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Original Article

Assessing the impact of PGPR and water retention agents on bread wheat growth: an experimental study

Khaoula Habbadi[1*], Meriem Guartoumi El Idrissi[1], Salma El Iraqui El Houssaini[1], Ilyass Maafa[2], Faical Aoujil[1], Abdellatif Benbouazza[1], El Hassan Achbani[1], Moha Ferrahi[3]

[1]Phytobacteriolgy and Biological Control Laboratory, Regional Center of Agricultural Research of Meknes, National Institute of Agricultural Research, Avenue Ennasr, BP 415 Rabat Principal, 10090 Rabat, Morocco
[2]International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat 10080, Morocco
[3]Department of Breeding and Conservation of Genetic Resources, National Institute for Agricultural Research (INRA), Rabat 10020, Morocco

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Abstract
Considering the imperative challenges posed by climate change, particularly the escalating instances of drought stress, optimizing wheat production stands as a critical goal for agricultural development. This experimental study delves into the pivotal role of Plant Growth-Promoting Rhizobacteria (PGPR) and Water Retention Agents (WRA) in bolstering the resilience of bread wheat crops to drought conditions. The study encompasses germination tests, field observations, and a comprehensive analysis of agronomic parameters, emphasizing the significance of PGPR and WRA in mitigating the adverse effects of water scarcity on wheat crops. The germination tests in Petri dishes reveal a substantial enhancement with the application of PGPR strains, showing higher rates (95% for *Serratia nematodiphila* strain GAB111 and 92% for *Pseudomonas koreensis* strain GAJ222) compared to the control. PGPR significantly increased root and shoot lengths, with significant increases observed. Field observations show the climatic impact on wheat development, with an accelerated growth cycle due to high temperatures and arid conditions. Further analyses reveal that WRA and PGPR significantly affect agronomic parameters such as the number of leaves, tillers, ears, and chlorophyll content. The study also assesses the impact on leaf temperature, wheat yield, plant-root development, and soil parameters such as organic matter and nutrient content. The combined use of WRA and PGPR shows promising results, highlighting their potential synergistic effects on wheat growth and development, especially in challenging environmental conditions. These findings offer practical solutions for enhancing wheat resilience and have broader implications for the sustainable development of agricultural systems confronting increasing climate-induced stresses.

Keywords: Climate change, Wheat resilience, Aagronomic parameters, PGPR, WRA

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**Corresponding author email:*
Khaoula.habbadi@inra.ma

Introduction

Agriculture, the foundation of human civilization, faces an ever-pressing challenge in the form of water scarcity, exacerbated by the increasing frequency and severity of drought events induced by climate change (Smith et al., 2018). Among the staple crops that bear the brunt of this challenge is wheat, a global dietary cornerstone, and a vital source of nourishment for billions of people (FAO, 2019).

Bread wheat, scientifically known as *Triticum aestivum*, plays a vital role in Morocco's agricultural landscape and is a staple crop that significantly contributes to the country's food security and economic stability (MAPMDREF, 2020). With its versatile applications in various food products, including bread, pasta, and pastries, bread wheat holds a prominent position in the Moroccan diet and food industry. Wheat grains mainly comprise carbohydrates (starch), proteins (gluten), lipids, fiber, vitamins, minerals, and phytochemicals. Their composition varies with genetics, growing conditions, and post-harvest handling, affecting nutritional value and processing properties (Moussaoui and El Atki, 2019).

However, the production of bread wheat in Morocco faces a growing challenge due to the adverse impacts of climate change and increasing occurrences of drought stress (WBG, 2021). As a predominantly arid and semi-arid country, Morocco has long grappled with water scarcity issues (ITA, 2023). In recent years, the effects of climate change have exacerbated these challenges, leading to more frequent and severe droughts (Hssaisoune et al., 2020). These changing climatic patterns are putting immense pressure on the country's agriculture sector, especially on crops like bread wheat, which are highly sensitive to water availability (Jäger et al., 2014).

In recent years, the integration of innovative agricultural technologies has emerged as a promising avenue to address the challenges posed by climate change and drought stress on bread wheat production (Francisco Ribeiro and Camargo Rodriguez, 2020). Among these technologies, the application of Plant Growth-Promoting Rhizobacteria (PGPR) and Hydrogel technology has gained recognition for their potential to enhance crop resilience and productivity in water-limited environments (Zheng et al., 2018; Azeem et al., 2023).

The use of PGPR in bread wheat production has garnered significant attention as a sustainable and eco-friendly approach to enhance crop yields and resilience (Alaskar and Al-Shwaiman, 2023). PGPR are beneficial soil bacteria that colonize the root zone of plants, forming mutually beneficial relationships that contribute to improved plant health and growth (Zishan and Manzoor, 2022). They can solubilize and mineralize essential nutrients in the soil, making them more available to bread wheat plants. This includes phosphorus, which is often limited in availability in many soils (Hasan et al., 2023). Some other PGPR strains have the capability to fix atmospheric nitrogen into a form that can be utilized by plants (Dheeman, 2021). This reduces the dependence on synthetic nitrogen fertilizers, contributing to cost savings and reduced environmental impacts (Bhattacharyya and Jha, 2012; Khan et al., 2020). PGPR contribute also on the hormone regulation (production of auxins and cytokinins), which play specific roles in bread wheat development. Auxins stimulate cell elongation, root initiation, and differentiation, while cytokinins promote cell division and shoot formation (Tsukanova et al., 2017). These hormones lead to increased biomass and higher yields in bread wheat (Grover et al., 2021). PGPR can enhance water use efficiency in bread wheat by promoting deeper root growth and reducing transpiration rates, allowing the plant to thrive with less water (Bouremani et al., 2023).

The use of hydrogel in wheat production represents a significant advancement in agricultural technology aimed at improving crop yields and sustainability, particularly under drought stress conditions (Bhatnagar et al., 2016). Hydrogels, also known as Water Retention Agents (WRA) or Superabsorbent Polymers (SAP), have gained attention for their capacity to enhance soil moisture retention, increase water use efficiency, and promote plant growth (Ahmed et al., 2018). They can absorb large quantities of water and retaining it within their structure. When incorporated into the soil, hydrogels act as reservoirs, storing water during periods of rainfall or irrigation and gradually releasing it to plant roots as the soil dries out (Tariq et al., 2023). This helps to maintain a consistent level of soil moisture, ensuring that wheat plants have access to water, even during drought stress (El Bergui et al., 2023). They controlled the release of water from hydrogels enhances water use efficiency by reducing water wastage through runoff and evaporation (Ahmed et al., 2018). This is particularly beneficial in regions with limited water resources, where maximizing the effectiveness of each drop of water is crucial for crop production. Wheat plants grown in soils enriched with hydrogels often exhibit improved growth characteristics. Hydrogel-amended soils provide a more stable and favorable environment for root development, nutrient uptake, and overall plant health. This can lead to increased wheat yields and better crop quality (Bhatnagar et al., 2016).

The combined effects of PGPR and WRA technology on drought-tolerant wheat cultivation represent a powerful and innovative approach to address the critical challenges posed by water scarcity, climate change, and sustainable food production (Seleiman et al., 2021). The importance of this integrated technology lies in its potential to revolutionize wheat cultivation by providing multiple benefits that contribute to the resilience and productivity of wheat crops in drought-prone environments (Ahmad et al., 2023).

In the following sections of this research study, we will explore the role of innovative agricultural practices, such as the use of PGPR and WRA, in enhancing bread wheat production. Through a comprehensive analysis, this study aims to provide valuable insights and practical recommendations for sustainable bread wheat cultivation in the face of a changing climate and increasing drought stress in Morocco.

Material and Methods

Study site

The experiment was carried in the Regional Center of National Institute of Agronomic Research of Meknes (latitude: 33.84065741, longitude: -5.47274890 and altitude: 665.39) from February to June 2021. The climate of the zone is of the type semi-arid Mediterranean climate, marked by consistently hot, dry summers and relatively cool winters, with temperatures ranging between 10° to 38°C. The climate data for this site was recorded and retrieved from a meteorological station during the experiment.

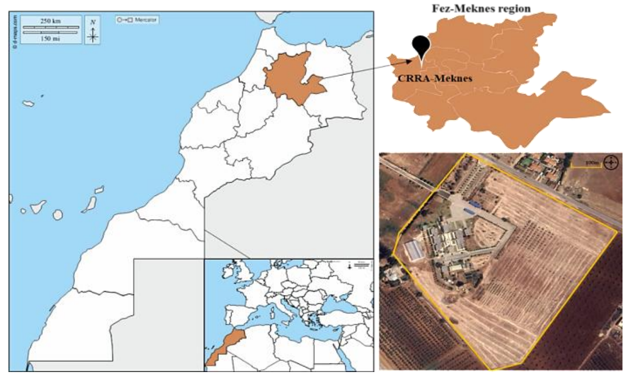

Figure-1. **Experimental field in the Regional Agricultural Research Center in Meknes (CRRA)** (D-map: https://d-maps.com/index.php?lang=en & Google-Earth: https://www.google.com/intl/fr/earth/)

Plant material

In our study, we utilized a Moroccan variety of bread wheat developed by INRA, known as the 'FAIZA' variety (synonym; FD 1720, breeder: Florimond Desprez). This variety was released in 2010 and is known for its sensitivity to some fungal diseases such as stem rust and yellow rust, as well as to water stress.

Bacterial strains and water retention agents

Two PGPR strains used in this study (Table 1) were obtained from the laboratory collection of Phytobacteriology and Biological Control at the National Institute of Agronomic Research in Meknes, Morocco. Our selection process is based on the strains proven abilities to promote and protect other crops in previous studies (El Allaoui et al., 2023; Ou-zine et al., 2023). PGPR were cultured for 2 days at 28°C on YPGA medium (yeast extract, 5 g/L; peptone, 5 g/L; glucose, 10 g/L; agar, 15 g/L). Our large-scale bacterial production process involves culturing the bacteria in the fermentor (Bioengineering pilot 2-7/RALF, USA-Massachusetts) while maintaining precise control over culture parameters, including temperature, pH, dissolved oxygen, and agitation. Bacterial growth is continuously monitored, and concentration adjustments were made at optical density (OD_{600}) intervals using a spectrophotometer. Once the desired cell concentration, which is 10^8 CFU/mL, is achieved, the bacterial culture can be harvested for subsequent use.

The WRA used in this study, called "Water Hope", it is a homopolymer with a cross-linked structure, composed of polyacrylic acid neutralized with potassium salt, enriched with catalytic minerals (Nitrogen 5.5%, Phosphor 8%, and Potassium 7%) (Water-Hope, Lasne, Belgium).

Table-1. Strains tested on potato tubers.

Strain code	Specie	Origin	N2	PS	KS	ZnS	IAA	Sider	Reference
GAJ222	*Pseudomonas koreensis*	Rhizospher of *Phoenix dactylifera*	+	+	-	+	++	++	Ou-zine et al., 2023
GAB111	*Serratia nematodiphila*	Rhizospher of *Phoenix dactylifera*	+	+	+	+	+	+	

N2: non symbiotic nitrogen fixation; PS, KS and ZnS: phosphorus, potassium, and zinc solubilization, respectively; Sider: Siderophores production; IAA: 3-indol acetic acid production. (+++): high activity, (++): moderate activity, (+): low activity, and (-): non-detected activity.

Effect of the PGPR on the germination of the bread wheat seeds

Wheat seeds of the FAIZA variety were subjected to surface sterilization and then immersed in a PGPR suspensions of 10^8 CFU/mL for each tested bacterium (GAB111 and GAJ222) for one hour to ensure a consistent coating on the surface under aseptic conditions. Seeds soaked in sterilized distilled water were used as the control. Ten seeds were placed in Petri plates containing autoclaved filter paper and were incubated at 22°C. The progress of germination was observed over a 7-day incubation period to analyze the kinetics of germination (Buntić et al., 2019).

Inoculum preparation and experimental approach

The experiment was conducted in the pots of 8 kg (26 cm in length and 21 cm in diameter) placed in open field and filled with a dried Hamry soil that has been sieved to remove debris and large particles. The trial laid out in a Randomized Complete Block Design (RCBD) with three replicates. All treatments were assigned randomly within the block and each treatment was represented by two pots in each block. Each pot was considered as an experimental unit. The combination of these two treatments (PGPR & WRA) resulted twelve treatments (Table 2) that were applied with two replications for each block, resulting in a total of 72 pots (Figure 2).

Firstly, the seeds were washed in bacterial suspensions containing 10^8 CFU/mL for 30 minutes. Following this, six wheat seeds were planted at a depth of 3 cm in pots. Subsequently, 52 mL of the bacterial suspension was introduced into the wheat pots for inoculation. In cases where the treatments involved both WRA and the bacterial strain, these two components were thoroughly mixed to ensure complete contact between them. Nutrients were administered in all treatments according to the recommended application rates for nitrogen, phosphorus, and potassium (NPK) in bread wheat cultivation: 100 kg/ha of nitrogen, 60 kg/ha of phosphorus, and 40 kg/ha of potassium, respectively.

For the Water Retention Agent (WRA) treatment, the granules were placed deep within the pots and then covered with soil to prevent them from resurfacing. The product was applied in two doses (3 and 1.5 grams), which were mixed with the required amount of water. In the other set of pots, containing only the hydrogel, Water Hope was deposited with the appropriate dose, and nutrients (N-P-K) were added simultaneously.

After planting, each pot was watered with one liter of water. Subsequently, rainfall values in millimeters were recorded using data from the meteorological station at CRRA Meknes.

Table-2. Treatments used in this study.

N°	Treatment
T0	Negative control (without PGPR and WRA)
T1	Dose 1 of WRA (3 g)
T2	Dose 2 of WRA (1.5 g)
T3	PGPR strain GAB111
T4	PGPR strain GAJ222
T5	Combination of the two PGPR (GAB111+GAJ222)
T6	PGPR strain GAB111+ Dose 1 of WRA (3 g)
T7	PGPR strain GAB111+ Dose 2 of WRA (1.5 g)
T8	PGPR strain GAJ222+ Dose 1 of WRA (3 g)
T9	PGPR strain GAJ222+ Dose 2 of WRA (1.5 g)
T10	Combination of the two PGPR (GAB111+GAJ222) + Dose 1 of WRA (3 g)
T11	Combination of the two PGPR (GAB111+GAJ222) + Dose 2 of WRA (1.5 g)

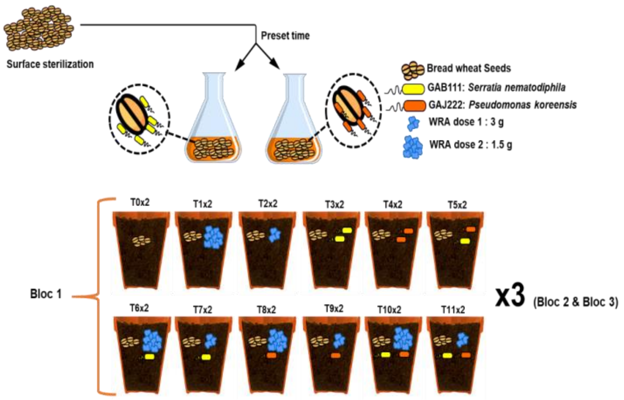

Figure-2. Experimental setup of the experiment (Randomized Complete Block Design (RCBD)).

Agronomic parameters collection

To assess the impacts of the two PGPR and the WRA under study on bread wheat cultivation, a range of agronomic parameters were considered. Measurements were conducted at four distinct time intervals: the initial sampling took place shortly after wheat seeding, the second sampling occurred after 60 seedlings had emerged (corresponding to the early stage of the growing season), the third sampling was performed at the end of the heading stage, and the final sampling took place during the harvest.

For the above-ground portion of the plant, the following parameters were measured for each pot (Alemu, 2018): stem length, number of stems, number of leaves, number of ears, chlorophyll content (measured using a SPAD meter, Reference: 20210036_0042, Maximum size: 63.5 × 42.33 cm/300 dpi), and leaf temperature. During the harvest, the focus was on assessing the weight of the plants, the number of seeds, and their respective weights.

For the underground portion of the plant, measurements included crown diameter, root length, and root weight, all of which were assessed during the harvest.

Chemical analysis

The samples tested belong to both treatment groups, including those with the two doses of WRA mixed with the fertilizers and those with only the water repellent. This entails assessing the polymer's capacity to retain fertilizers and facilitating a comparison between the two application methods of the water retainer (with and without fertilizers). Soil samples treated with bacterial preparations were also subjected to chemical analysis for the purpose of comparing the two strains used, namely GAB111 and GAJ222, which are distinguished by their respective abilities to fix atmospheric nitrogen and solubilize phosphorus.

The determination of potash content follows a specific protocol (Delaunois et al., 2008). This involves weighing 5g of dried and sieved soil from each sample. Subsequently, 50 ml of the Ammonium Acetate ($C_2H_7NO_2$) extraction solution is added to the samples, which were then stirred for 30 minutes and filtered. Calibration of the flame photometer is achieved using a range prepared with Potassium chloride (KCl).

The quantification of phosphorus content requires the creation of an extraction solution by dissolving 42g of Sodium dichromate ($Na_2Cr_2O_7$) in 1000 mL of distilled water. Each soil sample is carefully weighed to obtain 2.5 g, and then 50 mL of the extraction solution is added. After thorough mixing, the mixture is filtered into 250 mL flasks to remove any solid particles. Following this step, 1 mL of sulfuric acid (H_2SO_4) and 8 mL of the coloring solution (SPC) were introduced into each sample. The final volume is adjusted to 50 mL by adding distilled water. Subsequently, the resulting solution is allowed to stand for 10 to 15 minutes to develop the blue coloration. This coloration's intensity is then measured at OD_{820nm} using a spectrophotometer. A calibration curve is established beforehand using a stock solution of monopotassium phosphate KH_2PO_4 (Delaunois et al., 2008).

The measurement of organic matter content follows a specific protocol originally developed by Walkley and Black (1934). This method is based on the oxidation of carbon in the soil using potassium dichromate. The color change observed during this process is indicative of the quantity of reduced products, and this change in color can be correlated to the amount of organic carbon present in the soil. The percentage of organic matter in the soil is then calculated using the following formula: OM (%) =[(V(t)-V titrated) x 0.3] x 0.5; where 0.5g corresponds to the amount of soil, and V(t) represents the initial titrated volume of the solution.

The determination of total nitrogen content in the soil was conducted following the Kjeldahl method (Aguirre, 2023). In this method, the sample is mineralized in a sulfuric acid medium in the presence of copper (II) and a catalyst, namely titanium oxide. During mineralization, organic nitrogen is converted into the ammonium form. Subsequently, ammonium ions are transformed into ammonia when passed into an alkaline medium. The ammonia (NH_3) is then carried by water vapor, and the resulting condensate is quantified through volumetric acid/base titration. This analysis comprises three key stages: the mineralization stage, the distillation stage, and the titration step. The percentage of total nitrogen in the soil was calculated using the formula: (%) N = (X - Y) × N × 14 × 100 / Dry test portion (1000 mg), where X represents the volume of 0.1 N H_2SO_4 required to neutralize ammonia, Y corresponds to the volume of 0.1 N H_2SO_4 needed to neutralize the nitrogen traces in the control, N represents the normality, and 14 stands for the atomic mass of nitrogen.

Statistical analysis

The data collected was entered using EXCEL software. Agronomic parameters were subjected to a two-way analysis of variance (ANOVA), mixed model (replicates and treatments) with interaction to assess the effects of rhizobacteria and the water retention on wheat growth.

Results

Seed germination in petri dishes

The germination test conducted in Petri dishes revealed a substantial enhancement in the germination of bread wheat with the application of the two PGPR strains, demonstrating a significant difference compared to the control (Figure 3). The inoculation of the seeds with bacterial suspension results in a germination rate of 95% for the GAB111 strain (*S. nematodiphila)* and 92% for the GAJ222 strain (*P. koreensis)*, surpassing the negative control insulated with sterile distilled water (79%). Additionally, the inoculated treatments exhibited an earlier initiation of germination compared to the control.

Furthermore, the application of PGPR positively influenced both root and shoot lengths. In the control, the root length measured 1.3 cm, while in the inoculated treatments, it increased significantly to 3.5 cm. Similarly, the shoot length also experienced growth due to the PGPR isolates, albeit to a slightly lesser extent. In the control, the shoot length was 1.2 cm, which increased to 3 cm in the inoculated treatments.

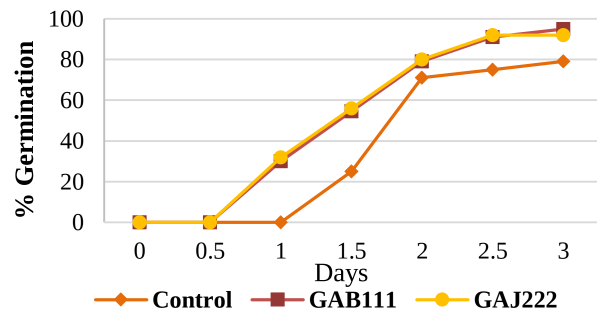

Figure-3. **Effect of PGPR on the germination kinetics of bread wheat seeds in Petri dishes.**
Control corresponds to the inoculation with SDW, GAB111 correspond to *Serratia nematodiphila* and GAJ222 correspond to *Pseudomonas koreensis*.

Field observation

The field observations from the trial highlight a substantial impact of the climatic conditions, spanning from February to June 2021, on the development of bread wheat. The most notable observation is a discernible shortening of the wheat development cycle when compared to the typical wheat growth cycle, as illustrated in Figure 4.

This deviation in the development cycle can be attributed to the specific climatic conditions prevalent in the region during the experiment. The summers are characterized by high temperatures and arid conditions, while the winters are cool. The average precipitation rate recorded during the trial period is 55 mm. Moreover, the minimal temperature is noted at 4°C, while the maximal temperature reaches 45°C. These climatic factors collectively contribute to an accelerated wheat development cycle. The higher temperatures and lower moisture levels, typical of the region, appear to play a significant role in shaping the growth patterns of bread wheat.

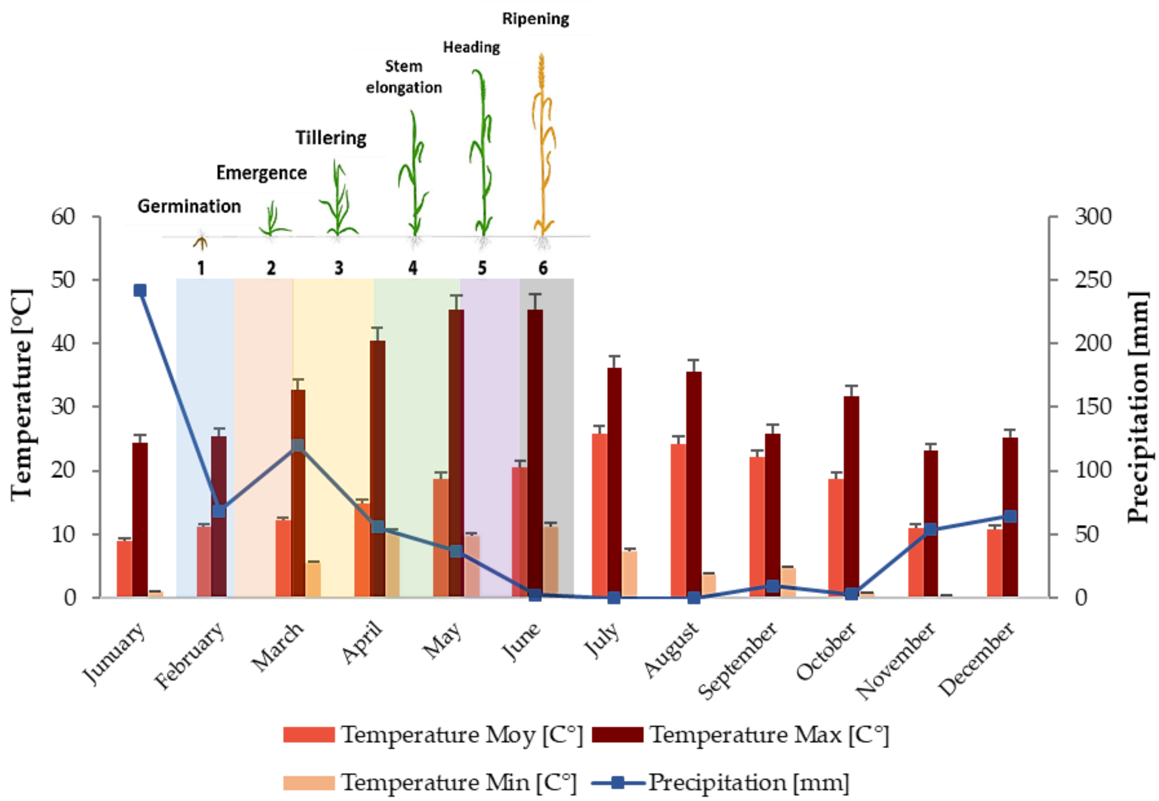

Figure-4. Phonological stage of bread wheat according to the climatic conditions in the 2021 agricultural campaign at INRA-Meknes.

Table 3. Descriptive analysis of all measured agronomic parameters.

Treatments	CC	CD	LT	LT°	NE	NL	NT	RL	WS
Max	57.07	7.50	85.33	18.83	20.00	107.33	26.00	19.67	29.48
Min	48.87	4.33	70.33	7.55	5.33	39.50	9.00	14.67	11.63
Average	53.05	6.52	78.81	12.61	12.57	73.81	18.43	17.58	21.55
STD	2.64	1.09	5.05	4.03	4.73	27.06	6.66	1.54	5.91

CC: Chlorophyll content; CD: Collar diameter (cm); LT: Length of tillers (cm); LT°: Leaf temperature (°C); NE: Number of ears; NL: Number of leaves; NT: Number of Tillers; RL: Root length; WS: Weight of seed (g)

Agronomic parameters

The investigation into the growth of bread wheat subjected to various treatments of PGPR and Water Retention Agents (WRA) demonstrates the significant impact of these components on the crop's morphological parameters. Analysis of the data, illustrated in Figure 5 and summarized in Table 3, which portrays the progression of measured parameters across different stages, underscores the considerable influence of all treatments (T1 to T11) on agronomic parameters compared to the negative control (T0). This finding is further substantiated by the results of the analysis of variance (ANOVA) presented in Table 4, which reveals statistically significant differences among the treatments.

The noteworthy effects observed on agronomic parameters highlight the potential of the tested treatments (WRA & PGPR) to enhance the growth and development of the crop. Specifically, the application of WRA at two different doses demonstrates a stimulating effect on wheat growth compared to the control. Moreover, the inoculation of the culture with the two strains GAB111 and GAJ222 manifests a positive impact across all measured parameters, further emphasizing the beneficial effects of these treatments on crop performance and productivity.

Table-4: **Analysis of Variance (ANOVA) for the treatments used in this study (WRA & PGPR).**

	df	CC	CD	LT	LT°	NE	NL	NT	RL	WS
Treatments	11	41.974	7.085*	153.146*	97.651*	134.499*	4392.389*	265.923*	14.318	209.358*
Rep	5	8.368	.350	16.556	1.869	8.847	45.789	17.114	35.300	51.708
Error	55	35.350	.281	12.580	2.040	6.181	109.419	6.708	12.136	26.920
R Squared		0.206	0.837	0.719	0.906	0.818	0.890	0.891	0.333	0.634

CC: Chlorophyll content; CD: Collar diameter (cm); LT: Length of tillers (cm) ; LT°: Leaf temperature (°C); NE: Number of ears; NL: Number of leaves; NT: Number of Tillers; RL: Root length; WS: Weight of seed (g). *****, Significance at *p-value*< 0.05.

Impact on number of leaves, tillers, and ears

The results reveal a significant positive impact of the WRA on various growth parameters throughout all sampling stages. Notably, in a marked increase in the length of tillers, the number of tillers, the number of leaves, and the number of ears in the crops.

In terms of dosage effects of WRA, the higher concentration (3 g/plant) exhibits a more pronounced influence on crop performance compared to the lower dose (1.5 g/plant). Into control plants, those treated with 3 g of WRA demonstrated substantial enhancements in the number of leaves (Figure 5a), registering a remarkable increase of 53.6% and 58.45%, while the 1.5 g dose exhibited growth percentages of 48.27% and 52% for the two sampling periods, respectively. This positive trend extends to the number of tillers, which consistently showed higher values compared to the control. The application of WRA at the 3 g dose resulted in percentage increases of 52%, 53.6%, 47.6%, and 44.4% across the four sampling stages. In contrast, the 1.5 g dose led to growth percentages of 40%, 40.9%, 33.3%, and 26.7% for the respective sampling periods.

Furthermore, the length of tillers (Figure 5b) exhibited a similar pattern, with the 3 g dose contributing to increases of 52%, 53.6%, 47.6%, and 44.4%, while the 1.5 g dose yielded growth percentages of 40%, 40.9%, 26.7%, and 33.3%, respectively, for the four samples. The WRA application also positively influenced the number of ears (Figure 5c), showcasing increases of 85.2%, 47.5%, and 46% for the 3 g dose, and 61.9%, 24.1%, and 18.2% for the 1.5 g dose across the four sampling periods.

Interestingly, the combination of the high dose (3 g) of WRA with the GAB111 strain (*S. nematodiphila*) demonstrated the most significant increase in length for all samples, with percentage increases of 27.1%, 14.9%, 9.1%, and 10.8%, while the 1.5 g dose showed growth percentages of 22.5%, 13.2%, 8.2%, and 8.7%, respectively. This suggests a potential synergistic effect between the high dose of WRA and the GAB111 strain on overall crop development.

The treatment involving the inoculation of wheat grains with both PGPR strains has demonstrated a notable improvement in the morphological parameters of wheat when compared to the non-inoculated control. Furthermore, the strain GAB111 (*S. nematodiphila*) exhibits a particularly significant impact compared to the GAJ222 (*P. koreensis*), and this effect is consistent across all sampled instances. Specifically, the application of strain GAB111 led to a substantial increase in the number of tillers, showing growth percentages of 25.0%, 31.6%, 23.1%, and 21.4%, whereas the application of strain GAJ222 resulted in comparatively lower growth percentages of 7.7%, 13.3%, 8.3%, and 9.1% (Figure 5d). This suggests that GAB111 has a more pronounced positive influence on tiller development compared to GAJ222.

Moreover, the combined treatment using both strains demonstrated a higher rate of increase than when each strain was applied separately. The percentage increase in the number of tillers was 25%, 23.5%, 26.7%, and 33.3% across the four samples. This indicates a potential synergistic effect when both strains are applied together, resulting in a more substantial improvement in the tiller count compared to individual strain applications.

Similarly, the combination of the two strains in the form of a bacterial suspension accentuated the number of leaves, showcasing growth percentages of 25.8% and 39.0%. These values surpass those obtained during separate inoculations, where the growth percentages were 23.3% and 31.9% for GAB111 and 6.3% and 3% for GAJ222. This underscores the potential cooperative effect of the two strains when applied together, resulting in a more robust enhancement of the leaf count compared to individual applications.

Impact on chlorophyll content

Following the application of the WRA agent in both doses (3 and 1.5 g) and the growth-stimulating strains (GAB111 and GAJ222) along with their combination, a substantial increase in chlorophyll content was recorded. Specifically, it reached 22.8% and 16.8%, successively, for doses 3 and 1.5 g. Additionally, the combination of the two PGPRs further elevated the chlorophyll content by 24.3%. Consequently, the data indicates that the impact of bacterial strains is fortified by the addition of Water Hope at a 3g dose, increasing the average from 24.2 to 27.6% for GAB111 and from 20.3 to 21.9% for GAJ222 (Figure 5).

Influence on leaf temperature

In terms of leaf temperature, there was an observable increase compared to the control. Water Hope facilitated a rise of 28.9°C for the 3 g dose and 16.8°C for the 1.5 g dose. Similarly, the combination of the two bacteria resulted in a temperature increase of 30.7%. On the other hand, the wheat semi with the composition: hydro-repellent-bacterium with a 3 g dose contributed to higher leaf temperatures, registering 31.9°C for strain GAB111, 27.6°C for GAJ222 strain, and 31.9°C for their mixture. In contrast, the temperatures were 26.8°C and 24.5°C, respectively, for both strains (Figure 5f). This suggests that the synergistic application of Water Hope and bacterial strains has a positive impact on both chlorophyll content and leaf temperature, indicating enhanced physiological processes in the wheat crops.

Effect on wheat yield and plant-roots development

The study highlights that the combination of WRA with PGPR yielded promising results, primarily attributed to the polymer's ability to retain bacteria at ground level. This is evident in the Figure 6, illustrating that the strains independently promote plant growth compared to the control, while their incorporation with WRA further amplifies the parameters under consideration. Taking strain GAJ222 as an example, it is observed that their integration with the water repellent significantly enhances yield components.

Concerning the components of yield, all the implemented treatments demonstrated a positive impact on the overall seed weight compared to the control group. Specifically, the application of WRA at both doses (3 g and 1.5 g) resulted in respective weight increases of 55.7% and 37.35%. Inoculating the two bacterial strains individually and their combination also enhanced the total seed weight, with percentage improvements of 32.29%, 17.14%, and 37.63%, respectively. Furthermore, combining the WRA with various bacterial suspensions further amplified grain weight, showcasing increases of 60.7% and 56.8% when combining GAB111 with both doses, and 52.8% and 48.2% for GAJ222 (Figure 6a).

The study also observed clear responses in root length and collar diameter to the different treatments. The application of the water retainer at a 3g dose generated increases of 19.7% and 41.2% for these two parameters, indicating a positive effect on root development. The introduction of PGPR mixed with the WRA also positively influenced root length and crown diameter, particularly for the GAB111 strain, where percentage increases were 14.6%, 12.2%, 42.7%, and 41.6% for the dose 3 g and 1.5 g, respectively. This improvement surpassed that achieved with the GAJ222 strain, which recorded increases of 9.7%, 6.5%, 42.3%, and 37.8% for the same parameters and doses. Similarly, the combination of both bacteria and WRA resulted in successive increases of 24.0%, 21.0%, 41.1%, and 39.3% for the two parameters and doses (Figure 6b).

The graph below illustrates the weight of the plants and roots at harvest based on the applied treatments. Notably, both parameters exhibited increases compared to the control, with percentage increases of 53.8% and 33.9% for plant weight, and 46.5% and 38.4% for root weight, respectively, for the two doses of WRA. Furthermore, the application of growth-stimulating strains in the form of a bacterial suspension demonstrated positive effects, with an increase of 29.8% and 36.1% recorded for the GAB111 strain, compared to 7.4% and 29.2% for GAJ222. The combination of these strains resulted in a significant increase of 36.8% and 44.3% for plant weight and root weight. Combining these PGPRs with the water retainer contributed to an improvement in both aerial and root weight, with increases ranging from 52.4% to 55.7% in the case of mixing GAB111 with both doses, 52.7% to 50.4% for GAJ222, and 52.4% to 51.5% for the combination of both bacteria. Likewise, the root part exhibited marked increases compared to the control treatments, with percentage increases of 65.9%, 52.0%, 60.6%, 46.5%, 63.7%, and 58.9% in treatments combining GAB111, GAJ222, and their mixture with both doses of WRA (Figure 6c).

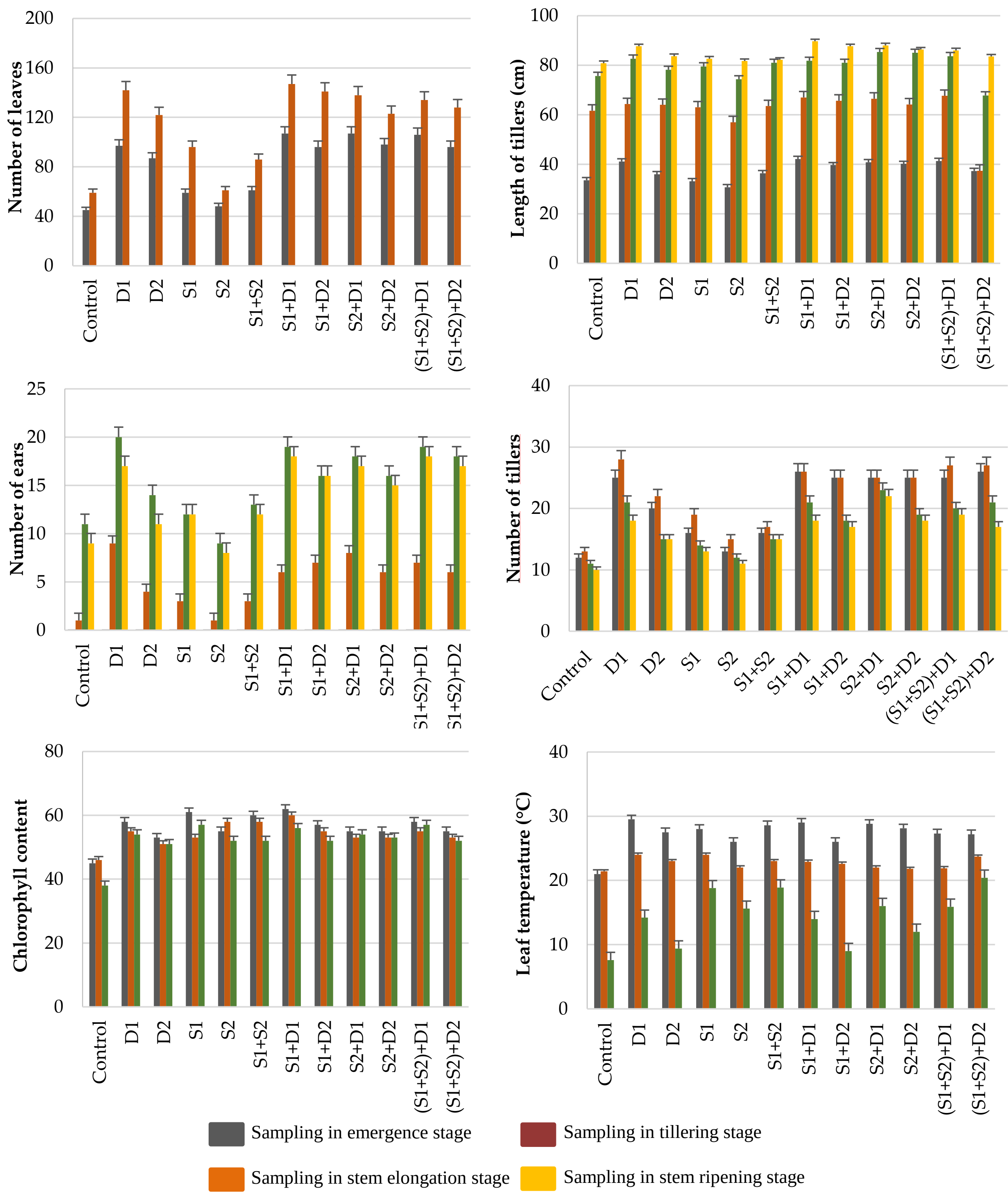

Figure-5. Effect of applied treatments on wheat growth by measuring agronomic parameters. The diverse colors of the histograms correspond to different sampling periods and the measurement of agronomic parameters at various wheat phenological stages. The code for various treatments is: D1: dose 1 of WRA (3 g); D2: dose 2 of WRA (1.5 g); S1: strain GAB111 (*S. nematodiphila*); S2: strain GAJ 222 (*P. koreensis*). The combination of the treatments corresponds to the synergic effect of their application.

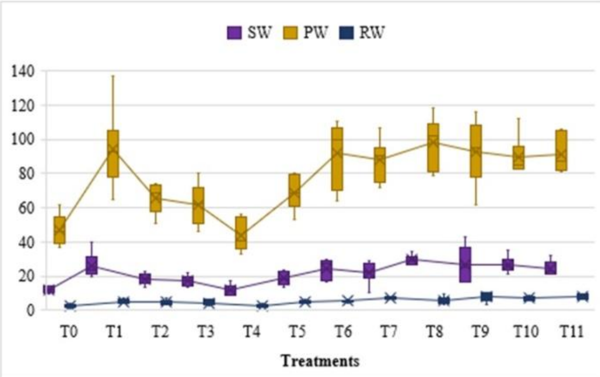

Figure-6. **Graphical presentation in boxplots of the evaluation of wheat yield and plant-roots in different treatment (T0 to T11). SW: seed weight; PW: plant weight; RW: root weight (RW). Treatments T0 to T11 correspond to the treatments mentioned in Table 2.**

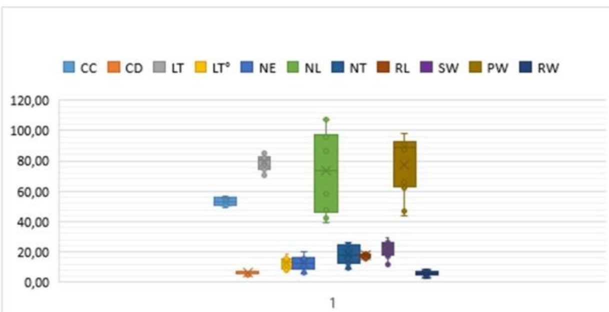

Figure-7. Comparative boxplots analysis of the variation of the different agronomic parameters measured in this study. CC: Chlorophyll content; CD: Collar diameter (cm); LT: Length of tillers (cm); LT°: Leaf temperature (°C); NE: Number of ears; NL: Number of leaves; NT: Number of Tillers; RL: Root length; SW: Weight of seed; PW: weight of the plant; RW: Weight of the roots.

Chemical analysis

The results of our data analysis unequivocally demonstrate the significant impact of treatments involving WRA and PGPR on various chemical properties of the soil, as delineated in Figure 8 and detailed in Table S1. Notably, this effect was observed within the soil depths of 30-60 cm in the sampled pots.

The utilization of both doses of WRA, along with the application of the two PGPR strains, either individually or in combination, led to a noteworthy enhancement in soil organic matter content. Statistical analysis revealed significant increases with F-values of 35.9 ($p<0.001$), 50.5 ($p<0.001$), and 49.9 ($p<0.001$), respectively. The observed concentrations of organic matter varied across treatments, with the negative control group (lacking WRA and PGPR) exhibiting the lowest content at 0.32%. Conversely, the highest dose of WRA (3 g) demonstrated the most substantial increase, reaching 0.93%. Similar trends were observed for the lower dose of WRA (1.5 g), strain GAB111, strain GAJ222, and various combinations thereof, underscoring the nuanced effects of each treatment regimen on soil organic matter enrichment.

Subsequent soil analysis following the incorporation of WRA and bacterial inoculation revealed a positive stimulation in nutrient contents compared to the control group, with statistically significant F-values of 40.35 ($p<0.001$) and 26.23 ($p=0.001$), respectively. Noteworthy findings include strain GAB111, renowned for its nitrogen-fixing ability, recording the highest nitrogen content at 7.5 g/kg, while strain GAJ222, with phosphorus solubilization capabilities, generated the highest phosphorus content at 2.1 mg/kg. Additionally, strain GAB111 significantly increased potash content to 0.9 mg/kg, compared to 0.75 mg/kg with GAJ222. Similarly, the addition of the absorbent polymer (at 3 g and 1.5 g doses) combined with PGPR (T10 & T11) resulted in substantial increases in nitrogen, phosphorus, and potash levels.

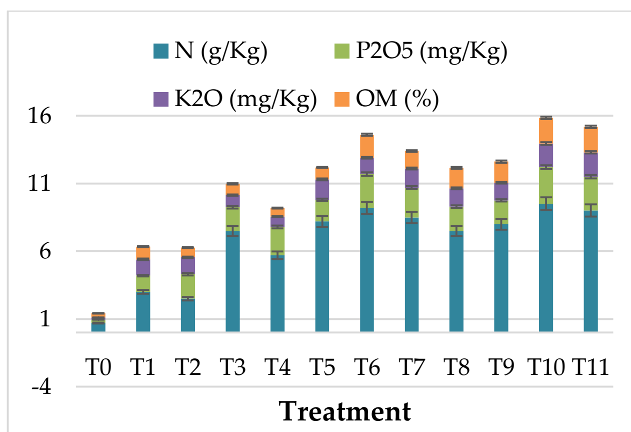

Figure-8. **chemical properties of the soil from different treatment. Treatments T0 to T11 correspond to the treatments mentioned in Table 2. N: nitrogen; OM: organic matter; P_2O_5: phosphorus; K_2O: Potassium.**

Discussion

The sensitivity of wheat bread to drought stress poses a substantial challenge to agricultural productivity, particularly in regions susceptible to water scarcity (WBG, 2021). Drought stress significantly hampers wheat growth and can result in yield losses (Jäger et al., 2014; Hssaisoune et al., 2020). In addressing this critical concern, the role of Plant Growth-Promoting Rhizobacteria (PGPR) and water retention agents emerges as a promising avenue. PGPR, known for their beneficial interactions with plants, can enhance drought tolerance by promoting root growth, nutrient uptake, and stress response mechanisms (Zheng et al., 2018). Additionally, the incorporation of water retention agents in soil management holds potential for mitigating the adverse effects of water scarcity by maintaining optimal soil moisture levels (Azeem et al., 2023). This discussion delves into the pivotal importance of PGPR and water retention agents as strategic tools to bolster the resistance of wheat bread against drought stress, ultimately contributing to the sustainability and resilience of agricultural systems.

In the present study, the utilization of Plant Growth-Promoting Rhizobacteria (PGPR) strains, specifically *Serratia nematodiphila* (GAB111) and *Pseudomonas koreensis* (GAJ222), has manifested a pronounced positive influence on the germination of wheat bread seeds. The germination test conducted in Petri dishes revealed a significant augmentation in germination rates compared to the control treated with sterile distilled water. Moreover, the PGPR-treated samples exhibited an earlier initiation of germination in comparison to the control, signifying a stimulating effect on the germination process. The facilitation of germination by these two PGPR strains involves diverse mechanisms. These bacteria contribute to an enhanced seed germination by producing phytohormones such as auxins, cytokinins, and gibberellins, thereby facilitating cell elongation and division (Orozco-Mosqueda et al., 2023). Additionally, both strains play a pivotal role in nutrient mobilization, involving the solubilization of phosphate and nitrogen fixation, ensuring optimal nutrient availability for germinating seeds (Timofeeva et al., 2023). The heightened water uptake facilitated by specific PGPR strains contributes to the effective rehydration of seeds, while their enzymatic activities assist in breaking down seed coat barriers. Interactions with plant growth substances additionally modulate physiological processes during germination (Paravar et al., 2022).

Numerous studies have highlighted the positive effects of these PGPR strains on seed germination, early seedling vigor, and overall plant health. For instance, in wheat cultivation, inoculation with *S. nematodiphila* and *P. koreensis* has been associated with increased germination rates, improved root development, and enhanced resistance to certain environmental stresses. Beyond wheat, similar beneficial impacts have been observed in various crops, including maize, rice, and soybeans, showcasing the broad applicability of these PGPR in diverse agricultural contexts. The application of *Serratia* sp. and *Pseudomonas* spp. significantly promoted root elongation and nutrient uptake in wheat plants (Zahir et al., 2009). The enhanced nitrogen-fixing and phosphate-solubilizing activities of these bacteria were correlated with increased crop yields and improved nutrient utilization efficiency. Additionally, the production of phytohormones by these PGPR strains was associated with positive modulation of plant growth and development (Mehmood et al., 2023).

The results of the study on the agronomic parameters and chemical analysis of bread wheat exposed to different treatments of Plant Growth-Promoting Rhizobacteria (PGPR) and Water Retention Agent (WRA) reveal significant effects on various growth and physiological aspects of the bread wheat. This investigation unveiled that each of the examined treatments exerts a notable influence on growth parameters.

Based on our findings, the two tested doses (3 g and 1.5 g) exhibited distinct effects on all evaluated parameters, surpassing the control treatment. The utilization of WRA in soil represents an innovative approach to enhance moisture management in agriculture. These agents, acting as reservoirs for water, contribute to increased soil moisture content, fostering optimal conditions for plant growth (Scholz, 2022). The augmented soil moisture, facilitated by water retention agents, plays a dual role in regulating leaf temperature by improving transpiration efficiency and promoting photosynthesis (Tariq et al., 2023; El Bergui et al., 2023). This integrated impact not only mitigates the risk of heat stress on plants but also enhances their carbon assimilation processes, ultimately leading to improved growth and productivity. The application of water retention agents exemplifies a holistic solution for sustainable agriculture, addressing both soil moisture concerns and promoting key physiological processes in plants (Bhatnagar et al., 2016).

These outcomes align with findings in corn cultivation, where the Super Absorbent Product (SAP) maintained soil surface temperatures at a depth of 0-20 cm. With these diverse attributes, SAP holds the potential to alleviate water scarcity issues, proving advantageous in arid and semi-arid regions (Yang et al., 2020). The application of WRA at a low dose of 1.5 g proved effective in enhancing wheat yield, even under suboptimal conditions. This observation is consistent with the results obtained by Abrisham et al. (2018), where a reduced input of the SAP improved soil moisture, raising the moisture content by up to 68.5%. This was achieved by concurrently decreasing the infiltration rate and soil apparent density by 21.5% and 25.5%, respectively, along with a reduction in economic loads.

Furthermore, our study demonstrates that WRA positively influences growth indices and germination percentage under water stress conditions. Seed germination, being a pivotal stage in crop growth and development, faces limitations in establishing vegetation cover in coarse-textured soils and arid areas due to low moisture levels (Akhter et al., 2004). In line with this, Yang et al. (2020) emphasized that an optimal amount of SAP enhances germination rate, budding time, flowering time, and overall plant growth. However, an excessively high amount of SAP inhibits germination, root elongation, and physiological functions, thereby reducing the emergence and survival rates of plants.

Hydrogels, such as the one used in this study, also exhibit versatility as they can be employed in wastewater purification, seed treatment, and serve as a biological insecticide against fungal infections. The incorporation of the hydrogel into loamy soil favored the germination of barley, wheat, and chickpea seeds, delaying seedling wilting by 4 to 5 days. This delay is induced by a minor loss of soil moisture, resulting in increased length, fresh and dry weight of wheat compared to the control. This growth trend is further pronounced with an elevation in frost levels (Su et al., 2017).

Our findings align with the study by Yang et al. (2014), confirming the positive impact of SAP content on the germination and survival rates of grasses. The enhanced soil moisture can be attributed to the water-repellent property of the hydrogel, allowing it to rapidly absorb rainwater or irrigation in scarce conditions and gradually release it according to the specific needs and levels of the crops (Tejada and Gonzalez, 2007). These results are in line with those obtained in our study; this can be explained by the essential contribution of water in maintaining cellular turgescence, the circulation of nutrients and organic compounds involved in the photosynthesis and transpiration of plants and protected against large temperature fluctuations (Mnyika, 2020). In addition, the incorporation of the soil with WRA resulted in a clear improvement of the indicators studied (dry weight, chlorophyll content, relative water content in the leaves) with an increased resistance to water scarcity (Panagea et al., 2021).

Our investigation indicates a substantial enhancement in wheat growth with the application of WRA, consistent with earlier research emphasizing WRA's significant role in promoting crop growth (Yazdani et al., 2007). Another noteworthy attribute of WRA is its capacity to retain nutrients and microorganisms. In this context, Li et al. (2014) conducted a study examining changes in soil physical properties, microbial activity, and soil biomass subsequent WRA application. The results revealed that WTA application facilitated the formation of 0.25 mm soil particles (macro aggregates) and increased the abundance of bacteria in soil cultivated with winter wheat. This aggregating capability arises from WRA's progressive penetration of aggregate pores, ensuring pore continuity (Li et al., 2019). Significantly, WRA induced a marked increase in soil moisture content and maximum hygroscopic humidity. Furthermore, WRA's positive impact on microbial activity was evident, as indicated by a significantly higher number of RNA gene copies compared to the control group.

According to Zheng et al. (2009), super-absorbent polymers present an effective solution to address the losses associated with conventional fertilizers, such as leaching, pollution, and eutrophication of lakes and reservoirs. Studies investigating the impact of incorporating natural substances and micro-absorbent gel antagonists on the germination of Phaseolus vulgaris seeds have demonstrated promising results.

The study's exploration of the long-term effects of agricultural interventions on soil health, microbial communities, and agricultural sustainability aligns with broader discussions in agricultural science literature. Sustainable agriculture endeavors to uphold or enhance soil quality and fertility while minimizing detrimental environmental impacts (Iqbal et al., 2023). The substantial use of agricultural inputs like fertilizers and pesticides can profoundly influence soil microbial communities, nutrient cycling processes, and overall ecosystem health (Jeyaseelan et al., 2024). Moreover, unsustainable farming practices may lead to soil degradation, resulting in reduced agricultural productivity, biodiversity loss, and environmental harm (Alam, 2014). Thus, it's imperative to assess the enduring impacts of agricultural activities on soil health and microbial communities to ensure the resilience and sustainability of agricultural systems (Nadarajah and Abdul Rahman, 2023). By prioritizing regenerative practices that support soil health, farmers can enhance crop yields, mitigate climate change effects, and foster environmental sustainability for future generations (Khangura et al., 2023).

Conclusion

This study illuminates the significant potential of integrating Water Retention Agent (WRA) and Plant Growth-Promoting Rhizobacteria (PGPR), specifically the *S. nematodiphila* and *P. koreensis* strains, to enhance wheat growth amid challenges posed by water scarcity and salinity. The observed synergistic effects, encompassing improved soil fertility, enhanced nutrient availability, and heightened crop performance, underscore the practical viability of this innovative approach. The dose-dependent efficacy of the water repellent polymer underscores the critical need for optimizing application rates to maximize benefits. The demonstrated multifaceted advantages of the two strains highlight the adaptability of rhizobacteria in addressing diverse challenges encountered by crops. Future research should focus on refining application protocols, exploring additional PGPR strains, and evaluating the long-term impacts on soil health and overall ecosystem sustainability. Moreover, scaling up these eco-friendly inputs for widespread field application and conducting economic analyses are pivotal steps toward realizing the practicality and feasibility of this integrated approach for sustainable and resilient agriculture. Overall, this study provides valuable insights and lays the foundation for further advancements in eco-friendly strategies to optimize wheat productivity in the face of challenging environmental conditions, particularly in the context of climate change-induced stress.

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Contribution of Authors

Habbadi K: Conceptualized study, designed research methodology and software analysis, data curation, validation and analysis and original draft preparation
Idrissi MGE: Designed research methodology and software analysis
Houssaini SEIE: Data validation and analysis
Maafa I: Formal analysis and data curation
Aoujil F: Manuscript Writing
Benbouazza A: Designed research methodology
Achbani EH: Conceptualization of study and manuscript editing
Ferrahi M: Literature review and provision of resources

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