Colonization Stability and Efficacy of Plant Growth Promoting Rhizobacteria in Creeping Bentgrass

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Introduction

Inoculation with plant growth promoting rhizobacteria (PGPR) is a novel approach to improve growth and abiotic stress tolerance of cool season turfgrasses. Several endophytic PGPR colonize plant roots and produce ACC deaminase, which reduces the production of stress-induced ethylene, effectively reducing leaf senescence. However, for this symbiosis to have the intended effects on improving stress tolerance, the roots must first be colonized by the PGPR using successful and confirmed inoculation methods. Additionally, these methods must be confirmed under fields conditions, which can be challenging due to fluctuating temperatures and moisture, as well as the presence of native soil organisms. In this study, field plots of creeping bentgrass (*Agrostis stolonifera* cv. Penncross) were inoculated with two novel strains of *Paraburkholderia aspalathi* bacteria that have demonstrated growth promoting properties using a foliar spray and soil drench inoculation method.

Objectives

1. To determine effective methods of PGPR inoculation to improve drought stress tolerance in creeping bentgrass under field conditions.
2. To quantify the presence and density of bacteria in creeping bentgrass roots that have been inoculated with PGPR strains *P. aspalathi* 'WSF23' and 'WSF14'.

Materials and Methods

Plant Materials and Growing Conditions

Creeping bentgrass plots (1 m x 1.3 m) were established in field soil and maintained at a height of 1.2 cm. Plants were inoculated twice before the start of drought stress and once again upon re-watering using a foliar spray or soil drench method. A rainout shelter was used to cover the plots to simulate drought stress. Each treatment was replicated four times.

Bacteria Inoculation Treatments

P. aspalathi strains 'WSF23' and 'WSF14' were applied individually and in combination at a concentration of 1.0 x 10^7 CFUs in a carrier volume of 2.0 gallons per 1,000 square feet.

1. Non-inoculated control
2. Foliar spray
3. Soil drench (inoculant watered in with 0.25 inches of water)

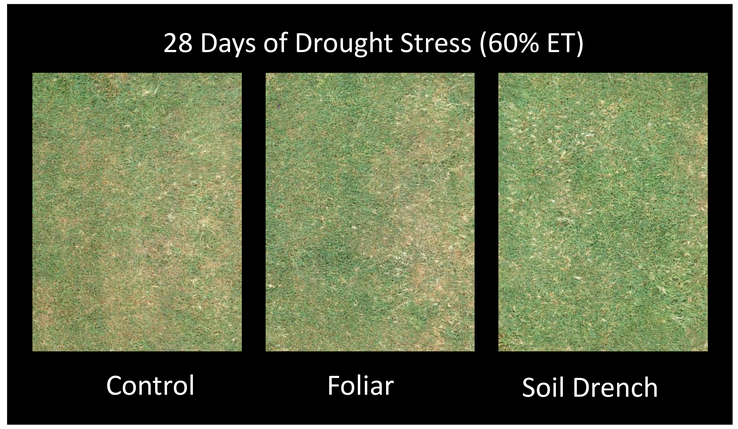
Deficit Irrigation Treatments:

- Well-Watered Control: Plants were irrigated (100% ET)
- Drought Stress: Deficit Irrigation (60% ET) for 28 days
- Post-stress Recovery: Drought-stressed plants were re-watered for 14 days

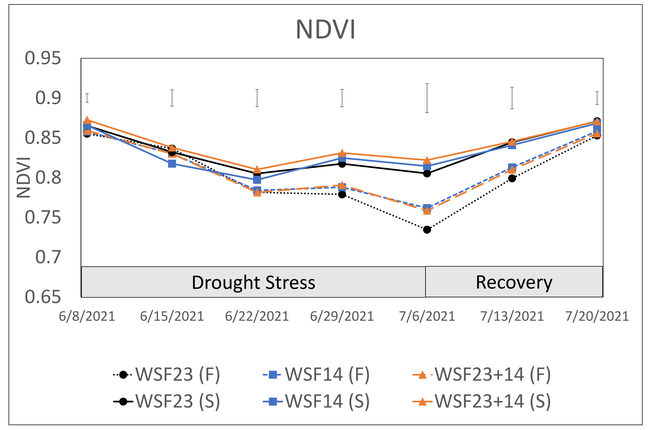
Bacteria Colonization Confirmation with PCR Analysis

Roots were sampled from the plots to examine the presence and quantity or density of the bacteria strains that were applied. The presence of bacterial inoculants in plant tissues was determined by bacterial isolation and PCR that was designed based on signature sequences of the 16S rDNA in *P. aspalathi*.

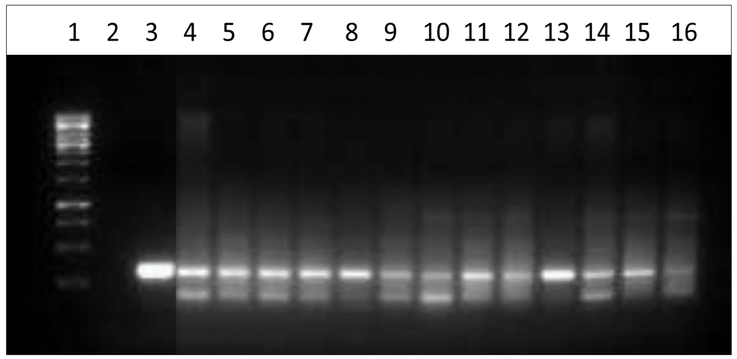
Results



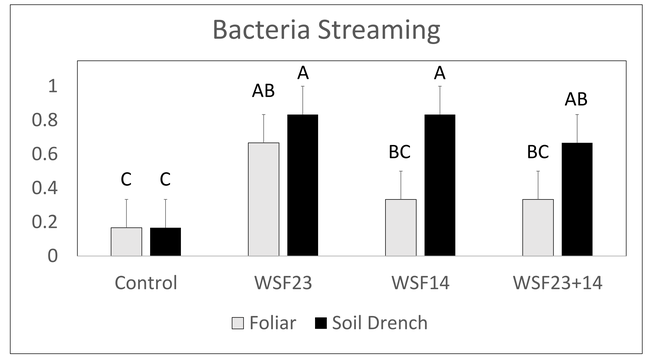
Inoculation with *P. aspalathi* improved drought tolerance in creeping bentgrass in field conditions using a foliar and soil drench application method.



The soil drench method (S) resulted in the highest normalized difference vegetation index (NDVI) values for creeping bentgrass plots throughout 28 days of drought stress and 14 days of rewatering compared to the foliar application (F).



PCR confirmation of *P. aspalathi* colonization efficacy and stability in creeping bentgrass roots in the field trial. Bands show the amplification of the bacterial 16S rDNA region of *P. aspalathi* 'WSF23' and 'WSF14' isolated from roots. Line 1-ladder; 2-negative control; 3 – positive control; 4-16 – inoculation treatments (foliar and soil drench).



Bacteria streaming from root samples of creeping bentgrass was highest when the soil drench inoculation method was used (*1.0 = profuse streaming; 0.5 = moderate streaming; 0 = no streaming*).

Conclusions and Discussion

- Inoculation of creeping bentgrass with *P. aspalathi* 'WSF23' and 'WSF14' bacteria improved drought tolerance and post-drought recovery by promoting turf quality, canopy density, and NDVI through 28 days of deficit irrigation (60% ET) and 14 days of rewatering (100% ET).
- PCR analysis confirmed the effective colonization of *P. aspalathi* 'WSF23' and 'WSF14' in the root tissues of creeping bentgrass plants inoculated using both the foliar spray and soil drench application method. Bacteria streaming counts indicated that *P. aspalathi* 'WSF23' and 'WSF14' inoculation by soil drenching enhanced the populations of bacteria in the roots of creeping beentgrass.