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Increasing The Tolerance of Grain Corn Plants (*Zea mays* L.) to High Temperatures by applying Rhizobacteria

Fiqih Arizah Purba[1], Wan Arfiani Barus[1,*], Mohd Aziz Rashid[2], Syuhaidah Abu Bakar[2]

Abstract

Corn (*Zea mays* L.) is a grain crop cultivated in Malaysia, where the kernels are primarily used as feed for animals. Malaysia's climate is generally hot and rainy throughout the year, which has a significant impact on the growth of corn plants (*Zea mays* L.). This study aims to compare the effects of rhizobacteria on the growth rate and physiological responses of corn seedlings. Rhizobacteria are bacteria that have a positive impact on plant growth. Two strains of heat-tolerant rhizobacteria were used to observe their impact on corn growth: Heat-Tolerant Rhizobacteria 1 (HTR1) and Heat-Tolerant Rhizobacteria 2 (HTR2). Corn seedlings were transplanted into polybags and placed in a controlled environment at 38°C for 14 days, with four bacterial treatment levels: T1 (without HTR), T2 (HTR1), T3 (HTR2), and T4 (a combination of HTR1 and HTR2). Physiological analyses and growth rate measurements showed no significant differences among treatments; however, photosynthesis rates were significantly higher in treatments with rhizobacteria (T2, T3, and T4) compared to the control (T1). These findings suggest that rhizobacteria can enhance plant physiological functions and have potential applications for long-term crop improvement.

Keywords: Climate Change, Heat Tolerance, PGPR, Plant Physiology

1. Introduction

Corn (*Zea mays*) is the world's most important food crop after wheat and rice. Corn offers benefits not only as a food ingredient but also as feed and raw material for various industrial products. It is estimated that more than 55% of domestic corn production is used for animal feed, 30% for human consumption, and the remainder for industrial purposes and seed production. Consequently, the demand for corn continues to increase (Kasryno et al, 2007).

Grain corn (*Zea mays*) refers to corn kernels that retain their husks. These kernels vary in shape from round to flat and exhibit colors ranging from yellow and white to orange. They typically measure between 6 and 12 mm in length, have a moisture content of 13–15% for optimal storage, and possess a specific gravity of approximately 1.2 g/cm³. The growth of grain corn occurs in two main, interrelated phases: the vegetative phase and the generative phase. During the vegetative phase, growth begins with seed germination, during which the seeds absorb water, triggering the emergence of primary roots and the first leaves. The anticipated rise in temperature due to climate change, along with the increased likelihood of extreme temperature events, is expected to impact plant productivity. During the generative phase, corn plants begin producing male flowers (tasseling) approximately 50–60 days after planting, followed by female flowers (silking), which capture pollen for fertilization. Corn is susceptible to elevated temperatures during tassel formation, flowering, pollination, and grain filling. Simulation results suggest that corn yield decreases by approximately 10% for every 1°C increase in global temperature. Additionally, corn plants exhibit varying responses to heat stress during different phenological stages in both the vegetative and reproductive phases (Rashid et al, 2023).

Plant Growth-Promoting Rhizobacteria (PGPR) are soil microbes that inhabit the rhizosphere, the region within 1–2 cm of plant roots, where they directly or indirectly stimulate plant growth and development. These

*Correspondence: wanarfianibarus@umsu.ac.id
1) Universitas Muhammadiyah Sumatera Utara - Jl. Mukhtar Basri No.3, Medan Timur, Kota Medan, Sumatera Utara, Indonesia
2) Malaysian Agricultural Research and Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia

microorganisms colonize the root surface and are capable of producing indole-3-acetic acid (IAA), a type of auxin that promotes cell proliferation, differentiation, and overall plant growth (Müller et al., 2019).

Rhizobacteria can also produce plant growth hormones and act as plant growth promoters (biostimulants), increase atmospheric nitrogen (biofertilizers), and produce substances such as siderophores that can suppress plant pathogens (bioprotectants), thereby increasing crop yields (Maulina et al., 2022).

In nutrient-deficient agricultural environments, particularly in tropical regions, plant growth is often hindered by inadequate nutrient availability. Most crops exhibit nutrient use efficiency of less than 50% in many agricultural regions, exacerbating this problem. Plant growth-promoting bacteria (PGPR) play a crucial role in regulating geochemical nutrient cycling and providing nutrients to plants and soil microbial communities. Incorporating these beneficial bacteria as bioinoculants can significantly increase nutrient availability in soil, reduce reliance on chemical fertilizers, minimize environmental pollution, and promote sustainable agricultural practices (Silveira et al., 2021).

The use of biofertilizers, which have been demonstrated to enhance plant nutrition and soil health, is one effective way to achieve this goal. In this context, the use of plant growth-promoting rhizobacteria (PGPR) has garnered significant attention for enhancing plant nutrient uptake and utilization. PGPR is known to promote plant growth through various mechanisms, including increasing nutrient availability, enhancing root biomass and area, and improving plant nutrient uptake capacity. Consequently, there is growing interest in exploring the potential of PGPR to increase plant nutrient supply and promote plant growth (Ramana et al., 2020).

The results of Lehar et al.'s (2018) study show that regular weekly application of PGPR can significantly increase crop yields. Applying PGPR directly to the soil is believed to accelerate the decomposition of organic matter and provide essential nutrients that are readily available for plant absorption. This result is because microorganisms in PGPR utilize organic matter as a food source, thereby multiplying and increasing nutrient availability. (Irfhan, 2013) added that PGPR application increases plant dry weight because rhizobacteria produce the hormone IAA, associate with plants, and help decompose organic matter in the soil, which ultimately improves nutrient absorption and has a positive impact on overall plant productivity. Biofertilizers containing PGPR are increasingly popular due to their economic and environmental benefits. The global market for plant growth stimulants, including biofertilizers, is estimated to grow at a rate of 12% annually (Rahman et al., 2012). This finding aligns with research on grain corn plants, which shows that these plants can survive in extreme conditions after being treated with rhizobacteria.

2. Material and Methods

This research was conducted in September 2024 at the Climate Change Program Greenhouse Complex, Agrobiodiversity and Environment Research Center (BE), Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor, Malaysia, located at coordinates 2.9905°N and 101.7015°E, with an altitude of approximately 46 meters above sea level.

The materials used are unhusked corn plants from the Climate Change Program Greenhouse, Research Center for Agrobiodiversity and Environment, Phosphate-Buffered Saline (PBS), and nutrient agar powder. The tools used include micropipettes, petri dishes, analytical balances, HVE-50 autoclaves, laminar flow hoods, ovens, SPAD-502 Plus, LI-6400XT, and a Plant Efficiency Analyzer (PEA). SPAD-502 Plus is a portable tool for measuring the relative content of leaf chlorophyll quickly and non-destructively by measuring differences in red and infrared light transmittance. The LICOR functions to measure photosynthetically active radiation (PAR) in the spectral range of 400–700 nm, which is the basis for calculating the availability of light for photosynthesis. The LI-6400XT is a portable system that can measure photosynthesis rate, transpiration, stomatal conductance, and environmental parameters in situ with controlled settings for CO_2 , light, temperature, and humidity, making it highly reliable in plant physiology research and the Plant Efficiency Analyzer (PEA) functions to analyze the efficiency of photosystem II through chlorophyll fluorescence measurements, with parameters such as Fv/Fm and OJIP transients that can indicate physiological stress conditions of plants.

The research method employed is a quantitative approach with an observational component, focusing on measurable parameters influenced by the high-temperature-resistant rhizobacteria treatment. Quantitative research is based on the collection and analysis of numerical data to explain, predict, and control events of interest. Quantitative research emphasizes the analysis of numerical data, which is processed using statistical methods and techniques. Quantitative methods are used to identify patterns and relationships between variables. This research was conducted using a non-factorial nested design, consisting of one treatment factor with four replications (eight plants per treatment). The treatment factor used was high-temperature-resistant (HTR) bacteria with four levels: T1 (without HTR), T2 (HTR-01), T3 (HTR-02), and T4 (a combination of HTR-01 and HTR-02).

Rhizobacteria were isolated from corn-growing areas in the northern part of Peninsular Malaysia, specifically in agroecological Zone 1, which is characterized by a distinct dry season each year. Two strains of rhizobacteria were identified, characterized by high-temperature resistance and the ability to influence tree growth, including the production of indole acetic acid (IAA), solubilization of phosphate, nitrogen binding, and the possession of ACC deaminase activity.

A high-temperature treatment of 38°C was conducted for 4 hours, from 10:00 to 14:00, in an air-conditioned room over 14 days. This high-temperature behavior aligns with the Malaysian Meteorological Department's definition of a heat wave. This control room is equipped with an automatic temperature control system. Plants were given artificial light from light-emitting diodes (LEDs) with an intensity of 400 μmol m -2 s -1 photosynthetically active radiation (PAR) for 15 hours and carbon dioxide (CO2) levels were set at 450 μmol m -2 s -1 . 2 s -1.

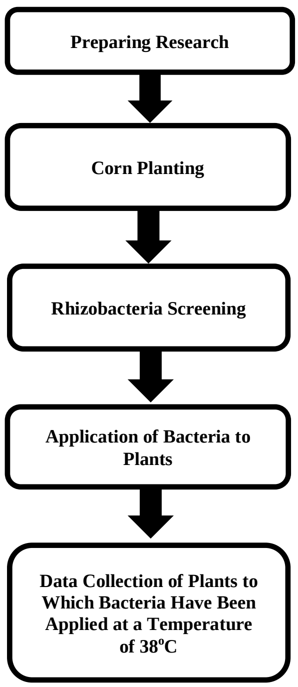

Figure 1. Research flow diagram

Research parameters are values or conditions used as benchmarks in discovering existing information, delving deeper into existing information, developing, expanding, and testing existing information whose validity is still questionable. Therefore, a study was conducted to retest several parameters that could demonstrate the effectiveness of using rhizobacteria as an adaptation strategy to the effects of high temperatures on grain corn plants. The parameters observed were rhizobacteria growth at high temperatures, plant growth rate, relative chlorophyll content (SPAD), and corn leaf physiology.

The research data were analyzed using ANOVA to test for significant differences between treatments. If the test results showed a significant difference, a Least Significant Difference (LSD) test was performed at a p-value of 0.05 to determine whether the treatments were significantly different from each other. All statistical analyses were performed using SAS software version 9.4 (SAS Institute Inc., Cary, North Carolina, USA).

Research Implementation

A total of 28 grams of nutrient agar powder was dissolved in 1 L of distilled water. The media solution was then autoclaved at 121°C for 15 minutes. Afterward, the media solution was poured into a petri dish and allowed to solidify. Afterward, the HTR was grown on nutrient agar medium using the streaking method for 24 hours at 50 °C in an incubator.

3. Results and Discussion

3.1. Rhizobacterial Growth at High Temperatures

Rhizobacteria were screened for viability testing at 50°C for 24 hours. The results showed that two bacterial strains grew at 50°C.

Based on research (Zia et al., 2021), heat-tolerant PGPR was isolated from the Cholistan Desert and its 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 subtropical, arid, and semi-arid, characterized by very high temperatures and low monsoon rainfall, which contributes to increased soil and environmental temperatures. Summer temperatures range from 46 to 51°C during the dry months, while winter temperatures vary between 0 and 1°C. The soil is not saline, low in organic matter, and has a pH that is alkaline. In addition, the properties of PGPR bacteria native to the desert, including heat resistance, and their possible role in inducing heat tolerance in maize during the early stages of vegetative growth, are investigated. Maize is more sensitive to heat stress than wheat and rice (Zhao et al., 2023). Research by Eida et al. (2018) suggests that under heat stress, plants undergo significant changes in carbon and sugar content, enabling their survival in diverse forms of soil carbon under heat-stress conditions. As shown in Figure 2.

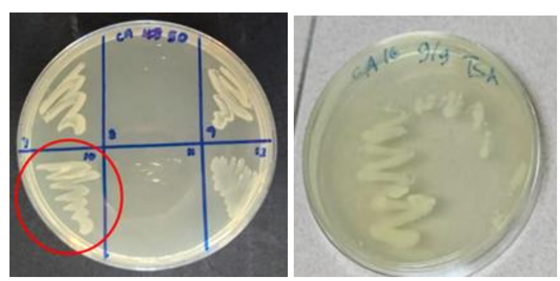

Figure 2. Growth of High Temperature Resistant Rhizobacterial Colonies HTR-01 (Left, Red Circle) and HTR-02 in an Incubator at 50°C.

3.2. Growth rate of grain corn

Although there were no significant differences in any parameters, the increase in leaf length in T3 and T4 showed a 50% and 54% increase, respectively, compared to plants without rhizobacteria treatment, although this increase was not statistically significant. However, if the data collection period were extended, the effect of HTR growth might have been more pronounced.

According to related research on PGPR, this study resulted in a greater increase in grain corn yield (22%-29%) in strip tillage. This increased effect may be related to the fact that the PGPR used in this study was isolated from the same region. Since inoculants selected from native microflora may be more adaptive and effective than exogenous bacteria, as previously reported for rhizobial populations (Jia et al., 2008), this could be a contributing factor. However, inoculation still significantly increased corn growth and yield, suggesting that specific plant growth-promoting mechanisms, independent of inoculant density, are at play. This result suggests that two types of mechanisms are involved in the effects of PGPR on plants: direct and indirect mechanisms (Martinez-Viveros et al., 2010). In addition to growth-promoting properties, inoculated plants also appear to regulate the number and structure of bacterial communities in the corn rhizosphere, which may be an additional indirect mechanism for increasing corn growth and yield. Considering the relationship between rhizosphere bacteria and plant health, given their ecological significance, it is crucial to explore the effects of PGPR inoculation on the rhizosphere microbiome (Berendsen et al., 2012). Similar findings to previous studies showing a positive correlation between corn biomass or production and bacterial species abundance or richness in the corn rhizosphere in this study suggest that regulation of the rhizosphere microbial community may be an indirect mechanism for PGPR to promote plant growth and production in strip tillage in response to PGPR inoculation (Garbeva et al., 2004).

Table 1. Growth rate of grain corn plants under the influence of high-temperature-resistant rhizobacteria (HTR).

Treatment (rhizobacteria)	Plant Height (cm)	Leaf length (cm)	Leaf Length Increase (mm day-1)	Leaf Width (cm)	Number of leaves	Stem diameter (mm)
T1 (without HTR)	34.20 ±2.60 a	40.45 ±0.23a	1.35 ±0.39 a	2.87 ±0.31 a	6.0 ±0.30 a	14.48 ±0.59 a
T2 (HTR-01)	34.00 ±0.87 a	42.60 ±0.57 a	1.49 ±0.52 a	2.95 ±0.28 a	6.0 ±0.17 a	14.35 ±0.52 a
T3 (HTR-02)	32.00 ±2.13 a	46.45 ±0.06 a	2.02 ±0.25 a	3.14 ±0.32 a	6.0 ±0.31 a	14.76 ±0.93 a
T4 (combination of HTR-01 and HTR-02)	34.75 ±2.55 a	45.38 ±0.14 a	2.08 ±0.03 a	2.88 ±0.30 a	6.5 ±0.42 a	15.70 ±0.93 a

Note: Numbers followed by the same letter in the same column are not significantly different at the 5% Duncan level.

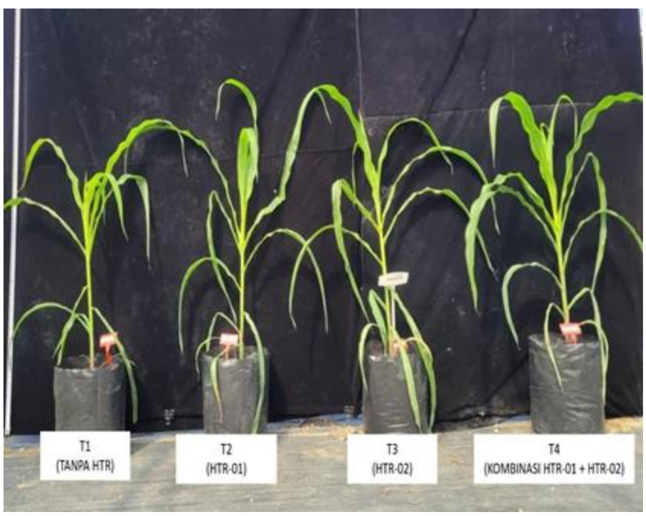

Figure 3. Comparison of Corn Seed Growth for High-Temperature-Resistant Rhizobacteria. Treatments Vary According to Different Temperatures.

3.3. Photosynthesis rate, stomatal conduction, and transpiration rate

Based on Figure 4, the effect of high-temperature-resistant rhizobacteria application was significantly different on the physiological parameters of corn leaves exposed to high temperatures of 38 °C for 14 days. Treatments T2 (HTR-01), T3 (HTR-02), and T4 (a combination of HTR-01 and HTR-02) increased leaf photosynthesis rates by 26.29%, 16.60%, and 15.12%, respectively, compared to T1 (without HTR).

Related research has shown that photosynthetic pigments, including chlorophyll and carotenoids, play a crucial role in harvesting and protecting the photosynthetic system from damage caused by excessive light. Their levels are important indicators of plant physiological status and can be used to estimate photosynthetic activity. Plant chlorophyll levels can be increased by inoculation with PGPR, which bind nitrogen, solubilize phosphate, or produce siderophores. PGPR-induced chlorophyll significantly correlates with the bacteria's ability to stimulate nutrient uptake in corn (Sabir et al., 2012).

Research by Binder et al. (2020) has shown that microbial application can enhance plant productivity by optimizing chlorophyll absorption, a process that plays a crucial role in various physiological processes, including germination, root initiation, leaf and flower senescence, fruit ripening, and mitigating abiotic stress. Quantitatively, these results are in line with Frontiers' (2024) research on corn, which showed that the combination of gibberellic acid (GA_3) and biochar (BC) was able to increase chlorophyll a levels by ±3.16 mg/g under non-stress conditions, and still provided an increase of 0.60–1.92 mg/g under conditions exposed to Cd up to 12 ppm. These findings are also supported by biofertilizer research on spinach involving Bacillus subtilis and Bacillus mucilaginosus, which, although not reporting absolute values, demonstrated a significant increase in total chlorophyll, photosynthetic parameters (F_0, Fm, Fv/F_0), and energy transfer efficiency. Thus, quantitatively, the research of Binder et al. strengthens previous evidence that microbial interventions can increase chlorophyll levels within a physiologically relevant range, especially under environmental stress conditions, thus potentially increasing plant efficiency.

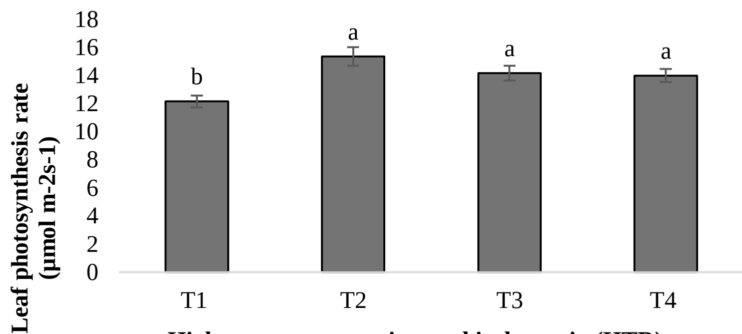

Figure 4. Photosynthesis Rate of Corn Leaves Treated with High Temperature Resistance. Rhizobacteria vary.

Table 2. Physiological Levels of Corn Leaves Under the Influence of High Temperature Resistant (HTR) Bacteria.

Treatment (rhizobacteria)	**Stomatal conductance (mol m^{-2} s^{-1})**	**Leaf transpiration rate (mmol m^{-2} s^{-1})**	**Chlorophyll fluorescence (Fv/Fm)**	**Relative Chlorophyll Content (SPAD)**
T1 (without HTR)	0.07 ±0.06 a	2.16 ±0.10 b	0.77 ±0.04 a	48.58 ±1.50 a
T2 (HTR-01)	0.07 ±0.05 a	2.78 ±0.17 a	0.78 ±0.07 a	48.86 ±2.49 a
T3 (HTR-02)	0.06 ±0.02 a	2.25 ±0.08 b	0.77 ±0.07 a	45.58 ±0.31 a
T4 (combination HTR-01 & HTR-02)	0.07 ±0.04 a	2.33 ±0.15 a	0.77 ±0.04 a	47.42 ±0.95 a

Note: Numbers followed by the same letter in the same column are not significantly different at the 5% Duncan level.

From the three parameters above, it can be concluded that the physiology of corn seed leaves is significantly different. This result is attributed to the optimal temperature, which is also supported by research conducted by Utami et al. (2018) using Plant Growth Promoting Rhizobacteria (PGPR) bacteria at a concentration of 1 × 10^8 CFU/mL. The quality of the bacterial content and the composition of the strains were not reported in detail. Before being applied to cultivated plants, the bacteria underwent a potential test stage that included the ability to produce the growth hormone Indole-3-Acetic Acid (IAA), bind nitrogen, and dissolve phosphate. However, the test results were not presented in the form of quantitative data, making it difficult to measure the effectiveness of the PGPR used with certainty and compare it with other studies.

In comparison, research by Marissa et al. (2024) offers more comprehensive quantitative data reporting. In this study, PGPR isolates obtained from post-nickel mining areas produced 6.4 mg/L of ammonia, an indicator of nitrogen-fixing capacity, and 53.1 mg/L of the hormone indole-3-acetic acid (IAA). This quantitative data provides a clear understanding of the physiological capabilities of the PGPR used.

The differences in data presentation between the two studies indicate that, although Utami et al. (2018) identified the physiological potential of PGPR, the absence of concrete numerical data limits quantitative comparative analysis with previous research. Therefore, reporting results in measurable units, such as mg/L or µg/mL, is essential to enhance scientific validity, enable more objective evaluation, and provide a solid foundation for developing more effective PGPR formulations to improve plant growth and yield.

4. Conclusion

The application of high-temperature-resistant rhizobacteria (HTR-01 and HTR-02) to corn plants at 38°C significantly increased the rate of leaf photosynthesis compared to untreated plants. Additionally, it showed a tendency to promote leaf length growth, indicating its potential as a long-term adaptation strategy for corn cultivation in hot climates.

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