W, Kunkel KE, Shao Q, Xing W, Wei J (2021) Heterogeneous response of global precipitation concentration to global warming. Int J Climatol 41:E2347–E2359. https://doi.org/10.1002/joc.6851
Ehmann, A. (1977b). The Van Urk-Salkowski reagent—a sensitive and specific chromogenic reagent for silica gel thin-layer chromatographic detection and identification of indole derivatives. J Chromatogr A 132**,** 267–276. https://doi.org/10.1016/S0021-9673(00)89300-0
Ehmann A (1977a) The Van Urk-Salkowski reagent—a sensitive and specific chromogenic reagent for silica gel thin-layer chromatographic detection and identification of indole derivatives. J Chromatogr A 132:267–276
Eida, A.A., Ziegler, M., Lafi, F.F., Michell, C.T., Voolstra, C.R., Hirt, H., and Saad, M.M. (2018). Desert plant bacteria reveal host influence and beneficial plant growth properties. *PLoS One* 13**,** e0208223. https://doi.org/10.1371/journal.pone.0208223
El-Sappah AH, Rather SA (2022). Genomics approaches to study abiotic stress tolerance in plants. *Plant abiotic stress physiology*, 2, pp. 25-46.
Elshafie HS, Racioppi R, Bufo SA, Camele I (2017) In vitro study of biological activity of four strains of Burkholderia gladioli pv. agaricicola and identification of their bioactive metabolites using GC–MS. Saudi J Biol Sci 24:295–301. https://doi.org/10.1016/j.sjbs.2016.04.014
García JE, Maroniche G, Creus C, Suárez-Rodríguez R, Ramirez-Trujillo JA, Groppa MD (2017) In vitro PGPR properties and osmotic tolerance of different Azospirillum native strains and their effects on growth of maize under drought stress. Microbiol Res 202:21–29. https://doi.org/10.1016/j.micres.2017.04.007
Gaweł S, Wardas M, Niedworok E, Wardas P (2004) Malondialdehyde (MDA) as a lipid peroxidation marker. Wiad. Lek (Warsaw, Poland: 1960) 57:453-455PMID (15765761)
Gomes LH, Duarte KMR, Andrino FG, Tavares FCA (2000) A simple method for DNA isolation from Xanthomonas spp. Scientia Agricola 57:553–555. https://doi.org/10.1590/S0103-90162000000300028
Gordon, S.A., and Weber, R.P. (1951). Colorimetric estimation of indoleacetic acid. *Plant Physiol.* 26**,** 192. https://doi.org/10.1104/pp.26.1.192
Halebian, S., Harris, B., Finegold, S., and Rolfe, R. (1981). Rapid method that aids in distinguishing Gram-positive from Gram-negative anaerobic bacteria. *J. Clin. Microbiol.* 13**,** 444–448. https://doi.org/10.1128/jcm.13.3.444-448.1981
Harrison JJ, Ceri H, Yerly J, Stremick CA, Hu Y, Martinuzzi R, Turner RJ (2006) The use of microscopy and three-dimensional visualization to evaluate the structure of microbial biofilms cultivated in the Calgary Biofilm Device. Biol Proced Online 8:194–215. https://doi.org/10.1251/bpo127
He, F. (2011). Laemmli-sds-page. *Bio-protoc.***,** e80-e80. https://doi.org/10.21769/BioProtoc.80
Hungria M, Franco AA (1993) Effects of high temperature on nodulation and nitrogen fixation by Phaseolus vulgaris L. Plant Soil 149:95–102. https://doi.org/10.1007/BF00010766
Iloh A, Omatta G, Ogbadu G, Onyenekwe P (2014) Effects of elevated temperature on seed germination and seedling growth on three cereal crops in Nigeria. Sci Res Essays 9:806–813. https://doi.org/10.5897/SRE2014.5968
Imran A, Mirza MS, Shah TM, Malik KA, Hafeez FY (2015) Differential response of kabuli and desi chickpea genotypes toward inoculation with PGPR in different soils. Front Microbiol., 6, p. 859. https://doi.org/10.3389/fmicb.2015.00859.
Imran A, Sardar F, Khaliq Z, Nawaz MS, Shehzad A, Ahmad M, Yasmin S, Hakim S, Mirza BS, Mubeen F (2022) Tailored bioactive compost from agri-waste improves the growth and yield of chili pepper and tomato. Front Bioeng Biotechnol 9:1366. https://doi.org/10.3389/fbioe.2021.787764
Izaurralde RC, Thomson AM, Morgan J, Fay P, Polley H, Hatfield JL (2011) Climate impacts on agriculture: implications for forage and rangeland production. Agron J 103:371–381. https://doi.org/10.2134/agronj2010.0304
Kachhap, S., Chaudhary, A., and Singh, S. (2015). Response of plant growth promoting rhizobacteria (pgpr) in relation to elevated temperature conditions in groundnut (Arachis hypogaea L.). The Ecoscan 9, 771–778. https://doi.org/10.35652/IGJPS.2014.68
Kajla, M., Yadav, V.K., Khokhar, J., Singh, S., Chhokar, R., Meena, R.P., and Sharma, R. (2015). Increase in wheat production through management of abiotic stresses: a review. J. Appl. Nat. Sci. 7, 1070–1080. https://doi.org/10.31018/jans.v7i2.733
Kaushal M, Wani SP (2016) Plant-growth-promoting rhizobacteria: drought stress alleviators to ameliorate crop production in drylands. Ann Microbiol 66:35–42. https://doi.org/10.1007/s13213-015-1112-3
Kavamura VN, Santos SN, Da Silva JL, Parma MM, Ávila LA, Visconti A, Zucchi TD, Taketani RG, Andreote FD, De Melo IS (2013) Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. Microbiol Res 168:183–191. https://doi.org/10.1016/j.micres.2012.12.002
Kohler J, Hernández JA, Caravaca F, Roldán A (2008) Plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungi modify alleviation biochemical mechanisms in water-stressed plants. Funct Plant Biol 35:141–151. https://doi.org/10.1071/FP07218
Mathur, S., Raikalal, P., and Jajoo, A. (2019). "Physiological responses of wheat to environmental stresses," in Wheat Production in Changing Environments. Springer), 31–61
Mhamdi A, Queval G, Chaouch S, Vanderauwera S, Van Breusegem F, Noctor G (2010) Catalase function in plants: a focus on Arabidopsis mutants as stress-mimic models. J Exp Bot 61:4197–4220. https://doi.org/10.1093/jxb/erq282
Moore S, Stein WH (1954) A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J Biol Chem 211:907–913
Mukhtar, T., Rehman, S.U., Smith, D., Sultan, T., Seleiman, M.F., Alsadon, A.A., Ali, S., Chaudhary, H.J., Solieman, T.H., and Ibrahim, A.A. (2020). Mitigation of heat stress in Solanum lycopersicum L. by ACC-deaminase and exopolysaccharide producing Bacillus cereus: effects on biochemical profiling. Sustainability 12, 2159. https://doi.org/10.3390/su12062159
Naqvi RZ, Zaidi SS-E-A, Akhtar KP, Strickler S, Woldemariam M, Mishra B, Mukhtar MS, Scheffler BE, Scheffler JA, Jander G (2017) Transcriptomics reveals multiple resistance mechanisms against cotton leaf curl disease in a naturally immune cotton species. Gossypium Arboreum Sci Rep 7:15880. https://doi.org/10.1038/s41598-017-15963-9
Nautiyal CS, Bhadauria S, Kumar P, Lal H, Mondal R, Verma D (2000) Stress induced phosphate solubilization in bacteria isolated from alkaline soils. FEMS Microbiol Lett 182:291–296. https://doi.org/10.1111/j.1574-6968.2000.tb08910.x
Niu S, Wu M, Han Y, Xia J, Li L, Wan S (2008) Water-mediated responses of ecosystem carbon fluxes to climatic change in a temperate steppe. New Phytol 177:209–219. https://doi.org/10.1111/j.1469-8137.2007.02237.x
Okon Y, Kapulnik Y (1986) Development and function ofAzospirillum-inoculated roots. Plant Soil 90:3–16. https://doi.org/10.1007/BF02277383
Pathak R, Paudel V, Shrestha A, Lamichhane J, Gauchan DP (2017) Isolation of phosphate solubilizing bacteria and their use for plant growth promotion in tomato seedling and plant. Kathmandu Univ. J. Sci. Engi Technol 13:61–70. https://doi.org/10.3126/kuset.v13i2.21284
Pikovskaya R (1948) Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiologiya 17:362–370
Pratt LA, Kolter R (1999) Genetic analyses of bacterial biofilm formation. Curr Opin Microbiol 2:598–603. https://doi.org/10.1016/s1369-5274(99)00028-4
Reischl S, Thake S, Homuth G, Schumann W (2001) Transcriptional analysis of three Bacillus subtilis genes coding for proteins with the α-crystallin domain characteristic of small heat shock proteins. FEMS Microbiol Lett 194:99–103. https://doi.org/10.1111/j.1574-6968.2001.tb09453.x
Richter A, Hecker M (1986) Heat-shock proteins in Bacillus subtilis: a two-dimensional gel electrophoresis study. FEMS Microbiol Lett 36:69–71. https://doi.org/10.1016/j.jchromb.2006.09.029
Sarker MMR, Islam KN, Huri HZ, Rahman M, Imam H, Hosen MB, Mohammad N, Sarker MZI (2014) Studies of the impact of occupational exposure of pharmaceutical workers on the development of antimicrobial drug resistance. J Occup Health 56:260–270. https://doi.org/10.1539/joh.14-0012-oa
Schauberger B, Archontoulis S, Arneth A, Balkovic J, Ciais P, Deryng D, Elliott J, Folberth C, Khabarov N, Müller C (2017) Consistent negative response of US crops to high temperatures in observations and crop models. Nat Commun 8:1–9. https://doi.org/10.1038/ncomms13931
Shekhawat, K., Saad, M.M., Sheikh, A., Mariappan, K., Al-Mahmoudi, H., Abdulhakim, F., Eida, A.A., Jalal, R., Masmoudi, K., and Hirt, H. (2021). Root

endophyte induced plant thermotolerance by constitutive chromatin modification at heat stress memory gene loci. EMBO Rep. 2, e51049. https://doi.org/10.15252/embr.202051049.
Somasegaran, P., and Hoben, H.J. (1994). "Quantifying the growth of rhizobia," in *Handbook for rhizobia*. Springer), 47–57
Stepanović S, Vuković D, Dakić I, Savić B, Švabić-Vlahović M (2000) A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J Microbiol Methods 40:175–179. https://doi.org/10.1016/s0167-7012(00)00122-6
Tariq M, Iqbal H (2010) Maize in Pakistan–an overview. Agric Nat Resour 44:757–763
Vincent J, Humphrey B (1970) Taxonomically significant group antigens in Rhizobium. Microbiology 63:379–382. https://doi.org/10.1099/00221287-63-3-379
Wahid A (2007) Physiological implications of metabolite biosynthesis for net assimilation and heat-stress tolerance of sugarcane (Saccharum officinarum) sprouts. J Plant Res 120:219–228. https://doi.org/10.1007/s10265-006-0040-5
Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. Environ Exp Bot 61:199–223. https://doi.org/10.1016/j.envexpbot.2007.05.011
Wellburn AR (1994) The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol 144:307–313. https://doi.org/10.1016/S0176-1617(11)81192-2
Zhang M, An P, Li H, Wang X, Zhou J, Dong P, Zhao Y, Wang Q, Li C (2019) The miRNA-mediated post-transcriptional regulation of maize in response to high temperature. Int J Mol Sci 20:1754. https://doi.org/10.3390/ijms20071754
Zhao C, Liu B, Piao S, Wang X, Lobell DB, Huang Y, Huang M, Yao Y, Bassu S, Ciais P, Durand J-L, Elliott J, Ewert F, Janssens IA, Li T, Lin E, Liu Q, Martre P, Müller C, Peng S, Peñuelas J, Ruane AC, Wallach D, Wang T, Wu D, Liu Z, Zhu Y, Zhu Z, Asseng S (2017) Temperature increase reduces global yields of major crops in four independent estimates. Proc Natl Acad Sci USA 114:9326–9331. https://doi.org/10.1073/pnas.1701762114
Zia R, Nawaz MS, Yousaf S, Amin I, Hakim S, Mirza MS, Imran A (2021) Seed inoculation of desert-plant growth-promoting rhizobacteria induce biochemical alterations and develop resistance against water stress in wheat. Physiol Plant 172:990–1006. https://doi.org/10.1111/ppl.13362

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