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Research Article

Effect of Different Bacillus Species on Morphological Parameters, Chlorophyll Content and Relative Water Content of Rice under Water Stress Condition

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Abstract

Multiple factors are threatening food security through climate change. Potential effect of this is seen in the variability of rainfall received with drought as the most critical factor that is affecting the growth and yield of plants. Plant growth promoting bacteria (PGPB) are taunted as one of the most promising organisms to counter the drastic effect of climate change especially in drought condition. They facilitate the growth of plants and helps alleviate the stresses brought by abiotic stress conditions. Three previously isolated Bacillus species labeled (SA1 (Bacillus subtilis), SA3 (Bacillus niacini) and SB1 (Bacillus cereus)) were tested for plants growth promoting enhancement in rice under water stress condition. Growth parameters; Root length, shoot length, fresh of root and dry weight of root were all tested using standard techniques. Chlorophyll content was measured using SPAD chlorophyll meter while relative water content of the leaves was calculated using a standard formula. Among all the three Bacillus species tested, Bacillus subtilis (SA1) was found to enhance all the measured growth parameters, relative water content and chlorophyll content in the rice under water stress condition to a significant extent when compared with other Bacillus species. Therefore, Bacillus subtilis (OM184294) could be a potential candidate as PGPB especially under drought conditions.

Nomenclature and units

1.0 Introduction

Water demand has been on increase in the past decades because of the increase in population as well as intense agricultural demands from irrigation services (Dau et al., 2021). Aggravated socio-economic development fueled by industrialization has further worsened the situation as a result of climate change. The climate change has drastically introduces variability in the amount of rainfall received at different parts of the world (Wadanambi et al., 2020). Furthermore, higher temperatures, desertification, erosion and rise in the sea level are all factors that have negative impact on the agricultural crops and thus threaten food security in the entire world (Abbas et al., 2022).

Drought is one of the major consequences of climate change especially in arid and semiarid regions of the world. Other factors that precipitates drought conditions includes lack of access to irrigation water, salinity and low and high temperatures (Abdelaal et al., 2021). However, even in the presence of sufficient amount of water, a phenomenon known as physiological drought occurs in which the plants are unable to absorb the water (Xiaolong Yang et at., 2019). Consequences of drought condition to plants are manifested in physiological, biochemical and morphological features. In response to drought situation, plants react by reducing the amount of water loss to the atmosphere through transpiration by closing their stomata, a process that negatively affects the amount of CO_2 uptake as well as photosynthesis (Xinyi Yang et al., 2021). Leaf development is hampered which further affects photosynthesis and overall development of the plants. General assimilation of mineral nutrients could not be attained because the medium through which they are absorbed is not available. The overall consequence of drought is the death of the plants (Elakhdar et al., 2022).

In order to alleviate the effect of various stresses, plants have adopted various mechanisms including the expression of stress response genes such as superoxide dismutase, catalase, ascorbate peroxidase and glutathione peroxidase (Ghouri et al., 2022). These genes products allow the plants to neutralize the negative consequences of various radicals produced during the stressed period. Micro and macronutrients such as Zinc, Selenium, Magnesium, Boron, Potassium, Calcium and Manganese help the plants attain high water status through stomata and physiological alterations of ion homeostasis in plants (Kumari et al., 2021). Moreover, group of free living and natural inhabitant of the soil known as plant growth promoting rhizobacteria (PGPR) are group of bacteria that were found to help plants overcome the negative effects of some abiotic stresses especially drought (Ahluwalia et al., 2021). PGPB were found to cut across different species of bacteria including *Bacillus*, *Klebsiella*, *Azotobacter* and many more (Goswami & Suresh, 2020).

We recently isolated and characterized plant growth promoting *Bacillus* species from soil in Kura Local Government, Kano State, Nigeria labeled SA1, SA3 and SB1 (Abdullahi et al., 2022). These isolates were fully characterized for growth promoting properties such as; Phosphate solubilization, Zinc solubilization, ammonia production, hydrogen cyanide production, Indole acetic acid production which made them ideal candidates as plants growth promoting rhizobacteria. They were further analyzed at molecular level for specie identification using 16SrRNA. Phylogenetic analysis was performed in order to unravel the specie of these three isolates and they were identified as *Bacillus subtilis*, *Bacillus niacini*, and *Bacillus cereus* with accession numbers OM184294, OM1842295 and OM184296 respectively (Abdullahi et al., 2022). In this research, we investigated the effect of these *Bacillus* species on chlorophyll and other morphological growth parameters of rice under water stress condition.

2.0 Materials and Methods

2.1 Study site

The experiment was conducted at Plant Science Department, Bayero University, Kano, Nigeria, 11.9645° N, 8.4309° E.

2.2 Methods

2.2.1 Soil Preparation and Sowing

Soil was collected about 0-30cm surface layer in the botanical garden of Plant Biology Department, Bayero University Kano. The soil was air-dried, crushed to pass through 4mm diameter sieve and mixed thoroughly. Plastic pots (5L) were then filled with the soil, transferred to screen house and irrigated to field capacity. Five seeds of rice (Faro 44) were then planted in the pots and after germination, thinned to three plants per pot (Ikhajiagbe et al., 2021).

2.2.2 Bacterial Treatment and Experimental Design

A total number of forty (40) pots were used in the study. The experimental plants were arranged in a completely randomized block design (CRBD) with three (3) replicates per treatment (Batool et al., 2020). Bacterial suspension (10^8 CFU) was applied directly on the soil (Soil Inoculation). A drought treatment was then imposed by withholding irrigation in the potted plants after the application of bacterial suspension at the vegetative stage of development. Morphological parameters and relative water content and chlorophyll contents were measured after stress was induced 30 days after germination of plant.

2.2.3 Measurement of Growth Parameters

At the end of the experiment, the shoots and roots were detached from each other and cleaned from the mud and their fresh weight was immediately measured after 0, 3, 6 and days of treatment (Batool et al., 2020).

2.2.4 Measurement of Relative Water Content

The relative water content of leaf was also calculated according to the following equation $(RWC) = 100\times (FW-DW)/(TW-DW)$.

Where FW is the fresh weight, DW is the dry weight, and TW the turgid weight (Yamasaki & Dillenburg, 1999).

2.2.4 Measurement of Chlorophyll content

Chlorophyll content was determined using SPAD chlorophyll meter for each of the stress levels (Uddling et al., 2007).

Statistical Analysis:

Graphs and tables were used for data presentation. Morphological parameters, Chlorophyll content and relative water content were represented using bar charts; analysis was done using one way ANOVA with replications ($p < 0.05$). The statistical package used was genstat.

3.0 Results

3.1 Morphological Properties of the Rice under Water Stressed Condition

Figure 1, 2, 3, and 4 shows the effect of different *Bacillus* species (SA1, SA3 and SB1) on some growth parameters in rice under water stress condition.

3.1.1 Shoot Length

The result of the effect of PGPR inoculation on the Shoot length of drought stressed rice plant was presented in figure 1. From the result, it was observed that rice plant treated with PGPR show a significant difference as compared with untreated control. 0 day stress shows no level of significance in both treated and untreated groups. 3 days treatment show significant difference compared with control. With SA1 having the highest value. The highest effect of PGPR is seen in 9 days treatment with SA1.

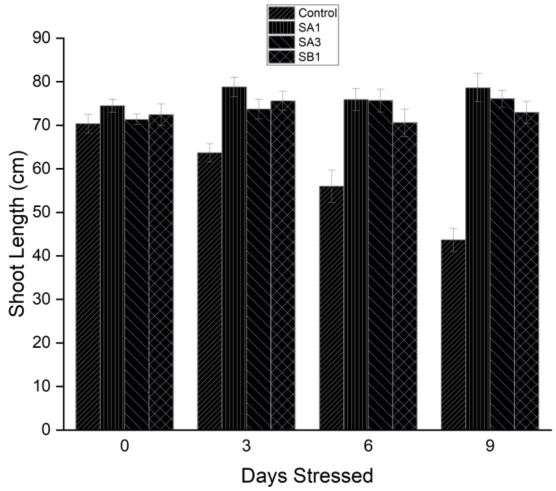

Figure 1: Shoot length of rice plant treated with PGPRs under 0, 3, 6 and 9 days of water stress.
Values are presented as Mean±SD

3.1.2 Root Length

The result of the effect of PGPR inoculation on the root length of the water stressed rice plant was presented in figure 2. From the result, it was observed that root length show significant difference in treated plant compared to untreated control. The highest effect of PGPR was recorded in 9 days stress, SA1 having the highest value (15.67 ± 0.4) followed by SB1 (15.34 ± 0.6).

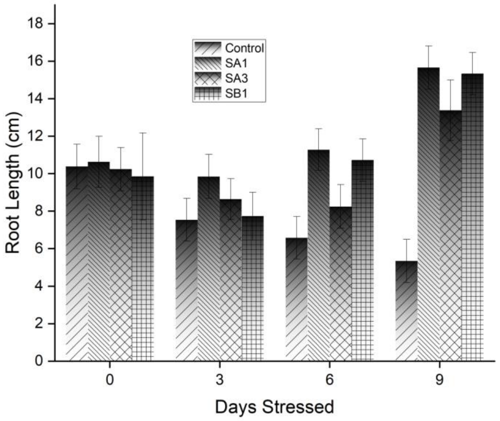

Figure 2: Root length of rice treated with PGPR under 0, 3, 6 and 9 days of water stress.
Values are presented as Mean ± SD.

3.1.3 Fresh Weight of Root

The result of the effect of PGPR inoculation on fresh weight of root of drought stressed rice plant was presented in figure 3. It was observed that PGPR treated rice plant is significantly different from untreated control. The highest effect is recorded in 0 day stress with SA1 treated group (4.0 ± 0.12) having the highest value of significance. SA1 treatment has the highest fresh weight of root in all the levels of stresses.

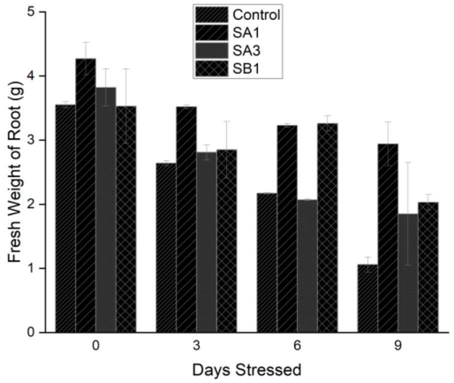

Figure 3: Fresh weight of root of rice plant treated with PGPR under 0, 3, 6 and 9 days of water stress.
Values are presented as Mean ± SD

3.1.4 Dry Weight of Root

The result of the effect of PGPR inoculation on root dry weight of water stressed rice plant is shown in figure 4. The result for dry weight of root show significant different in treated group as compared with untreated control. The highest effect was recorded in 0 day stress with SA1 treatment having the highest value (2.36 ± 0.2) followed by 3 days treatment (1.73 ± 0.13). 3 days stress showed SA1 having the highest dry weight of root as compared with other treatment group. SA1 traetment has the highest dry weight in all the stress levels.

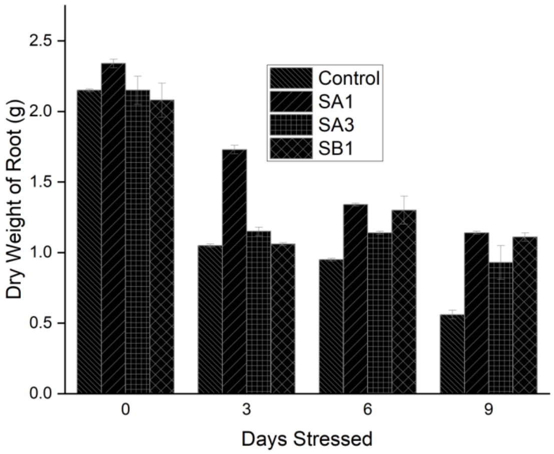

Figure **4:** Dry weight of root of rice plant treated with PGPR under 0, 3, 6 and 9 days of water stress.
Values are presented as Mean ± SD

3.1.5 Relative water content

Figure 5 presents the result of relative water content of rice under variable drought stress conditions. Highest effect was recorded in 3 days stress treated with SA1 PGPR.

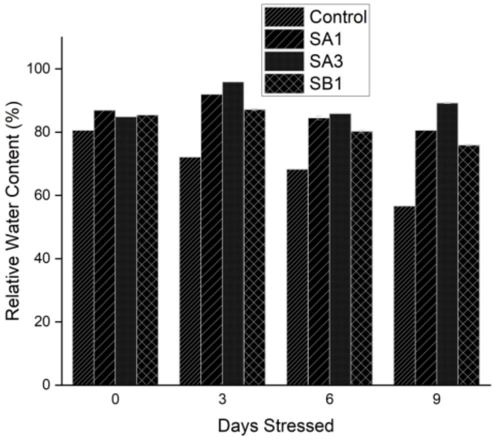

Figure 5: Relative water content of rice plant treated with PGPR under 0, 3, 6 and 9 days of water stress.
Values are presented as Mean± SD

3.1.6 Chlorophyll content

The effect of inoculation of PGPR on chlorophyll content of water stressed plant is shown in figure 6. Chlorophyll content shows highest level of significant difference in 0 day+SA1, 0 day +SA3, 6days +SA3 and 0 day +SBI, compared with untreated control. 6 days stress (11.11 ± 0.40) treated with SA3 have the highest mean value as compared with control. There was significant increase in the chlorophyll of stressed plant inoculated with bacteria compared with stress without inoculation.

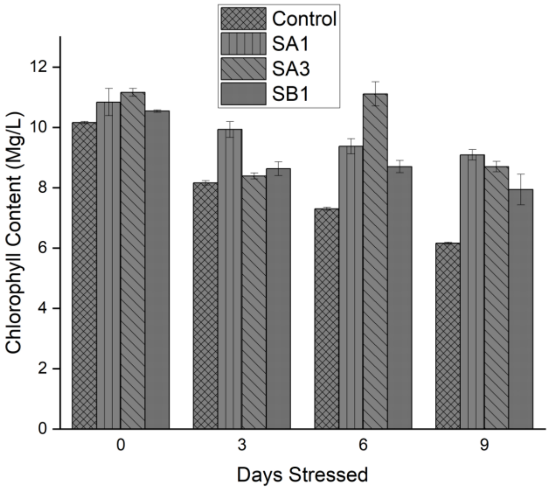

Figure 6: Chlorophyll content of rice leaf treated with PGPR under 0, 3, 6 and 9 days of drought stress. Values are presented as Mean ±S D

4.0 Discussions

Drought is the most devastating abiotic factor threatening the growth of rice in different parts of the world and also a major threat to global food security (Panda et al., 2021). Various efforts towards mitigating drought stress in rice are being utilized, including the use of plant growth promoting rhizobacteria (PGPR). Out of the 12 bacterial strains isolated in the previous study, three were found to possess most of the properties needed to influence the growth of plants under certain stresses such as drought (Abdullahi et al., 2022). PGPR influences the growth of plants by making nutrients such as nitrogen and phosphorous available to the plants by solubilization, an effect termed direct mechanism (Shultana et al., 2020). This is expected to mitigate the effect of the drought and influence the growth of the plant even under stressful condition. In this study, the effect of isolated PGPRs on chlorophyll content and morphological parameters was investigated on rice exposed to drought stress. All the physiological parameters tested including; shoot length, root length, fresh weight of root, and dry weight of root show positive effect in PGPR inoculated groups when compared to the control group.

Increased in shoot and root length was observed in rice exposed to drought stress for varying periods and then treated by inoculation with different PGPR isolates when compared with the control (Figures 1 and 2). This enhancement in length of root and shoot could possibly be attributed to the phytohormone production by the PGPR which led to more root exudates making a favorable habitat for the microorganisms to grow. It is seen possibly that the inoculated PGPR strains have affected the root hormone levels by producing enhanced IAA and/or other plant hormones in the rhizosphere, which were then absorbed by the root (Rehman et al., 2020). This result showed that *Bacillus spp*, especially the *Bacillus subtilis* (SA1), was able to promote the shoot and root growth of rice plants. Similar result was obtained in maize treated with *Bacillus* species (Azeem et al., 2022). Analysis of fresh root weight and dry root weight is another important parameter to evaluate the effect of PGPR on plants. Similar results as found in root length and shoot length were obtained with *Bacillus subtilis* (SA1) having a greater effect than *Bacillus niacini* (SA3) and *Bacillus cereus* (SB1) (Figures 4 and 5). Similar effect of PGPR was obtained in a study carried out on Lentil by Erman et al., (2022).

Furthermore, it is related that plants associated with PGPR display better relative water content, especially in abiotic stress situations (Borzoo et al., 2021). In this research, rice cultivar inoculated with *B. subtilis* showed highest relative water content than non-inoculated plants under water stress (Figure 5). Relative water content reflects the leaf water status and the enhancement in this variable, due to inoculation with *B. subtilis.* , can represent an important strategy to tolerate water stress (Al-Garni et al., 2019).

Chlorophyll, a photosynthetic pigment, is involved in light absorption and plays an important role in plant photosynthesis. As drought stress can accelerate chlorophyll decomposition, chlorophyll content has been reported as the most frequently used metrics for ascertaining the severity of drought stress (Ali et al., 2021). In this study, a gradual decrease in chlorophyll content was found when water stress was induced. But there was increase in the chlorophyll content in inoculated rice plants especially in *B. niacini* and *B. subtilis* treated groups. The enhanced chlorophyll may increase the photosynthetic efficiency of inoculated plants, and thus may be a reason for the tolerance of abiotic stress.

5.0 Conclusion

The growth promoting properties of previously isolated strains of *Bacillus* (SA1, SA3 and SB1) were tested in rice under drought stress conditions. Out of the three strains tested, *Bacillus subtilis* (SA1) was found to enhance all the morphological parameters, relative water content and chlorophyll content more than the two other strains. Therefore, this strain can be an ideal candidate as biofertilizer and for enhancing the growth of crops especially under drought stress condition.

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Declaration of conflict of interest

The authors declare that there is no conflict of interest.

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