Plant growth promoting bacteria-induced physiological changes in Rice (*Oryza sativa* L.) Variety CO 51 under moisture stress

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Abstract

Drought is a huge global barrier to the growth of agriculture. Utilizing microorganisms that enhance plant growth is becoming more important among the various drought mitigation techniques. In this study, bacterial cultures previously isolated from the rhizosphere of xerophytes were used to reduce the impact of moisture stress in rice and the biochemical changes in the plant were observed. Among the different treatments, the plants treated with Bacillus altitudinis MLSB2 (MT729964) exhibited better stress tolerance as the relative water content was higher and the leaf water loss was lower than the other treated plants. The chlorophyll 'a' content was maximum in both Bacillus altitudinis MLSB2 (MT729964) treated plants and Bacillus velezensis VKSB5 (MT729963) treated plants and chlorophyll 'b' was maximum in the former one. The bacterial cultures with their ability to improve the physiological adaptation of plants under moisture stress can be developed as a bioinoculant to improve the growth of plants under drought conditions.

Keywords: Plant growth-promoting rhizobacteria, moisture stress, Bacillus, drought, proline

1. Introduction

Drought is one of the major environmental issues that the world's population is now dealing with [1]. It is projected that it will become more prevalent and severe in many regions as a result of decreased rainfall and higher evaporation brought on by global climate change [2]. Additionally, the world has frequently experienced prolonged dry periods as a result of the unpredictable rainfall patterns brought on by global warming [3]. The consequences of drought on agriculture are getting worse because of the world's increasing population expansion and the dwindling number of available water resources [4]. The damaging effects of drought could be reduced by using a variety of strategies, including water management, seed priming, and the use of drought-tolerant cultivars [5]. However, the financial, technical, and environmental drawbacks of these methods have sparked interest in finding more natural, affordable, and environmentally friendly solutions, such as rhizobacteria that promote plant development (PGPR) [6]. PGPR is an important and environmentally favourable source of sustainable agriculture production. The induction of systemic resistance to abiotic stressors in many crops has grown dramatically as a result of PGPR inoculation [7]. The bacteria associated with the plant may be better able to overcome the limits of drought with the assistance of a particular mechanism known as microbe-induced systemic tolerance [8]. It stimulates the bacterial assembly of phytohormones such as cytokinin, indoleacetic acid (IAA), and abscisic acid (ABA), as well as antioxidants [9].

In the present study, the effect of xerophyte-derived bacteria inoculation on the physiology of Rice (*Oryza sativa* L.) Variety CO 51 under moisture stress was analysed which focussed on the changes in relative water content, leaf water loss and chlorophyll content.

2. Materials and Methods

2.1. Bacterial strains

The bacterial strains *Bacillus aryabhattai* APSB18 (MT729997), *Bacillus velezensis* VKSB5 (MT729963), and *Bacillus altitudinis* MLSB2 (MT729964) were utilized in this investigation and were obtained from the Insects ecology laboratory, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore which was previously isolated from the rhizosphere of xerophytes [10]. *Bacillus altitudinis* FD48, acquired from the Biocatalysts Laboratory, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, was utilised as the standard in this investigation.

2.2. Plant growth and Induction of Moisture Stress

A pot culture experiment was conducted in a greenhouse at the Department of Agricultural Microbiology, TNAU, Coimbatore, India. The effects of plant growth-promoting bacteria on the physiological responses of the rice genotype CO 51 under moisture stress were studied. For germination, surface-sterilized seeds were biotized with bacteria (10^9 cells mL^{-1}) and were placed in a sterile Petri plate. Seeds were uniformly germinated and planted in sterile soil in pots with two seedlings per pot. For each treatment, three replicates were maintained.

The plants were exposed to moisture stress by withholding irrigation at the panicle initiation stage (after 45 DAS). To ensure data integrity, the experiment was performed in triplicates.

Treatments

Culture treatments	**Irrigation treatments**
C1- Uninoculated Control	I1- 100% irrigation
C2- *Bacillus altitudinis*	I2- Withholding irrigation for 7 days
C3- *Bacillus aryabhattai*	I3- Withholding irrigation for 10 days
C4- *Bacillus velezensis*	
C5- *Bacillus altitudinis* FD48	

2.3. Relative Water Content (RWC) and Leaf Water Loss

The RWC was calculated using the approach outlined by Barrs and Weatherley [11]. The leaf samples were collected and weighed right away to determine their fresh weight. The weight of the dried samples was calculated, and the percentage of leaf water loss was calculated using the formula below.

Leaf water loss = (Fresh weight-weight after 6h)/Dry weight*100

2.4. Evaluation of Photosynthetic Pigments

The approach described by Sumanta, et al. [12] was used to estimate chlorophyll 'a' and chlorophyll 'b'. About 500 mg of leaf samples were homogenized in 80 per cent acetone, centrifuged for 10 minutes at 6000 rpm, and the supernatant was utilized for pigment analysis. Using a spectrophotometer, absorbance was measured at 645 nm, and 663 nm. The photosynthetic pigment was measured in milligrams per gram of fresh weight (FW).

3. Results and Discussion

Water availability is the most important abiotic factor that greatly affects plant evolution in general, and the Rice crop in particular [13]. Many studies have found that inoculating plants with beneficial rhizobacteria increases drought tolerance [14]. The present study focussed on the effect of xerophyte-derived bacteria inoculation on the physiology of Rice (*Oryza sativa* L.) Variety CO 51 under moisture stress. The relative water content of plants decreased with an increase in moisture stress (Fig.1). Begum, et al. [15] discovered that when the amount of drought stress increases, the concentration of proline and other osmolytes increases, assisting in the maintenance of relative water content in maize plants. Similarly in this study, the plants treated with bio inoculants were able to maintain a relative water content higher than the non-treated plants. At moisture stress conditions, maximum relative water content was found in plants treated with *Bacillus altitudinis* (C2) (77.48% at I2 and 70.56% at I3) which was significantly on par with the standard culture (C5). The leaf water loss increased with an increase in days of withholding of irrigation (Fig.2). At moisture stress conditions I2 and I3, the plants inoculated with *Bacillus altitudinis* (C2) showed the least leaf water loss of 14.17% and 16.28% respectively.

In a study by Naveed, et al. [16], Wheat (*Triticum aestivum*) treated with *Burkholderia phytofirmans* showed improved chlorophyll content under moisture stress. Similarly in this study, when chlorophyll ‘a’ and chlorophyll ‘b’ levels were measured, we discovered that PGPR strains positively influenced plant photosynthetic activity (Fig.3). However, as the level of moisture stress increased, photosynthetic pigments decreased. In the case of chlorophyll ‘a’, both the *Bacillus altitudinis* (C2) primed plants and *Bacillus velezensis* (C3) primed plants showed the maximum production of 4.21 mg g^{-1} FW and 3.79 mg g^{-1} FW at nonstressed conditions (I1). But at I3, the production was significantly reduced but was highest produced by *Bacillus velezensis* (C3) primed plants (1.59 mg g^{-1} FW). However, there was no significant difference in chlorophyll ‘b’ synthesis between plants treated with bacterial inoculants. However, at I3 PEG concentration, plants treated with *Bacillus altitudinis* (C2) produced the highest amount of 1.59 mg g^{-1} FW.

4. Conclusion

The present investigation exhibited the potential of the xerophyte-derived bacterial inoculants to increase moisture stress tolerance in Rice (*Oryza sativa* L.) Variety CO 51. Among these, *Bacillus altitudinis* MLSB2 (MT729964) and *Bacillus velezensis* VKSB5 (MT729963) greatly increased the chlorophyll content and relative water content thereby reducing the leaf water loss of rice under moisture stress. These bacterial cultures can be effectively utilised to reduce drought stress in rice.

CONFLICT OF INTEREST

The authors declare that no conflict of interest, financial or otherwise.

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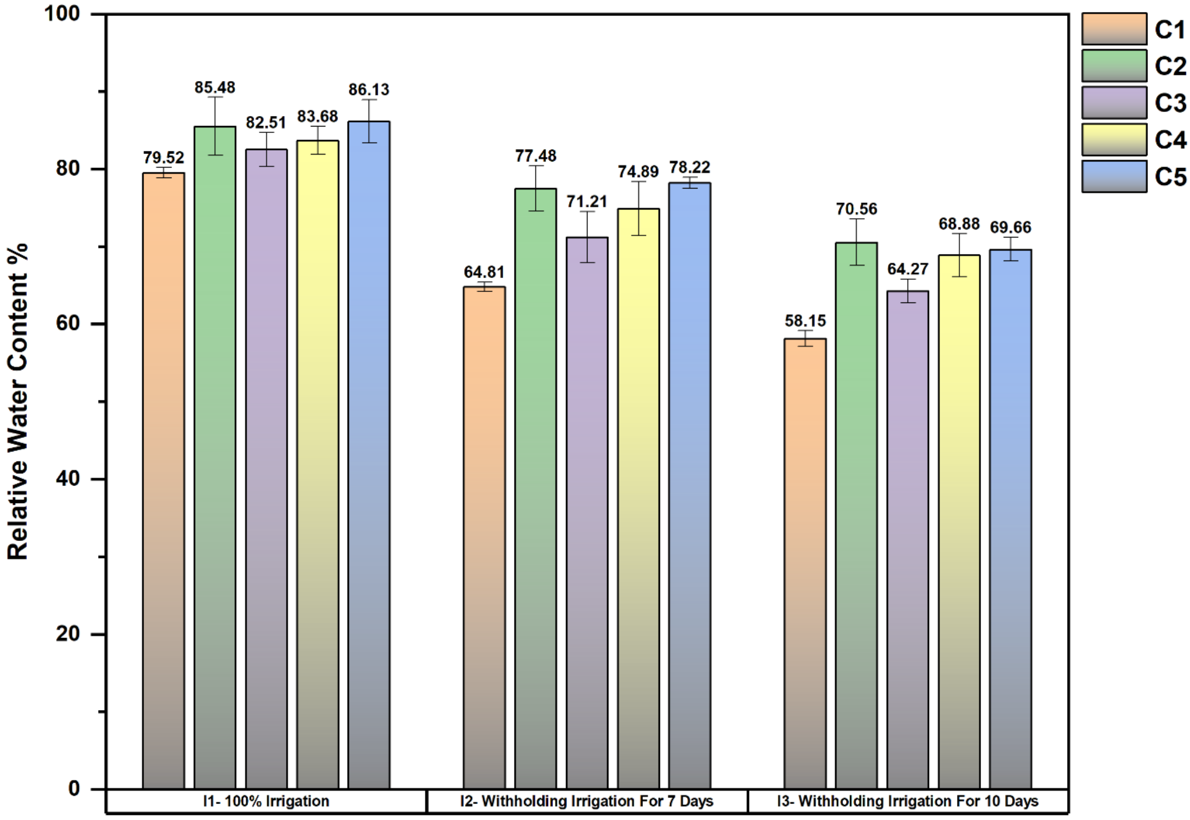

Figure 1. The relative water content of Rice CO 51 under Moisture Stress.
C1- Uninoculated plants C2- *Bacillus altitudinis*, C3- *Bacillus aryabhattai*, C4- *Bacillus velezensis*, C5- *Bacillus altitudinis* FD48.

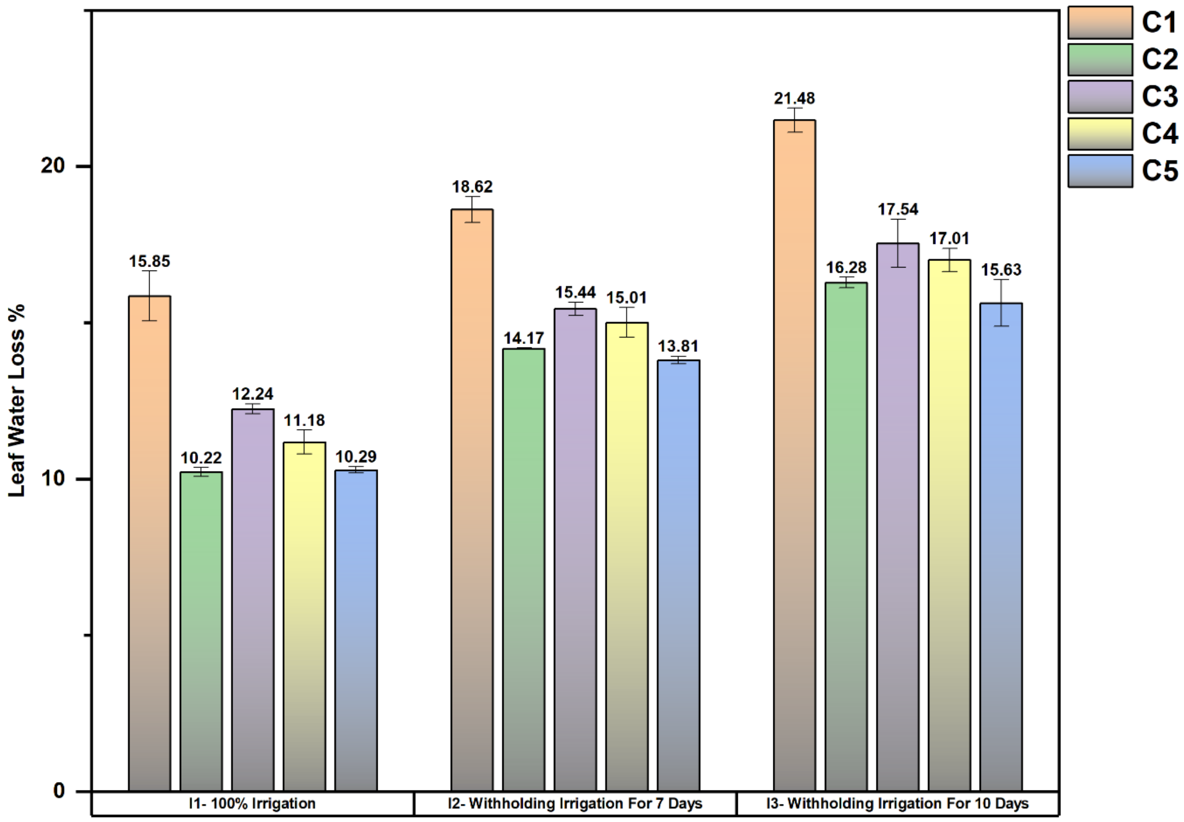

Figure 2. Leaf Water Loss of Rice CO 51 under Moisture Stress.
C1- Uninoculated plants C2- *Bacillus altitudinis*, C3- *Bacillus aryabhattai*, C4- *Bacillus velezensis*, C5- *Bacillus altitudinis* FD48.

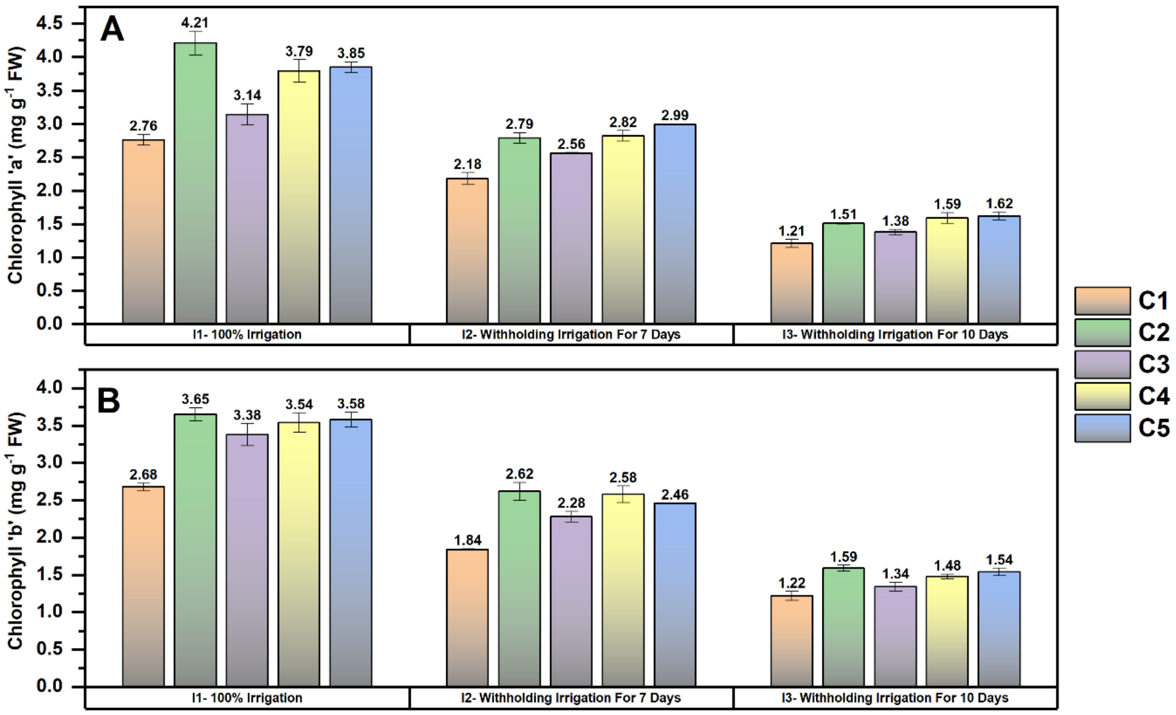

Figure 3. Chlorophyll content in Rice CO 51 under Moisture Stress.

A. Chlorophyll ‘a’, B. Chlorophyll ‘b’. C1- Uninoculated plants C2- *Bacillus altitudinis*, C3- *Bacillus aryabhattai*, C4- *Bacillus velezensis*, C5- *Bacillus altitudinis* FD48.