

## Studies on Effect of PGPR on Wheat (*Triticum aestivum* L.) for Mitigation of Water Stress

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**ABSTRACT:** Wheat (*Triticum aestivum*) is one of the world's most important staple crops, is significantly impacted by water stress, which hinders its growth and productivity globally. Various strategies have been developed to mitigate water stress, but the use of PGPR-based formulation remains underexplored. This study aimed to assess the morphological and biochemical differences between wheat genotypes (HI1636, GW513, HI8823, and MP1358) under water stress and those treated with PGPR. The experiment involved treatments: 1) normal irrigation, 2) water-stressed, and 3) water stress with seed inoculation of *Bacillus subtilis* and *Pseudomonas fluorescens* separately. Ten morphological and two biochemical parameters were analysed, revealing significant changes in traits like germination percentage, plant height, productive tillers, root length, relative water content (RWC), chlorophyll index, proline accumulation, and grain yield. Water stress significantly reduced grain yield, with MP1358's yield declining by 55.1% from 20.65 g to 9.31 g. Notably, root length increased in HI8823 under stress, demonstrating a 60.44% increase, suggesting adaptive responses. Chlorophyll content decreased under water stress, particularly in HI1636 (51.6% reduction). Proline content rose significantly in GW513, indicating stress acclimatization. PGPR demonstrated notable enhancements in the growth and physiological parameters of the wheat genotypes. In the case of HI8823, chlorophyll levels increased from 1.893 mg/g (normal) to 2.766 mg/g with *Bacillus subtilis*, marking an enhancement of 46.24%. HI1636's grain yield improved from 8.84 g (water stress) to 16.76 g when treated with *Pseudomonas fluorescens*, indicating an increase of approximately 89.80%. Among the genotypes, HI1636 and MP1358 showed good stress tolerance, especially when treated with *Bacillus subtilis* and *Pseudomonas fluorescens*, improving yield, RWC, and proline accumulation. On the other hand, GW513 and HI8823 were more susceptible to water stress, exhibiting significant declines in yield, RWC, and chlorophyll content, even though inoculation provided partial recovery.

**Keywords:** *Triticum aestivum*, water stress, PGPR, *Bacillus subtilis*, *Pseudomonas fluorescens*.

### INTRODUCTION

Wheat is a crucial cereal crop and staple food for billions, known for its versatility and adaptability. It ranks as the second most cultivated grain globally, after maize, with international trade exceeding that of all other crops. In 2020, global wheat production reached 760 million tons, with China, India, and Russia contributing about 41% of the total yield. Nutritionally, wheat consists of 68-70% carbohydrates, 10-12% proteins, and 1-2% lipids, along with vitamins like thiamine, riboflavin, niacin, and folate, and contains approximately 3-4 mg of iron per 100 grams. India is the second-largest wheat producer in the world,

following China. Because of its fertile agricultural lands and favorable climate, India has achieved an average annual wheat yield of 104.983 million tonnes over the past five years (2018/19 – 2022/23). This substantial production is vital for sustaining its population, with wheat cultivated across approximately 30.38 million hectares, highlighting India's significant role in global wheat production.

In the 2021-2022 fiscal year, India exported about 7.85 million metric tons of wheat, generating approximately \$2.12 billion in foreign exchange. Major export destinations included Bangladesh, Nepal, the United Arab Emirates, and Sri Lanka (APEDA-2022). The peninsular region of India struggles with wheat

cultivation due to unsuitable climatic conditions, including higher temperatures and lower rainfall, as well as less fertile soils and inadequate irrigation. Wheat requires a cool climate for growth and warm, dry weather for ripening, making these regions unfavourable for its production also in the Gangetic plain, winters are short, and heat stress sets in early. This leads to high temperatures causes moisture stress during the grain filling stage, which significantly hampers wheat yield (More *et al.*, 2022). Water scarcity during the rabi season, high evapotranspiration rates, and erratic rainfall further intensify water stress on crops, leading to reduced yields. Water stress, which affects plant morphology, physiology, and biochemistry, is a major constraint on productivity, impacting not only Indian states but also other Asian countries facing similar challenges. The wheat crop has critical growth stages, each with specific water requirements that, if not met, can significantly affect yield and quality. Moisture stress can lead to reductions in biomass, tillering capacity, the number of grains per spike, and grain size. Therefore, the impact of moisture stress on wheat is influenced by both the intensity and duration of the stress experienced (Bukhat, *et al.* 2005). Water deficit stress during critical growth stages can lead to a significant decline in crop yields. It impairs leaf expansion and development, disrupts water and nutrient relations, hinders dry matter accumulation, and affects its partitioning, ultimately resulting in reduced yields (Nagar *et al.*, 2015; Dhakar *et al.*, 2018). Water stress can delay the flowering stage, negatively impacting the reproductive cycle and reducing the number and size of grains, ultimately leading to lower yields. It often decreases chlorophyll content, which diminishes photosynthetic efficiency. In response to water stress, wheat plants accumulate osmoprotectants like proline, glycine betaine, and soluble sugars to maintain cell turgor and protect cellular structures, with proline being a key biochemical response. Plant growth-promoting rhizobacteria (PGPR) offer a promising strategy for enhancing sustainable agriculture despite environmental stress. Historically, the increased demand for crop production has largely been met through the use of chemical fertilizers and pesticides (Godfray *et al.* 2010; Kumar *et al.* 2017). The extensive reliance on synthetic chemical fertilizers has resulted in serious consequences, such as the degradation of soil health and environmental quality (Kumar *et al.*, 2017; Meena *et al.*, 2015; Nath *et al.*, 2017). Plant growth-promoting bacteria (PGPB), when used instead of synthetic chemicals, have the potential to enhance plant growth while supporting environmental health and maintaining soil productivity (O'Connell, 1992; Esitken *et al.*, 2010). Integrating microorganisms into agricultural systems offers a cost-effective and eco-friendly approach to boost productivity during severe water shortages. Plant growth-promoting rhizobacteria (PGPR) from various genera can enhance plants' defense mechanisms, helping them to better withstand abiotic stresses (Fadiji *et al.*, 2022). The interactive effect of PGPR-formulation enhances water stress tolerance in wheat by

boosting its defense mechanisms. These PGP-rhizobacterial formulations combine effective microorganisms with carrier-based inoculants. Bacterial inoculants, such as rhizobia and nitrogen-fixing rhizobacteria, play a crucial role in improving plant resilience against water stress (Franch *et al.*, 2009). Plant growth-promoting rhizobacteria (PGPR), including phosphate-solubilizing bacteria, are key contributors to growth enhancement in bioformulations (Podile and Kishore 2006). These beneficial microorganisms help improve nutrient availability and stimulate plant growth, making them valuable for sustainable agricultural practices (Marschner, 1995). It was presumed that the application of PGPR formulation can cause a delay in stress symptoms in wheat under water-stressed conditions. Therefore, this investigation was done to evaluate the effect of PGPR formulation on wheat [*Triticum aestivum* L.] crop under water stress, using *Bacillus subtilis* and *Pseudomonas fluorescense* strains which were inoculated with talcum powder-based carrier by seed treatment and surface application to improve root and shoot length, chlorophyll index, proline accumulation and other growth parameters.

## METHODOLOGY

The present investigation entitled: Effect of PGPR on wheat (*Triticum aestivum*) for mitigation of water stress will be carried out at department of Plant Biotechnology, Vilasrao Deshmukh Collage of Agriculture Biotechnology, Latur (M.S.) (India), during the year 2023-24. The material and laboratory procedure followed during this course of investigation are described in this section.

The experimental material which is used in the present study consisted of 4 Wheat (*Triticum aestivum*) genotypes, were obtained from VNMKV, Parbhani. The experimental material which is used in the present study consisted of 4 Wheat (*Triticum aestivum*) genotypes, were obtained from VNMKV, Parbhani, (India).

**Table 1: List of 4 wheat genotypes.**

Sr. No.	Name of wheat genotype
1.	HI1636
2.	GW513
3.	HI8823
4.	MP1358

**PGPR- Treatment.** Plant growth promoting rhizobacterial culture consisted of liquid broth culture of i) *Bacillus subtilis* and ii) *Pseudomonas fluorescense* which is used in study, were obtained from Biomix research & production unit, VNMKV, Parbhani, (India).

**Methodology for water stress and PGPR-treatment and normal replication.** Four wheat genotypes obtained from VNMKV, Parbhani, (India) were grown in pots under normal and water stress conditions. Initial watering was provided every two days for establishment. Water stress treatments began 14 days post-sowing, with partial water stress imposed during various growth stages, supplemented with irrigation at 60 and 90 days after sowing (Bayoumi *et al.*, 2008).

Before applying stress, all pots were irrigated to field capacity. Regular irrigation was maintained for the normal replication using a watering can. The genotypes were assessed for morphological traits under normal conditions, water stress, and PGPR treatment at different growth stages. The mean performance for morpho-physiological traits was calculated to minimize experimental errors, followed by molecular screening. Talc-based formulation was prepared following Vidhyasekaran and Muthamilan (1995). Liquid cultures of *Bacillus subtilis* and *Pseudomonas fluorescens* ( $10^9$  cfu/ml) were grown in LB media. 100g of sterilized carrier was inoculated with 10 ml of liquid broth and incubated for 24 hours, then packed and sealed. Wheat seeds were sterilized using 70% ethanol for 2-3 minutes and washed with autoclaved distilled water. PGPR-formulation (30g) was mixed with 10% jaggery solution (as a sticking agent) and used to coat 300g of seeds. Seeds were soaked for 1 hour and shade dried for 15 minutes before sowing.

**Table 2: Experimental work plan.**

Sr. No.	Symbol	Treatment
1.	T <sub>0</sub>	Normal irrigation (non-inoculated seeds grown under normal irrigation)
2.	T <sub>1</sub>	Water stress (non-inoculated seeds grown under water stress)
3.	T <sub>2</sub>	Seeds inoculated with <i>Bacillus subtilis</i> formulation + surface application of <i>Bacillus subtilis</i> 45 DAS
4.	T <sub>3</sub>	Seeds inoculated with <i>Pseudomonas fluorescens</i> formulation + surface application of <i>Pseudomonas fluorescens</i> 45 DAS

**Morphological Observation:** Observations on morpho-physiological traits will be recorded at different growth stages. In each pot, five random plants will be tagged to record these observations. Average of treatment will be taken. The traits studied and techniques adopted to record the observations are given below;

**1. Germination percentage:** Germination percentage was calculated after eight days of sowing by using the following formula;

$$\text{Germination percentage (\%)} = \frac{\text{Number of seeds germinated} \times 100}{\text{Total number of seeds sown}}$$

**2. Plant height:** The plant height will measure from the bottom of the plant *i.e.* from soil level to the base of the spike and represented in centimetres.

**3. Shoot length (cm):** Shoot length will be measured with the help of scale by randomly selecting the five seedlings on the eighth day of germination.

**4. Root length:** Length will be measured with the help of scale by randomly selecting the five seedlings on the eighth day of germination

**5. Number of tillers:** Number of tillers recorded at heading stages to avoid any ambiguity in the counting.

**6. Spike length:** The average spike length of five plants on the main Culm from the base of the spike to the top of the last spikelet excluding awns will record in centimetres.

**7. RWC (Relative water content):** Relative water content (RWC) Of leaf will be determined by using a method developed by Barrs and Weatherly (1962);

$$\text{RWC (Relative water content)} = \frac{\text{Fresh weight} - \text{Dry weight} \times 100}{\text{Turgid weight} - \text{Dry weight}}$$

**8. Biological Yield:** After maturity the crop was harvested in each pot and tied in bundles and allowed to dry in respective pots for 2 to 3 days for sun drying. After thorough drying the weight of total produce (grain + straw) from each pot was recorded in the pot containing field itself. The weights obtained in gram were expressed as biological yield in g.

**9. Harvesting index (%):** It is the ratio of economic yield to biological yield of the plant on a dry weight basis and expressed as a percentage. This will be worked out from randomly selected five plants from each plot at the time of harvest and their average will be recorded.

H. I = Seed yield per plant / Biological yield per plant  $\times 100$ .

**10. Grain yield per plant (g):** Weight of seeds harvested from each of the randomly selected plants will be recorded.

**Biochemical analysis:** For Biochemical analysis, Chlorophyll pigments and Proline content (PC) will be determined for the variation among normal and drought-stressed wheat genotypes;

**1. Chlorophyll pigments (content):** Chlorophyll pigments will be determined after extraction of 100 mg fresh leaves using 80% pre-refrigerated acetone solution overnight at 4°C. chlorophyll a (Chla) and b (Chlb) will be measured by taking the optical densities (OD) of the extracted solution at 470, 663.2, 646.8 nm respectively using UV spectrophotometer. The total chlorophyll content (mg g<sup>-1</sup> FW) was calculated according to Lichtenthaler (1987);

Chlorophyll a (Chla) =  $(12.25A_{663.2} - 2.79A_{646.8}) \times V$

$$\text{Chlorophyll a (Chla)} = \frac{(12.25A_{663.2} - 2.79A_{646.8}) \times V}{1000 \times W}$$

$$\text{Chlorophyll b (Chlb)} = \frac{(21.50A_{646.8} - 5.10A_{663.2}) \times V}{1000 \times W}$$

$$\text{Total chlorophyll (ChlT)} = \frac{(7.15A_{663.2} + 18.71A_{646.8}) \times V}{1000 \times W}$$

Where V = volume of the extracted chlorophyll; W = weight of the sample used.

**2. Proline Content:** Proline content (PC) was determined following the acid-ninhydrin method of Bates *et al.* (1973). The total amount of PC in a given sample is expressed as a fresh weight (FW) basis using the following formula,

$$\text{PC} = \frac{X \times \text{volume of the extract}}{\text{The volume of aliquot} \times \text{weight of the samp}}$$

where, y = absorption at 520 nm; X = unknown concentration determined from the standard curve; m = slope and b = y intercept.

## RESULTS AND DISCUSSION

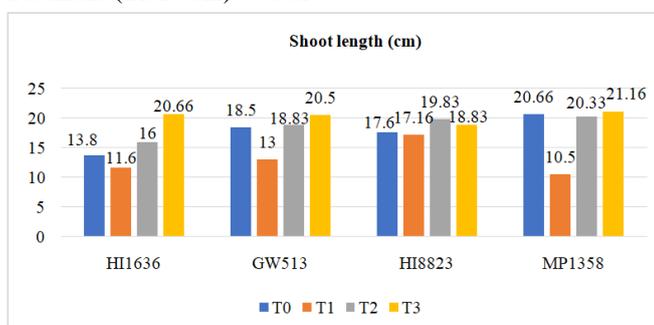
*A. Morphological parameters enhanced by PGPR under water stress*

Mean performance of morphological parameter; The recorded data on the average performance of wheat genotype accessions across morphological and biochemical traits at Vilasrao Deshmukh College of Agricultural Biotechnology, Latur (MS) is presented below;

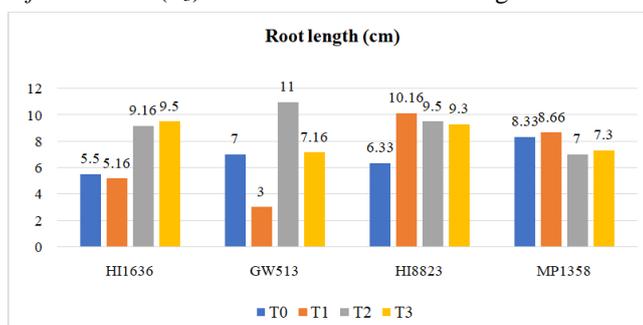
**(i) Germination (%) analysis:** Highest germination was observed in HI1636 (100%) and HI8823 (100%) under *Pseudomonas* treatment. Water stress reduced germination slightly across all genotypes, with GW513 and HI1636 showing a decline to 86% and 84%, respectively. Shankar and Prasad (2023) reported the results of germination percentage align to this study that; Bacterial treatment significantly improved seed germination compared to non-treated seeds. *Enterobacter cloacae* BHUAS1 (T1) had the highest germination rate at 88.89%, followed by *Bacillus cereus* BHUAS2 (T3) at 88.78%, and *Bacillus megaterium* BHUIESDAS3 (T2) at 85.56%. In contrast, the control group showed 77.78% germination after 7 days. Wheat genotypes show reduced drought tolerance, affecting germination. PGPR, like *Bacillus subtilis*, improves seed germination and seedling vigor under water stress, leading to better growth and yield (Ansari *et al.*, 2021; Ilyas *et al.*, 2020).

**(ii) Other yield contributing parameters analysis:** Microbial treatments, especially *Bacillus* and *Pseudomonas*, significantly improved plant performance under water stress across various parameters such as shoot length, root length, and grain yield, biomass, RWC, chlorophyll content, proline content highlighting their potential as effective biostimulants for water stress resilience in wheat genotypes (Fig. 1), MP1358 had the longest shoot length under *Pseudomonas* treatment (21.16 cm). Water

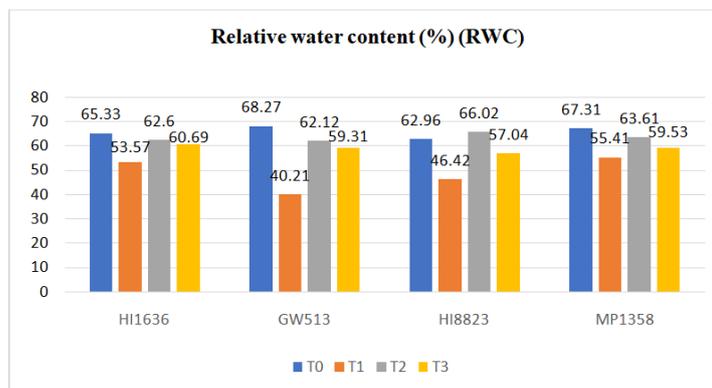
stress caused a notable decrease in shoot length, particularly in MP1358 and GW513 which is 49.17% and 29.73% respectively. *Pseudomonas* treatment had the most significant positive effect on shoot length in HI1636 and GW513 which enhanced by 49.71% and 10.81% respectively as compared to water stress. Bangash *et al.* (2013), Shoot length increased by more than 20% and 34% in response to PGPR inoculation with rhizobacterial isolates CC7 and RT7, respectively, compared to the uninoculated control, which align with the present studied data of shoot length. Root length increased in HI8823 under water stress (10.16 cm) compared to normal conditions (6.33 cm), and both bacterial treatments kept the length consistently high, showing that HI8823 is resilient under stress. In MP1358, Root length shows a small increase under water stress (8.66 cm compared to 8.33 cm under normal), but bacterial treatments led to slightly lower values (7.00–7.30 cm) (Fig. 2). *Bacillus* and *Pseudomonas* treatments significantly increased root length in HI1636 and HI8823 by 66.55% and 50.08% for *Bacillus* and 72.73% and 46.92% for *Pseudomonas* respectively. Ilyas *et al.* (2020) found that treating wheat varieties Pak 13 and NARC 09 with *Bacillus subtilis* and *Azospirillum brasilense* increased root length by 29.79% and 27.22%, respectively, under drought conditions compared to uninoculated plants. Also, Shankar and Prasad (2023) reported root length increased by 49%, 45%, and 47% in plants inoculated with *Enterobacter cloacae* BHUAS1, *Bacillus megaterium* BHUIESDAS3, and *Bacillus cereus* BHUAS2 treatments, respectively, compared to non-inoculated (control) plants under water stress, which support's this analysis for root length data.



**Fig. 1.** Indicating shoot length (cm) under Normal (T<sub>0</sub>), Water Stress (T<sub>1</sub>), *Bacillus subtilis* (T<sub>2</sub>), and *Pseudomonas fluorescens* (T<sub>3</sub>) formulation treatment along with stress.



**Fig. 2.** Indicating root length (cm) under Normal (T<sub>0</sub>), Water Stress (T<sub>1</sub>), *Bacillus subtilis* (T<sub>2</sub>), and *Pseudomonas fluorescens* (T<sub>3</sub>) formulation treatment along with stress.

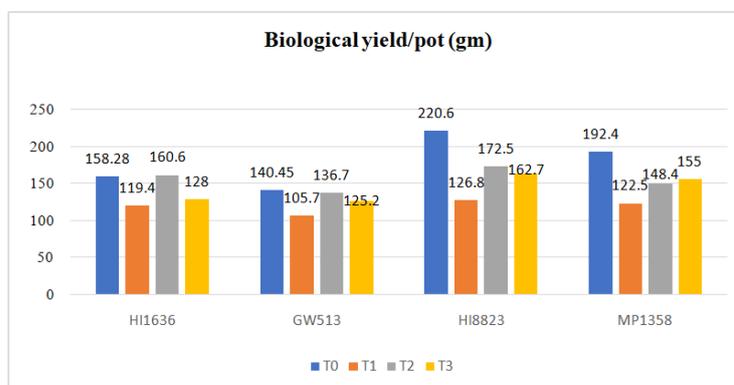


**Fig. 3.** Indicating relative water content % under Normal (T<sub>0</sub>), Water Stress (T<sub>1</sub>), *Bacillus subtilis* (T<sub>2</sub>), and *Pseudomonas fluorescens* (T<sub>3</sub>) formulation treatment along with stress.

GW513 showed the highest RWC (Relative water content) under normal conditions (68.27%) (Fig. 3). Under water stress, RWC decreased across all genotypes, particularly in GW513 (40.21%). *Bacillus* and *Pseudomonas* treatments improved RWC values, with HI8823 achieving the highest RWC under *Bacillus* (66.02%). Shankar and Prasad (2023) showed that water stress decreased relative water content (RWC), but bacterial treatments increased RWC by 44%, 31%, and 38% in wheat plants inoculated with *Enterobacter cloacae*, *Bacillus megaterium*, and *Bacillus cereus*, respectively.

HI8823 achieved the highest biological yield under normal conditions (220.60 g/pot), (Fig. 4). Water stress caused significant reductions of 24.74% in GW513, dropping from 140.45 g to 105.70 g. *Bacillus* treatment helped improve the yield, particularly in HI1636 with a

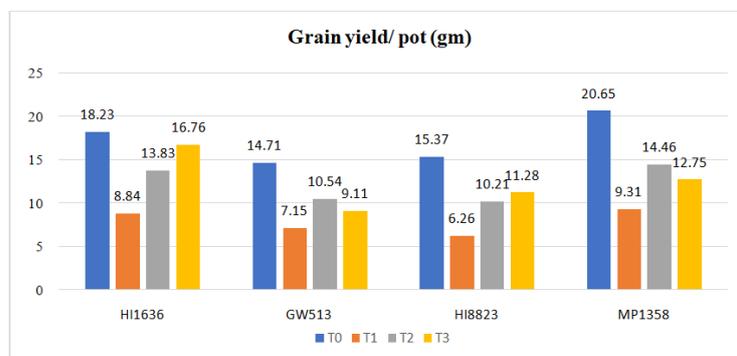
yield of 160.6 g/pot which is increased of 34.55% as compared to water stress. The *Bacillus subtilis* treatment had a significant positive impact on biological yield across all genotypes when compared to water stress, showing improvements ranging from 21.13% to 36.05%. Similar enhancement in biological yield using PGPR under water stress were reported by Latif *et al.* (2022), Inoculation with the EPS-producing rhizobacterial strain LEW16 *Klebsiella* sp. significantly increased the total dry biomass in wheat varieties, Johar-16 and Gold-16, under water stress. The highest biological yield, was recorded in Gold-16 representing a 44% increase over the control. These findings align with the observed mitigative effect of *Bacillus* treatment in present study, though the percentage improvements were relatively small.



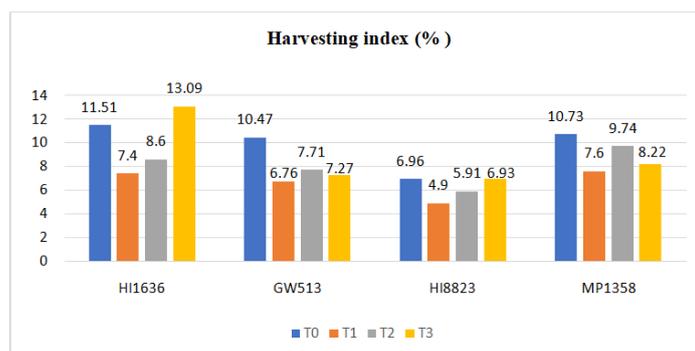
**Fig. 4.** Indicating Biological yield/pot (gm) under Normal (T<sub>0</sub>), Water Stress (T<sub>1</sub>), *Bacillus subtilis* (T<sub>2</sub>), and *Pseudomonas fluorescens* (T<sub>3</sub>) formulation treatment along with stress.

MP1358 had the highest grain yield under normal conditions (20.65 g/pot), (Fig. 5) HI1636 saw a marked increase under *Pseudomonas* treatment, reaching 16.76 g/pot compared to 8.84 g/pot under stress. Rahimi *et al.* (2024) found that Plant Growth-Promoting Rhizobacteria (PGPR) formulations, including *Bacillus subtilis* and *Pseudomonas fluorescens*, can alleviate the negative impacts of water stress. These PGPRs enhance drought tolerance by promoting root growth, improving nutrient uptake, and increasing water use efficiency.

The highest harvesting index was observed in MP1358 under *Bacillus* treatment (97.40%). Water stress reduced the harvesting index in all genotypes, with the lowest recorded in HI8823 (49%), (Fig. 6). Karimi *et al.* (2022) reported that biofilm-forming rhizobacterial isolates positively influenced the harvest index of wheat seedlings from the Kohdasht (C1) and Chamran (C2) varieties under moderate and severe water deficit conditions.



**Fig. 5.** Indicating grain yield/pot (gm) under Normal (T<sub>0</sub>), Water Stress (T<sub>1</sub>), *Bacillus subtilis* (T<sub>2</sub>), and *Pseudomonas fluorescens* (T<sub>3</sub>) formulation treatment along with stress.

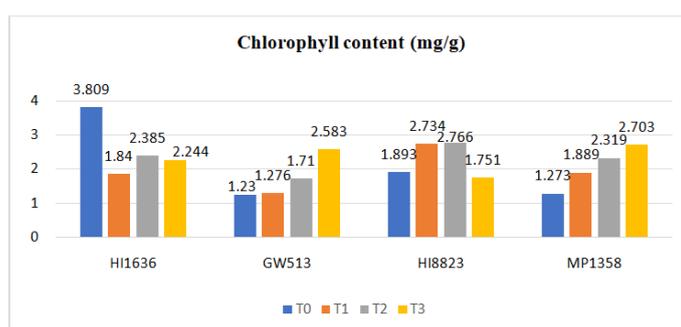


**Fig. 6.** Indicating Harvesting index (%) under Normal (T<sub>0</sub>), Water Stress (T<sub>1</sub>), *Bacillus subtilis* (T<sub>2</sub>), and *Pseudomonas fluorescens* (T<sub>3</sub>) formulation treatment along with stress.

**B. Biochemical parameters enhanced by PGPR under water stress**

**(i) Chlorophyll Content (mg/g):** HI1636 showed a significant reduction in chlorophyll under water stress (1.84 mg/g) compared to normal conditions (3.81 mg/g), (Fig. 7). The application of *Bacillus* and *Pseudomonas* treatments increased chlorophyll content, with *Pseudomonas* improving levels to 2.24 mg/g in HI1636 and 2.70 mg/g in MP1358. HI8823 recorded the highest chlorophyll content under *Bacillus* treatment (2.77 mg/g), indicating its effectiveness in maintaining

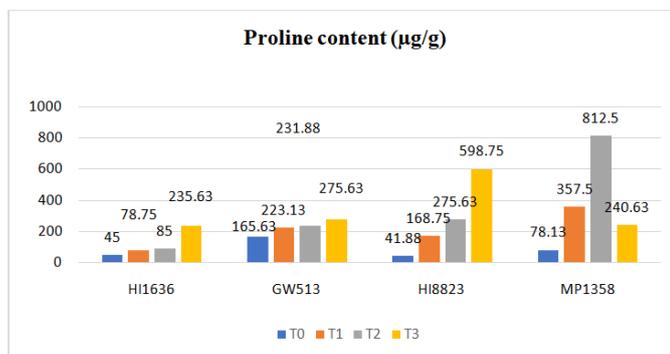
chlorophyll under stress. *Pseudomonas fluorescens* significantly enhanced chlorophyll content in most varieties, particularly in GW513 and MP1358 which is 102.4% and 43.1% increase over water stress respectively. The study aligns with Yadav *et al.* (2022), showing a 46% decrease in chlorophyll content in drought-stressed wheat. In contrast, inoculated plants with *Bacillus paramycoides* and *Bacillus paranthracis* had increases of 142% and 182% in chlorophyll content, respectively.



**Fig. 7.** Indicating Chlorophyll content (mg/g) under Normal (T<sub>0</sub>), Water Stress (T<sub>1</sub>), *Bacillus subtilis* (T<sub>2</sub>), and *Pseudomonas fluorescens* (T<sub>3</sub>) formulation treatment along with stress.

**(ii) Proline Content (µg/g): (Fig. 8)** HI8823 showed a dramatic increase in proline content under *Pseudomonas* treatment, reaching 598.75 µg/g compared to 41.88 µg/g under normal conditions, highlighting the role of *Pseudomonas* in proline accumulation. MP1358 also exhibited high proline accumulation under *Bacillus* treatment (812.5 µg/g), showing its strong response to the microbial inoculant

under stress. Proline content in HI1636 increased under *Pseudomonas* treatment to 235.63 µg/g, further confirming the protective role of this treatment in managing osmotic stress. Azmat *et al.* (2020) found that in wheat variety PAK 2013, using a combination of plant growth-promoting rhizobacteria increased proline synthesis by 149% compared to drought-stressed, uninoculated plants.



**Fig. 8.** Indicating Proline content ( $\mu\text{g/g}$ ) under Normal ( $T_0$ ), Water Stress ( $T_1$ ), *Bacillus subtilis* ( $T_2$ ), and *Pseudomonas fluorescens* ( $T_3$ ) formulation treatment along with stress.

## CONCLUSIONS

HI1636 and MP1358 demonstrated good stress tolerance, especially when inoculated with *Bacillus subtilis* and *Pseudomonas fluorescens*, improving yield, RWC, and proline accumulation. *Pseudomonas fluorescens* showed a stronger overall recovery for grain yield and proline accumulation, helping HI1636 return to near normal performance. In MP1358, *Bacillus subtilis* had a stronger positive impact on proline content and grain yield recovery for MP1358. *Pseudomonas fluorescens* led to full recovery and even improvement in chlorophyll content, indicating a positive effect on photosynthetic activity. Both PGPR-treatments helped to recover RWC, with *Bacillus subtilis* showing a slightly better effect. GW513 and HI8823 showed susceptibility to water stress, with significant reductions in key traits like yield, RWC, and chlorophyll content under stress, despite partial recovery with inoculation. Genotype GW513 under *Bacillus subtilis* showed better recovery in grain yield and RWC, improving overall plant performance under stress. *Pseudomonas fluorescens* helped significantly improve chlorophyll content and proline accumulation but was less effective for yield recovery, while HI8823 under *Bacillus subtilis* was better for maintaining RWC and chlorophyll content under stress, while *Pseudomonas fluorescens* improved grain yield and proline accumulation, despite negatively affecting chlorophyll. Despite the positive impacts of PGPR, some genotypes (particularly GW513 and HI8823) exhibited incomplete recovery, especially in terms of RWC and yield, indicating continued susceptibility to water stress even with PGPR inoculation. Water stress severely affected key phenotypic traits, leading to reductions in chlorophyll content, grain yield, RWC, and overall plant growth in all four genotypes. Although inoculation with PGPR like *Bacillus subtilis* and *Pseudomonas fluorescens* helped mitigate some of the negative effects, full recovery to normal conditions was not achieved. *Pseudomonas fluorescens* was generally more effective than *Bacillus subtilis* in enhancing stress tolerance, but genotypes like GW513 still struggled under stress, particularly in terms of water retention and yield.

## FUTURE SCOPE

The future scope of this study includes exploring the synergistic effects of microbial treatments on various

wheat genotypes under stress conditions, as well as expanding studies to include other crops and long-term impacts on soil health and agricultural sustainability. Additionally, investigating the molecular mechanisms behind enhanced stress tolerance will provide deeper insights into the effectiveness of microbial inoculants in agriculture.

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