PREPARING FOR BATTLE: RHIZOBACTERIA'S ROLE IN PRIMING PLANTS FOR DROUGHT STRESS

by

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ABSTRACT OF THE THESIS

Preparing for Battle: Rhizobacteria's Role in Priming Plants for Drought Stress

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Drought is an issue that is prevalent in our world today, becoming an unavoidable constraint to crop productivity imperiling agricultural-based economies and food availability. Therefore, the development of environmentally-friendly and sustainable approaches to ameliorate the damage caused by drought is of primary importance. Soil-dwelling microorganisms found in the rhizosphere of a plant play prominent roles in the livelihood and fitness of plants, therefore possibly aiding plants in battling abiotic stress. In efforts to establish the role of rhizobacteria in the priming of plants for drought stress, we studied the effect of the leguminous-associating *Bradyrhizobium japonicum* and multi-host associating *Bacillus amyloliquefaciens* on the model plant, *Arabidopsis thaliana* with a focus on parameters spanning their physiological and development differences attributing to better plant fitness in drought conditions. The data presented demonstrates that the presence of these rhizobacteria in the rhizosphere of the plants have significant effects on their germination rate, root architecture, biomass, reproduction, rate of water loss and soil moisture retention in *A. thaliana*, in comparison to the untreated control. The effect of these rhizobacterial species on *A. thaliana* establish an avenue for the development of environmentally-friendly and sustainable agricultural applications,

utilizing interactions that occur in nature to promote plant growth and protect against abiotic stresses.

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1. INTRODUCTION

Drought is an inevitable and encompassing event that emerges with the continuous surges of environmental shifts (Adeloye 2010; Fig. 1). A projected global population increase by 2.3 billion in the next 40 years, calls for food production malleable to its rising demand in denigrated environments (Foresight 2011; Chapman 2012; Smol 2012; UNFPA 2015). Therefore, a sustainable solution is not only relevant, but necessary.

A need for methods in establishing sustainable agricultural practices such as studies on the microbial interactions with plants to improve crop productivity have been implemented (Hayat et al. 2012; Glick 2012). An environmentally-friendly and naturalistic approach to remediation of issues littered by anthropogenic impacts, microbes, particularly plant growth promoting rhizobacteria (PGPR), have been used as a viable avenue for development of biofertilizers (Fuentes-Ramirez & Caballero-Mellado 2006) and biocontrol agents (Siddiqui 2006). PGPR are a group of microorganisms, which colonize the rhizosphere , the area around the root, due to chemoattractants exuded by the root system with known functions in plant growth (Kloepper & Schroth 1978; 1981). Most PGPR known today span the genera of *Bacillus* and *Pseudomonas* (Kloepper et al. 2004; Barea et al. 2005), but are found in numerous other genera such as *Bradyrhizobium*, *Azospirillum*, *Rhizobium*, *Azotobacter* amongst several others (Gomes et al. 2010). Originally coined as PGPR by JW Kloepper and MN Schroth (Kloepper & Schroth 1978), these microbes have been shown to promote plant growth using both direct and indirect mechanisms.

Direct mechanistic approaches include enhanced resource acquisition and phytohormone stimulation and/or biosynthesis. If particular nutrients are readily available to plants, they are no longer a limiting factor to their growth. PGPR promote plant growth via facilitation of difficultly acquirable nutrients from the soil such as fixed nitrogen, insoluble phosphorus and iron (Clarkson 1985; Glick 2012). Some strains of PGPR such as *Bradyrhizobium* japonicum and *Azospirillum lipoferum* are nitrogen-fixing, which promote root nodulation in leguminous plants converting N_2 into a favorable nitrogen source for plants, ammonia (NH_3) (van Rhijn & Vanderleyden 1995; Malik et al. 1997; Gachomo et al. 2014b). Other strains e.g. *Serratia marcescens* and *Burkholderia cepacia* partake in phosphate solubilization by producing organic acids and enzymes, which aid in the mineralization of both inorganic and organic bound phosphates (Lipton et al. 1987; Rodriguez and Fraga 1999; Vassilev et al. 2006; Perez et al. 2007). Additionally, Sheng and He (2006) noted that the PGPR, *Bacillus edaphicus* solubilizes potassium-bearing minerals found in the soil via the production of organic acids and capsular polysaccharides.

Nutrient acquisition of a plant is dependent on the proliferation of the root, the functionality of transporters, nutrient ion diffusion and water mass flow to the root surface, exudation chemistry and frequency, which influence the symbioses between soil-dwelling microorganisms and the plant (Chapman et al. 2012). Microbial diversity and populations are strikingly plentiful in the rhizosphere in comparison to the bulk of the soil (Grayston et al. 1996; Gans et al. 2005; Roesch et al. 2007). This is largely due to 5-21% of plant-produced organic compounds known as root exudates (Marschner 1995; Yuan et al. 2015). These root exudate compounds, however, undergo modifications over time

further influencing the rhizobacterial populations that colonize the roots of the plant at different growth and developmental stages (Chaparro et al. 2013). These microbial populations affect the proliferation, patterning and morphological architecture of the roots, which possess a role in the quality and quantity of root exudates produced, as well as the nutrient acquisition efficiency (Bowen & Rovira 1999; Zobel & Waisel 2010; Chapman et al. 2012; Gachomo et al. 2014a).

Indirect mechanisms in PGPR-mediated plant growth promotion, thematically span biological control. PGPR strains like *Pseudomonas chlororaphis* and *Pseudomonas fluorescens* are antagonistic in nature via antibiotic production paired with facilitation of competitive exclusion for nutrients whilst developing niches on the root creating an opportune platform for antibiotic delivery to the root system (Chin-A-Woeng et al. 2000; Jousset et al. 2006; Pliego et al. 2008). PGPR strains produce different compounds, such as hydrogen cyanide (HCN) produced by *Pseudomonas fluorescens* (Haas & Blumer 2000; Haas & Keel 2003), volatiles such as 2,3-butanediol produced by *Bacillus amyloliquefaciens* and *Bacillus subtilis* (Ryu et al. 2003), phenazine and its derivatives produced by *Pseudomonas chlororaphis* (Thomashow & Weller 1996, Chin-A-Woeng et al. 2000), lytic enzymes produced by *Bacillus subtilis* (Podile &Prakash 1996, Ko et al. 2009), organic acids such as 4-hydroxyphenylacetic acid produced by *Lysobacter antibioticus* (Ko et al. 2009), among others.

PGPR strains such as *Rhizobium leguminosarum* and *Bradyrhizobium japonicum*, promote siderophore production (Antoun et al. 1998). Siderophores are microbial iron chelators, that deprive competing microbes of their ferrous nutritive needs by complexing the iron in the soil conducive to their thriving but not other present microflora (Kloepper

et al. 1980; O'Sullivan & O'Gara 1992). PGPR like *Ralstonia eutropha* and *Pseudomonas syringae* can also partake in signal interference of homoserine lactones that some microorganisms need in order for their pathogenesis to occur, producing cell wall degrading enzymes and hindering biofilm production (Lin et al. 2003; Shephard & Lindow 2008). PGPR provide plants with resistance against pathogenic microorganisms using jasmonic acid and ethylene signaling, such as in the case of *Bacillus amyloliquefaciens* (van Loon 2007; Tan et al. 2013). This is known as induced systemic resistance. It is often triggered by bacterial cellular components (biological inducers) and metabolites (chemical inducers), such as N-Acyl homoserine lactone (AHL) signal molecules and volatiles (Kuc 1982; Lugtenberg & Kamilova 2009; Pietrese et al. 2014).

PGPR are very dynamic in activity. They have been shown to have a role in modulation of environmental stress responses. Although plants are sessile organisms with a knack for developing coping mechanisms for stark changes in their environment, often the damage of progressive droughts are higher than the plant's elasticity of survival. Plant-microbe interactions have been shown to confer abiotic stress tolerance. PGPR species such as *Kluyvera ascorbata* have been shown to enhance the rate of mobilization of heavy metals by interacting with phytoremediative plants resulting in an altered and functionally conducive root architecture (Burd et al. 2000; Tak et al. 2013).

In terms of drought stress tolerance, PGPR-inoculated plants have been shown to have higher relative water content and leaf water potential attributing to their drought stress tolerance capability (Liu et al. 2013). Certain PGPR-plant interactions, such as with *Arabidopsis thaliana* and *Paenibacillus polymyxa*, also stimulate the expression of stress-related genes such as *Cadhn, VA, sHSP, DREB2, COR15, ABR1* and *RD29* (Timmusk

and Wagner 1999; Lim & Kim 2013; Gachomo et al. 2014b), which function in the stabilization of cellular structures in the face of water deprivation. Similarly, with salt stress, PGPR-plant interactions have resulted in enhanced expression of stress-related genes (Timmusk and Wagner 1999) as well as production of 1-aminocyclopropane-1-carboxylate deaminase, volatile compounds and tissue specific regulation of sodium transporters as seen with *A. thaliana* associating *Bacillus subtilis* promoting the regulation of HKT1 (Mayak et al. 2004; Zhang et al. 2008). Another mechanistic employment of abiotic stress tolerance is the accumulation of osmoprotectant compounds, such as glycine betaine, induced by *Bacillus subtilis* in *Arabidopsis thaliana* (Zhang et al. 2010) in salinized conditions.

Endophytic and rhizospheric bacteria, such as *Pseudomonas pseudoalcaligenes*, have been shown to accumulate glycine betaine-like quarternary compounds that have a role in salt stress tolerance in rice (Jha et al. 2011; Vacheron et al. 2013). PGPR also induce the production of non-enzymatic and enzymatic antioxidants, which in turn reduces the accumulation of reactive oxygen species hindering membrane lipid peroxidation (Ozturk & Demir 2002; Kavi Kishor et al. 2005; Jha & Subramanian 2014).

In the ever-changing world we exist in, climate change adaptability is of importance particularly for resources we are dependent on. Beneficial microbes have shown to confer plants with several capabilities that they cannot solely accomplish, or at the very least are enhanced with these interactions. The tools used today to identify the possibilities presented with plant-microbe interactions are omnipresent, but their application and thorough mechanistic elucidation lacking. This project aimed to investigate if two rhizospheric bacteria, gram negative *Glycine max* (soybean)-associating

Bradyrhizobium *japonicum* IRAT FA3 and the gram positive, endospore-forming *Bacillus amyloliquefaciens* prime the model plant, *Arabidopsis thaliana* for progressive drought stress tolerance as well as promote plant growth. Such questions provide a compass to the establishment of plant-microbe interactions as an environmentally friendly approach to the amelioration of environmental issues that taunt our world today.

2. MATERIALS AND METHODS

Inoculum Preparation and Plant Inoculation of PGPR

Pure inoculum of *Bradyrhizobium japonicum* IRAT FA3 and *Bacillus amyloliquefaciens*, were acquired from 3-day old Luria-Broth (LB) liquid cultures grown at room temperature (24^0 C) in shaking conditions at 120 rpm. The bacterial inocula concentration was maintained at 10^8 CFU/ml, by dilution with sterile distilled water as measured spectrophotometrically at 600 nm.

Plant Material

Arabidopsis thaliana (ecotype Col-0) seeds from the Arabidopsis Biological Research Center (ARBC) were used in this study. The seeds were surface sterilized via sequential rinsing in a 2% bleach solution followed by a 70% ethanol solution, for a span of 30 seconds each and then rinsed an additional 5 times with sterile distilled water.

in vitro MS plate assays

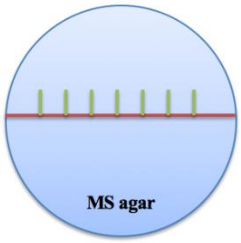


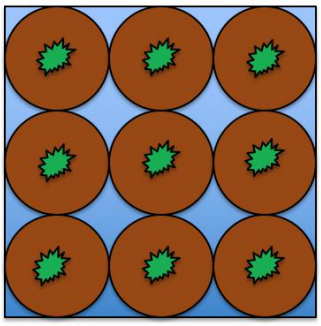
Plate assay schematic

The seeds were soaked overnight in a 10^8 CFU/mL bacteria and sterile distilled water mixture They were then sown on sterile Murashige and Skoog (MS) agar plates and grown in 26°C/23°C (day/night) variable temperature conditions under 12h/12h dark/light conditions.

Soil sterilization

The soil used in this study was sterilized by autoclaving it on a fluid cycle, at 121°C for 30 minutes. The soil was then left to cool and autoclaved a second time, using the same settings, within 12 hours of the initial autoclaving of the soil.

***in vivo* soil assays**



in vivo soil assay schematic

Germination pots filled with sterilized soil for each bacteria treatment were soaked overnight in their respective 10^8 CFU/mL bacteria solutions, whilst the control was soaked in water. The seeds were sown and left to germinate in 26°C/23°C (day/night) 12h/12h night/day conditions. At 10 days, they were transplanted into their respective bacteria-soaked pots, with the exception of the control whose soil was soaked in water. Growth chamber conditions were 26°C/23°C (day/night) variable temperature conditions with 12h/12h dark/light conditions.

Total chlorophyll content

At 31 days, leaves were collected from each treatment, cut into smaller pieces and ground using a ceramic mortar and pestle and liquid nitrogen. The powdered leaves were put in their respective, separate 15 mL sterile falcon tubes. 80% acetone was added to

each one and shaken in the dark for 15 minutes. The mixture of each treatment was centrifuged at 4 ^{o}C for 15 minutes at 3000 rpm. The supernatants were transferred to new, sterile centrifuge tubes and kept in the dark. The supernatants' absorbance values were measured using a spectrophotometer at 645 nm and 663 nm, with 80% acetone used as the blank control. The formula used to determine total chlorophyll content was as follows (Arnon 1949):

$$C_{a+b} = \frac{[8.02 * A_{663} + 20.20 * A_{645}]* V}{1000\ W}$$

V= volume of the extract (mL)

W= weight of fresh leaves (g)

Leaf surface area determination

Photographic images taken of all the plants, treated and untreated, had their surface area determined during different stages using the Java-based image processing program, Image J (U.S. National Institutes of Health, Bethesda, MD, USA. http://imagej.nih.gov/ij/ (1997-2005). A 1 cm x 1 cm piece of paper was used as a reference scale. The images were analyzed singularly, in fear of inconsistency of focal point differences during photographing. Each image was cropped, converted to 8-bit, thus becoming grayscale. The threshold was then adjusted, yielding red images. Using the *Despeckle* function, specks causing noise in the background of the image was minimized. Measurements were taken by using the *Set Measurements* function, whereby the Area, Standard Deviation, Display label and the Decimal place value was set to 1. Using the *Analyze Particles* function, a minimum area range was set using the smallest leaves as the

desired value. Using the *Display results* function, the results appeared in a separate small window (Glozer 2008).

Flowering time determination

When the flowering stalks reached 1 cm and their floral buds visible, flowering time was determined by counting the number of rosette leaves (Gachomo et al. 2014a).

Relative Water Content

3 leaves per plant treatment were cut, tightly sealed in a plastic bag and placed in a dark box. Each leaf was weighed to determine the fresh weight. Using a syringe, a drop of 5 mM $CaCl_2$ was added to the petiole end of the leaves and left in the dark for 2 hours. The leaves were weighed again to determine their turgid fresh weight. The leaves were then placed in a 70^{o}C drying oven for 24 hours and weighed, providing the dry weight value. Similarly, was done with soil samples with the turgid fresh weight of soil samples being soaked in distilled water for 8 hours. The formula used to determine the relative water content (RWC) was as follows:

$$RWC\ (\%) = \left(\frac{W - DW}{TW - DW} \right) \times 100$$

W: Fresh weight

TW: Turgid weight

DW: Dry weight

Drought stress treatment

At 38 days, the plants' flowering stalks were cut and the plants subjected to drought stress by withholding of water i.e. progressive drought exposure for 2 weeks, then rehydrated.

Water Loss Percentage Determination

Calculation for water loss of plants in both treated and untreated conditions, were determined by the following calculation:

$$\% \text{ Water Lost} = \frac{\text{FW-DW}}{\text{PSA}}$$

FW: Fresh weight of plant

DW: Dry weight of plant

PSA: Plant Surface Area

To establish the fresh weight value, the plants were weighed after a regular biweekly watering regiment leading up to 40 days. The plants were then weighed daily for seven days, charting their water loss. The plant surface areas of the samples were determined similarly to the leaf surface area determination, using Image J.

Statistical Analyses

The experiments were completely randomized in nature and each experiment comprised of nine replicates. Data were expressed as mean values ± SE. P values were determined by 2-way ANOVA, Unpaired t-tests with Welch's correction and One-way ANOVA tests using GraphPad Prism 6 Software for Mac OS X.

3. RESULTS

Screening of the effect of PGPR library on *Arabidopsis thaliana*

The screening of PGPR library on *A. thaliana* seedlings as described in Table 1 shows that the PGPR 405 and 407 are the best performing PGPR in this study, due to their earlier germination time, longer average of seedling root length and larger seedling biomass. Therefore, throughout the study PGPR 405 and 407 were selected for further analysis in this study.

Rhizobacteria promote faster germination rates in *Arabidopsis thaliana*

Arabidopsis thaliana plants typically germinate 3-5 days, matching the result of the untreated control with an average germination rate of 3.75 days. However, *A. thaliana* plants treated with *B. japonicum* IRAT FA3 and *B. amyloliquefaciens* each exhibited earlier germination rates with a mean of 2.50 days for each respective treatment. One-way ANOVA analysis yielded a p value of <0.05. In comparison to the control the *B. japonicum* IRAT FA3 treated plants, were significantly faster germinating yielding a p value <0.05 with the unpaired t-test with Welch's correction (Fig. 2). F test for variance whereby the F value yielded was $F_{(3,3)} = 1.333$. Additionally, the *B. amyloliquefaciens* treated plants had a significantly faster germination rate yielding a p value <0.05 with the unpaired t-test with Welch's correction (Fig. 2). F test for variance whereby the F value yielded was $F_{(3,3)} = 1.333$.

Rhizobacteria promote root length and lateral root development in *A. thaliana* seedlings

Both rhizobacterial species displayed promotion of longer root lengths in *A. thaliana* seedlings in the *in vitro* MS agar cell assays. The untreated control yielded an

average root length of 0.42 cm (Fig. 3). In juxtaposition, the *B. amyloliquefaciens* treated seedlings yielded an average root length of 1.10 cm, whereas the *B. japonicum* treated seedlings had a mean root length of 1.44 cm (Fig. 3). One-way ANOVA analysis displayed a significant increase in root length across the treatments resulting in p values <0.05. F test for variance yielded. Unpaired t-tests with Welch's correction resulted in p values <0.05, when each bacteria treatment was compared to the untreated control. F tests for variance yielded F values of $F_{(4,4)}$ =1.037 for control vs. 405; $F_{(4,4)}$ =1.302 for control vs. 407 and $F_{(4,4)}$ =1.349 for 405 vs. 407.

In the *in vitro* MS agar cell assays, seeds treated with both rhizobacterial species displayed significant lateral root development in comparison to their untreated counterparts. The untreated control had an average of 0.33, whereas those treated with *B. amyloliquefaciens* had an average of 3.67 lateral roots and those treated with *B. japonicum* had an average of 3.33 lateral roots (Fig. 4). One-way ANOVA statistical analysis yielded a p value of <0.05. Unpaired t-tests with Welch's correction displayed that both treatments against the untreated control significantly promote faster leaf development with p values <0.05 (Fig. 4). F tests for variance yielded F values of F values of $F_{(4,4)}$ =1.5 for control vs. 405; $F_{(4,4)}$ =1.5 for control vs. 407 and $F_{(4,4)}$ =1 for 405 vs. 407.

Rhizobacteria increases the biomass of *A. thaliana* seedlings

The untreated control yielded an average mass of 0.58 μg per seedling in the *in vitro* MS agar cell assays (Fig. 5). One-way ANOVA analysis yielded p values of <0.05. Those treated with *B. amyloliquefaciens* resulted in an average of 3.02 μg, which when compared to the untreated control generated a p value of <0.05 using the unpaired t-test

with Welch's correction (Fig. 5). Plants treated with *B. japonicum* had an average mass of 3.98 μg. In comparison to the untreated control, using the t-test with Welch's correction, those treated with *B. japonicum* were significantly higher as indicated by the p value of <0.05. Additionally, an unpaired t-test with Welch's correction between the bacteria-treated groups, yielded a statistically significant value of <0.05, as denoted by an octothorp (#) in Fig. 5. F tests for variance, however, yielded F values of F values of $F_{(4,4)}$ =2.846 for control vs. 405; $F_{(4,4)}$ =4.385 for control vs. 407 and $F_{(4,4)}$ =1.541 for 405 vs. 407.

Rhizobacteria promote faster leaf development in *A. thaliana*

At reproduction, *A. thaliana* plants typically have 6-10 leaves. The rhizobacteria treated plants not only resulted in faster leaf development, but in increased leaf production as exhibited by the leaf profile in Fig. 6A. The untreated control at reproduction, had an average of 6.25 leaves per plant (Fig 6B). Plants treated with *B. amyloliquefaciens*, however, yielded an average of 10.25 leaves per plant, with *B. japonicum* treated plants generating an average of 12 leaves per plant at reproduction (Fig. 6B). One-way ANOVA analysis yielded p values of <0.05. Unpaired t-tests with Welch's correction displayed that both treatments against the untreated control significantly promote faster leaf development with p values <0.05 (Fig 6B). F tests for variance yielded F values of $F_{(4,4)}$ =2.3 for control vs. 405; $F_{(4,4)}$ = 3.2 for control vs. 407 and $F_{(4,4)}$ =1.433 for 405 vs. 407.

Rhizobacteria significantly increase the leaf surface area of *A. thaliana*

The untreated control at 17 days had an average leaf surface area of 7.75 mm^2 (Fig. 7A), 21.86 mm^2 at 24 days (Fig. 7B), 70.19 mm^2 at 31 days (Fig. 7C) and 181.85 mm^2 at 38

days (Fig. 7D). Plants that were treated with *B. amyloliquefaciens* had an average leaf surface area of 13.83 mm^2 at 17 days (Fig. 7A), 80.47 mm^2 at 24 days (Fig. 7B), 270.97 mm^2 at 31 days (Fig. 7C) and 664.96 mm^2 (Fig. 7D). *B. japonicum* treated plants had an average leaf surface are of 49.23 mm^2 at 17 days (Fig. 7A), 382.82 mm^2 at 24 days (Fig. 7B), 743.91 mm^2 at 31 days (Fig. 7C) and 1210.63 mm^2 at 38 days (Fig. 7D). For all treatments there was a significant increase in leaf surface area at all time points (Fig. 8), as affirmed by the one-way ANOVA analysis generated p values of <0.05. Unpaired t-tests with Welch's correction displayed that both treatments against the untreated control significantly promote leaf surface area with p values <0.05 (Fig. 7A-D). Additionally, unpaired t-tests with Welch's correction, at all time points comparing both treatment groups, yielded p values of <0.05 as denoted by octothorps in Figs. 7A-D. At 17 days, F tests for variance yielded F values of F values of $F_{(2,3)}$ =1.142 for control vs. 405; $F_{(3,2)}$ = 14.09 for control vs. 407 and $F_{(3,3)}$ =16.1 for 405 vs. 407. At 24 days, F tests for variance yielded F values of F values of $F_{(3,3)}$ =37.99 for control vs. 405; $F_{(3,3)}$ = 228.8 for control vs. 407 and $F_{(3,3)}$ =6.02 for 405 vs. 407. At 31 days, F tests for variance yielded F values of F values of $F_{(3,5)}$ =3.679 for control vs. 405; $F_{(4,5)}$ =3.425 for control vs. 407 and $F_{(3,4)}$ =1.074 for 405 vs. 407. At 38 days, F tests for variance yielded F values of F values of $F_{(5,4)}$ =3.53 for control vs. 405; $F_{(6,4)}$ =2.325 for control vs. 407 and $F_{(6.5)}$ =6.586 for 405 vs. 407.

Rhizobacteria promote chlorophyll production in *A. thaliana*

At 31 days, the untreated *A. thaliana* yielded an average total chlorophyll content of 0.68 mg/g (Fig. 9). Plants that were treated with *B. amyloliquefaciens* had a total chlorophyll content average of 1.17 mg/g and *B. japonicum* treated plants had a total chlorophyll

content mean of 0.75 mg/g (Fig. 9). One-way ANOVA statistical analysis yielded a p value of <0.05. Additionally, unpaired t-tests with Welch's correction comparing the treatment groups individually with the untreated control both yielded statistically significant values of $p<0.05$ (Fig. 9). Unpaired t-tests with Welch's correction comparing both treatment groups generated a p value <0.05. F tests for variance, however, yielded F values of F values of $F_{(4,4)} = 1.119$ for control vs. 405; $F_{(4,4)} = 1.024$ for control vs. 407 and $F_{(4,4)} = 1.039$ for 405 vs. 407.

Rhizobacteria promote plant height increase in *A. thaliana*

At 46 days, the untreated control's plant height was at an average of 5.4 cm (Fig. 10A & B). *B. amyloliquefaciens*-treated plants, however, had an average plant height of 17.57 cm and *B. japonicum*-treated plants had an average plant height of 27.47 cm (Fig. 10A & B). One-way ANOVA statistical analysis yielded a p value of <0.05. Unpaired t-tests with Welch's correction, comparing the untreated control with each treatment group yielded statistically significant values of $p<0.05$. F tests for variance, however, yielded F values of F values of $F_{(4,4)} = 8.769$ for control vs. 405; $F_{(4,4)} = 2.434$ for control vs. 407 and $F_{(4,4)} = 3.603$ for 405 vs. 407.

Rhizobacteria promote earlier flowering times in *A. thaliana*

The PGPR-treated plants had shorter flowering times in comparison to the untreated control (Fig. 11). The untreated control flowered at an average of 33.17 days, *B. amyloliquefaciens*-treated plants flowered at 28 days and *B. japonicum* treated plants flowered at 23.33 days (Fig. 11). One-way ANOVA statistical analysis generated a p value of <0.05. Unpaired t-tests with Welch's correction, comparing the untreated control

with each treatment group yielded statistically significant values of $p<0.05$. Additionally, unpaired t-tests with Welch's correction comparing both treatment groups against each other generated p values of <0.05 as denoted by the octothorp in Fig. 11. F tests for variance, however, yielded F values of F values of $F_{(5,5)} = 1.491$ for control vs. 405; $F_{(5,5)} = 1.258$ for control vs. 407 and $F_{(5,5)} = 1.875$ for 405 vs. 407.

Rhizobacteria promote vegetative biomass accumulation

At 33 days, the fresh weight of the different treatment groups of *A. thaliana* were recorded. The vegetative biomass of the untreated control was an average of 0.27 g (Fig. 12). Whereas, those treated with *B. amyloliquefaciens* had an average mass of 0.6 g, and the *B. japonicum*-treated plants at an average mass of 0.5 g (Fig. 12). One-way ANOVA statistical analysis yielded a p value of <0.05. Unpaired t-tests with Welch's correction, displayed that both treatments compared to the untreated control yielded p values of <0.05 and therefore are statistically significant (Fig 12). F tests for variance, however, yielded F values of F values of $F_{(2,2)} = 1.1612$ for control vs. 405; $F_{(2,2)} = 1.551$ for control vs. 407 and $F_{(2,2)} = 1.039$ for 405 vs. 407.

Rhizobacteria promote the weight of moisture and soil moisture retention in *A. thaliana*

The average weight of moisture of untreated control plants is 0.22 g (Fig. 13). Plants treated with *B. amyloliquefaciens* had an average mass of 0.5 g, and the *B. japonicum*-treated plants' average mass of 0.41 g (Fig. 13). One-way ANOVA statistical analysis yielded a p value of <0.05. Unpaired t-tests with Welch's correction, displayed that both treatments compared to the untreated control yielded p values of <0.05 and therefore are

statistically significant (Fig 13). F tests for variance yielded F values of F values of $F_{(2,2)}$ = 5.286 for control vs. 405; $F_{(2,2)}$ = 2.837 for control vs. 407 and $F_{(2,2)}$ =1.863 for 405 vs. 407.

At reproduction, the soil moisture retention was recorded by determining the relative water loss of soil samples of the different treatment groups. In the untreated control, there was an average relative water loss per plant surface area of 5.71% (Fig. 14). Soil treated with the *B. amyloliquefaciens* had an average relative water loss at 1.05% and those treated with *B. japonicum* had an average relative water loss of 0.49% (Fig. 14). One-way ANOVA statistical analysis yielded a p value of <0.05. Unpaired t-tests with Welch's correction, displayed that both treatments compared to the untreated control yielded p values of <0.05 and therefore are statistically significant (Fig 14).

Rhizobacteria influence the amount of water lost in *A. thaliana* exposed to drought stress

Charted over a course of seven days, the amount of water lost per surface area was significantly higher in the untreated control, than those treated with the rhizobacteria species (Fig. 15A). Untreated control began with losing approximately an average of 5.51% in the first day, to 5.99% by the seventh day (Fig. 15A & B). The *B. amyloliquefaciens* treated *A. thaliana* lost approximately an average of 0.023% in the first day, to 0.27% by the seventh day (Fig. 15A & B). Whereas, the *B. japonicum* treated plants lost approximately 0.064% in the first day to 0.07% by the seventh day (Fig. 15A & B).All these results were relative to the plants' surface area. 2nd-way ANOVA statistical analysis yielded a p value of <0.05.

4. DISCUSSION

Rhizobacteria shorten germination time

The colonization of the roots of *A. thaliana* with both rhizobacteria yielded significant changes in their growth and development from their early stages to reproductive maturity. Both *B.japonicum* IRAT FA3 and *B. amyloloquefaciens* promoted earlier germination rates both at an average of 2.5 days respectively, significantly shorter than the untreated control's average of 3.75 days (Fig. 2). Early germination influences the plant's performance and is an integral tool in establishment of the plant in both unbothered and agricultural ecosystems (Weitbrecht et al. 2011). Early germination rates for both *B. amyloliquefaciens* and *B. japonicum*-treated *A. thaliana* could be attributed to the synthesis and stimulation of phytohormones (Sturtevant & Taller 1989; Boiero et al. 2007).

Phytohormone stimulation is one of the mechanisms tin which rhizobacteria-plant interactions influence the growth and development of plants. Cytokinins are typically found in very low concentrations in plants, however have a significant role in the growth and development of plants. Roles that they possess include the stimulation of reproductive development, delayed senescence, leaf size expansion, *de novo* bud release and formation, as well as seed germination promotion (Mok 1994; Timmusk et al. 1999; Kefela et al. 2015).

GAs play significant roles in triggering the processes of development including those of meristem to shoot growth, vegetative to reproduction (flowering) and juvenile to adult leaf stages (Gupta & Chakrabarty 2013). Additionally, GAs are heavily involved in germination stimulation (Gutierrez-Manero et al. 2001) and determination of sexual

expression, largely due to their major bioactive site being the stamens of flowers (Gupta & Chakrabarty 2013).

The phytohormone auxin, however, promotes seed dormancy due to the stimulation of abscisic acid signaling via the ARF-mediated, specifically the AUXIN RESPONSE FACTOR 10 and AUXIN RESPONSE FACTOR 16 pathways, activation of ABSCISIC INSENSITIVE 3 (*ABI3*) in *A. thaliana* which regulates seed dormancy (Liu et al. 2013). *B. japonicum* has been shown to promote plant growth via the production of auxins, particularly indole-3-acetic acid (Boiero et al. 2007; Gachomo et al. 2014b). Therefore, *B. japonicum*'s ability to synthesize cytokinins most likely attributed to the early germination in comparison to the untreated control (Fig. 2). According to Boiero et al. (2007), the amount of auxins produced in comparison to GAs was higher. Therefore, GAs most likely did not break the dormancy of the seeds, but rather cytokinins resulting in an earlier germination time. Similarly, *B. amyloliquefaciens* strains have been found to utilize auxin production as modes of plant growth promotion (Idris et al. 2007; Shao et al. 2015). Due to auxin's role in prolonging of seed dormancy, cytokinin production (Mok & Mok 2001) could most likely attribute to breaking the seed dormancy resulting in a faster germination rate in comparison to the untreated control (Fig. 2).

Rhizobacteria promote primary root length and lateral root development

Studying the impact of strains of PGPR on the primary root length, is typically done through the inoculation of PGPR seeds and monitoring the results *in vitro* (Vacheron et al. 2013). However, several studies have shown that the rate of the primary root growth decreases in comparison to control treatments (Dobbelaere et al. 1999) while promoting lateral root development and length (Combes-Meynet et al. 2011; Chamam et

al. 2013) and play a stimulatory role in the elongation of root hairs as was in the case of *Azospirillum brasilense*-inoculated wheat (Dobbelaere et al. 1999; Remans et al. 2008; Contesto et al. 2008). In comparison to the untreated control at an average of 0.42 cm, the *A. thaliana* seedlings that were treated with *B. amyloliquefaciens* and *B. japonicum* both had significantly longer primary roots with an average of 1.1 cm and 1.44 cm respectively (Fig. 3). In terms of lateral root development, *B. amyloliquefaciens*-treated *A. thaliana* seedlings had an average of 3.67 lateral roots, whereas the *B. japonicum*-treated plants had an average of 3.33 lateral roots which both were significantly more in comparison to the untreated control's 0.33 (Fig. 4). PGPR that have been found to promote changes of root morphological architecture e.g. *Bradyrhizobium japonicum* and *Azospirillum brasilence*, do so by the facilitation of phytohormone production. The auxin, indole-3-acetic acid (IAA), has been prevalently characterized as the most common phytohormone produced by rhizobacteria (Kapulnik et al. 1985; Spaepen et al. 2007; Vacheron et al. 2013). IAA is the most plenteous auxin in nature and is produced via *de novo* synthesis and the release of conjugates (Bartel 1997). Auxins are tryptophan-like potent growth regulators (Delker et al. 2008; Vanneste & Friml 2012). Their functions include regulation of cell division, expansion and differentiation, as well as lateral root formation, mediation of tropic responses and flowering (Davies 2004; Mashiguchi et al. 2011). They are notably an integral inductive signal necessary for lateral root formation and emergence (Casimiro et al. 2003; Swarup et al. 2008).

Lateral root formation is an integral part of the root system architecture's plasticity which in turn plays a role in providing plants with efficient coping mechanisms against abiotic stresses, hence their study being of importance as we exist in a world

subjected to immense climate change and increasing soil degradation (Lavenus et al. 2013). Lateral roots stem from the parental root due to a small population of founder cells found in the vascular tissue's periphery, which due to auxin-mediated pathways such as the auxin influx carrier LAX3, emerge through the layers of tissue due to reprogramming of adjacent cells (Swarup et al. 2008; Lavenus et al. 2013). LAX3 is found in the cortical and epidermal cells which are directly above primordia, therefore the presence of auxin, which can be enhanced due to PGPR colonization, results in the induction of the selective nature of certain cell-wall remodeling enzymes (e.g. polygalacturonase (Wen et al. 2006), xyloglucosyl transferase (Vissenberg et al. 2005), expansin (Cosgrove 2000) among others). These enzymes then stimulate cell separation, providing a lee way for lateral root emergence and development (Swarup et al. 2008).

To further affirm the possibility of cytokinin production breaking the dormancy of the PGPR studied, lateral root development is largely auxin's game. In that, GA-deficient and GA-insensitive *Populus* displayed promoted lateral root formation, which was swiftly reverted with the exogenous application of bioactive GA (Busov et al. 2006; Guo et al. 2010). The promotion of lateral root development was largely responsible to the upregulation of an auxin efflux carrier, PIN9. The study concluded that GAs do decrease the lateral root density of *Populus* plants (Guo et al. 2010). Therefore, for both lateral root development and early germination to occur, GAs ought to be lower in comparison to auxins.

Rhizobacteria promote biomass in *Arabiopsis*

Plant growth promotion of plants, as a result of rhizobacterial colonization, can be attributed to both direct and indirect processes. One of the most prevalent mechanisms of

rhizobacteria-mediated plant growth promotion are increased nutrient availability and uptake, as well as phytohormone production (Ryu et al. 2005). For younger *A. thaliana* seedlings, the increased biomass is most likely due to the phytohormones produced by the rhizobacteria. *B. japonicum* strains have been shown to produce numerous phytohormones, such as indole-3-acetic acid (an auxin), zeatin (a cytokinin), gibberellic acid, ethylene and abscisic acid (Antoun et al. 1998; Boiero et al. 2007). *B. amyloliquefaciens* have been shown to produce auxins, gibberellins and cytokinins (Turner & Backman 1991; Mok & Mok 2001; Reva et al. 2004; Talboys et al. 2014). *A.thaliana* seedlings treated with *B. amyloliquefaciens* resulted in an average fresh weight of 3.02 μg (Fig. 5), whereas *B. japonicum* treated seedlings had an average fresh weigh of 3.98 μg (Fig. 5). Both these rhizobacterial treatments significantly juxtaposed the untreated control's average of 0.58 μg (Fig. 5), also displaying a statistically significant difference between themselves. This could be due to the role of certain phytohormones produced by these rhizobacteria in cell proliferation, division and expansion.

Cyclins and cyclin dependent kinases (CDKs) form complexes that play significant roles in the regulation of the cell division and endoreduplication cycles in plants (Sabelli et al. 2007; Tank et al. 2014). Cytokinins and auxins play a stimulatory role in the G1 phase of cell division due to their activation of the A-type cyclin-dependent kinase (CDKA)/D-type cyclin complexes which are heavily involved in the cell division and reproduction of plants (Cockroft et al. 2000; Iwakawa et al. 2006; Perrot-Rechenmann 2010; Novikova et al. 2013). Due to auxins and cytokinins role in the stimulation of cell division (Novikova et al. 2013) and both phytohormones being

produced by *B. japonicum* and *B. amyloliquefaciens*, they most likely attribute to the increased seedling biomass (Fig. 5). However, both ought to be produced to stimulate cell division as was observed with cultured cells and plant tissues, whereby auxin alone was not sufficient unless cytokinins were present (Trehin et al. 1998; Stals & Inze 2001; Inze & De Veylder 2006; Perrot-Rechenmann 2010).

Certain nutrients such as nitrogen, phosphorous and iron are limiting factors to a plant's biomass (Feng et al. 2004; Hermans et al. 2006). Some of these rhizobacteria are N_2-fixing, promoting root nodulation in leguminous plants, which then convert N_2 into the favorable plant nitrogen source, ammonia (van Rhijn & Vanderleyden 1995). Nitrogen availability influences plant biomass partitioning, whereby plants that are in poor N environments allocate a large portion of their total biomass to their root system (Cambui et al. 2011), whereas in a N rich environment both the belowground and vegetative biomass contributes largely to the total biomass, in conditions where organic N is the dominant nitrogenous source (Cambui et al. 2011).

Phosphorus is not easily acquirable. Despite its limited acquisition, the element has a role in the generation of energy, photosynthesis, enzyme activation and inactivation, carbohydrate metabolism, nucleic acid synthesis among numerous other developmental and regulatory functions in plants (Vance et al. 2003). Low levels of soluble phosphate prove a hindrance to plant growth, certain species of PGPR however can ameliorate this issue. PGPR-mediated phosphate solubilization occurs due to the production of low molecular weight acids organic acids e.g. citric acid and acid phosphatases that assist in the mineralization of inorganic and organic bound phosphates (Tarafdar & Claassen 1988; Bolan et al. 1994; Rodriguez & Fraga 1999; Lipton et al.

1987; Vassilev et al. 2006). Another mechanistic acquisition of organic phosphorous by PGPR, such as *Bacillus cereus* and *Bacillus megaterium*, is the mineralization of it due to the production of phosphatases which stimulate the hydrolytic breakdown of esters (Tao et al. 2008). Additionally, PGPR such as *Bacillus edaphicus* solubilize potassium-bearing minerals via production of organic acids and capsular polysaccharides (Sheng & He 2006).

Iron may be one of the most abundant elements in the soil, however not necessarily in a form that is easily assimilated by plants, but remains an element that is integral for plant growth (Ma 2005; Glick 2012). This is largely due to it largely existing in the form of ferric ions (Fe^{3+}), which is not easily soluble (Ma 2005). PGPR, such as *Pseudomonas putida* and *Azobacter species* have been shown to produce low-molecular weight siderophores, which are molecules that have a high affinity for ferric ions (Scher & Baker 1982; Ahmad et al. 2008; Hider & Kong 2010). Additionally, some rhizobacteria create membrane receptors which have the ability to bind to ferric ion/siderophore complexes making assimilation for iron into the microorganisms easier (Neilands 1981).

At 33 days, *A. thaliana* plants of the untreated control had an average fresh weight of 0.27 g, which was significantly lower than that of the *B. amyloliquefaciens*-treated plants' average of 0.6 g and *B. japonicum*'s average of 0.5 g (Fig. 12). Qualitatively, these plants are notably larger when treated with rhizobacteria in comparison to the control (Fig. 8). This could be attributed to the fact that strains of *B. amyloliquefaciens* have been found to express nitrogen-fixing genes (Krober et al. 2014), production of extracellular phytase which solubilizes phosphorus (Idriss et al. 2002) and siderophore

production for iron assimilation (Chen et al. 2007). *B. japonicum* is well understood as a nitrogen-fixing symbiont typically via promotion of nodulation in leguminous plants (Bedmar et al. 2005). Strains have been noted to produce siderophores, typically as a citrate (Guiernot e al. 1990), making iron readily available to their host plant. This enhanced resource acquisition could attribute to increased biomass in the PGPR-treated plants.

Rhizobacteria promote leaf surface area and development

The rhizobacteria-treated plants yielded more numerous leaves in comparison to the untreated control. The untreated control had an average of 6.25 leaves at reproduction, whereas the *B. amyloliquefaciens*-treated plants had an average of 10.25 leaves, surpassed by plants treated with *B. japonicum* which had an average of 12 leaves per plant (Fig. 6B). Qualitatively, this is evident with the leaf profile in Fig. 5A, a week prior to reproduction. Notably, both *B. amyloliquefaciens* and *B. japonicum* stimulate the synthesis and signaling of auxins. Therefore, the auxin accumulation most likely promotes a crosstalk with ASYMMETRIC LEAVES 1 (AS1) pathways, working together to repress the KNOTTED1-like homeobox (KNOX) gene expression *BREVIPEDICELLUS*, promoting leaf production and development (Hay et al. 2006).

Across different time points, there was a consistent pattern in that, both rhizobacteria treatments yielded leaves of larger surface areas in comparison to the untreated control (Fig. 7A-D). Additionally, *B. japonicum* had the larger surface areas, followed by *B. amyloliquefaciens* (Fig. 7A-D). There was a significant difference between the average leaf surface areas between the untreated control and rhizobacteria, as well as between the different PGPR species (Fig. 7A-7D). The large leaf surface areas are

further affirmed by Fig. 8, whereby the leaves are significantly larger for the rhizobacteria-treated plants, in comparison to the untreated control. Phytohormones are often attributed to observations in increased cell size, expansion, division and proliferation (Ryu et al. 2003). Auxin, being a prime example, as its multifaceted functions such as cell division and differentiation despite cell expansion stimulation being its primary role (Trewavas 2000). According to Garrenton et al. (2002) and Ryu et al. (2003) due to auxin induction, proton pumps found in the plasma membrane are activated resulting in cell wall acidification and enzymatic breakdown by hydrolases loosening the cell wall. This creates a leeway for water to enter the cytosol resulting in an increase in cell size. Other possibilities could be promotion of cell division induced by phytohormones.

Rhizobacteria enhance plant height and early flowering time

The plant height of the rhizobacteria-treated *A. thaliana* at 46 days was measured and compared to the untreated control. The control had an average height of 5.4 cm, whereas the *B. amyloliquefaciens* had an average height of 17.57 cm, surpassed by *B. japonicum*-treated plants at a mean of 27.47 cm (Fig. 10A-B). These yielded statistically significant differences between the untreated control and the rhizobacteria treatments (Fig. 10A). Fig. 10B further affirms that the heights of the different treated plants were significantly higher than the untreated control, with *B. japonicum* being the tallest, followed by *B. amyloliquefaciens* then the untreated control. Additionally, earlier flowering times were noted in the rhizobacteria-treated plants in comparison to the untreated control. *B. japonicum*-treated plants had a flowering time of 23.33 days after transplantation, followed by *B.amyloliquefaciens*-treated plants' with an average

flowering time of 28 days. Both were shorter than the untreated control's flowering time of 33.17 days after transplation (Fig. 11).

Increased plant height and earlier flowering could be attributed to phytohormone stimulation/signaling, particularly gibberellins and cytokinins. In *A. thaliana*, plant height and flowering go hand in hand, as the plant's height is determined by the flowering stalk (Smyth et al. 1990). Stimulation of cytokinins were found to promote shoot development in cytokine-insensitive tobacco plants (Werner et al. 2004). They have known functions in the induction of shoot meristem formation (Fletcher & Meyerowitz 2000), whereby they promote cell proliferation and differentiation via interaction of transmembrane receptor proteins and transcription factors which occur in regulatory loops (Skoog & Miller 1957, Werner et al. 2004). Cytokinins and GAs overlap to serve numerous functions particularly to induce flowering, trichome initiation, senescence, among others (Matias-Hernandez et al. 2016). Although GA concentrations are not entirely specified, promotion of stimulation of cytokinins in both *B. japonicum* and *B. amyloliquefaciens* could possibly facilitate the synergistic relationship between the two phytohormones to induce early flowering and promote shoot meristem development, yielding taller plants than the untreated control.

Rhizobacteria increase chlorophyll, water accumulation and soil moisture retention

At 31 days, the total chlorophyll content of the *A. thaliana* plants was taken. The untreated control had a significantly lower average of chlorophyll accumulation at 0.68 mg/g, in comparison to the *B. japonicum*-treated plants value of 0.75 mg/g, which was surpassed by the *B. amyloliquefaciens*-treated plants with an average of 1.17 mg/g (Fig. 9). Not only was there a statistical significance between the untreated control and the

rhizobacteria-treated plants, but between the different rhzobacteria strains as well (Fig. 9). Both *B. amyloliquefaciens* and *B. japonicum* have been shown to promote nitrogen fixation (Bedmar et al. 2005; Krober et al. 2014). Therefore, the rhizobacteria's capability to promote nitrogen fixation could contribute to the increased chlorophyll content. This is largely due to the correlation between chlorophyll content and nitrogen availability in the soil (Tam & Magistad 1935; Sideris & Young 1947). This relationship influences the carboxylation capacity and electron transport rate (Hikosaka 2004). Additionally, a lack of nitrogen, or inefficient nitrogen uptake results in yellowing of leaves, decreased leaf surface area and lower photosynthetic output (Bojovic & Markovic 2009).

The rhizobacteria notably increased the amount of moisture accumulated in the leaves of the *A. thaliana.* The untreated control had an average of 0.22 g, followed by *B. japonicum*-treated plants at an average of 0.5 g of moisture, which were both surpasses with *B. amyloliquefaciens*-treated plants that had an average moisture weight of 0.5 g (Fig. 13). Moisture accumulation was significantly higher in the rhizobacteria-treated plants possibly due to the increased chlorophyll content (and therefore enhanced Rubisco activity) which attributes to increased photosynthetic output (Flexas et al. 2006). Soil moisture retention was observed at the point of reproduction, whereby *B. japonicum* retained 72.53% of moisture, followed by *B. amyloliquefaciens* retention at 71.71% both significantly higher than the 64.93% of the untreated control over a span of 24 hours (Fig. 14). This could be attributed to the fact that soil moisture is of importance to respiration of soil microbes (Moyano et al. 2013).

Due to the crucial role that moisture plays in the livelihood of soil microbes, modulation of water accessibility is a primary goal, which can be accomplished by the

production of extracellular polymeric substances heightening their survivability (Roberson & Firestone, 1992, Or et al. 2007, Deng et al. 2015). An example of an extracellular polymeric substances are biofilms (Deng et al. 2015). Majority of microbes found in the soil exist in biofilm form, which typically contain proteins, polysaccharides and DNA (Or et al. 2007, Deng et al. 2015). Structurally, biofilms tend to have the ability to shrink and expand based on their microenvironments' potentials (Or et al. 2007). Their mucoid like appearance contributes to a possible restructuring of the soil (Alami et al. 2000) and promotion of stable soil aggregation which makes moisture retention more favorable (Kets et al. 1996; Amellal et al. 1998; Park et al. 2007; Gajic et al. 2010). *B. japonicum* is known to form biofilms, typically around the rhizosphere of soybean plants (Perez-Gimenez et al. 2009; Lee et al. 2012). *B. amyloliquefaciens* have also been seen to exist in soil as biofilms (Chen et al. 2007). Both rhizobacteria's capability to form biofilms could contribute to their more enhanced moisture retention in comparison to the untreated control.

Rhizobacteria decrease the rate of water loss

Majority of flowering plants do not suffer a relative water content loss surpassing 5-15% (Gaff 1971). Water loss of the different treatments were observed over a 7-day period, in relation to the surface area of the plant. The untreated control had a sharp spike within one day, losing at its peak 0.8% of water, followed by a gradual decrease (Fig. 15 A). All treatments experienced a sharp loss, followed by a gradual decrease. Notably, despite having the larger surface areas, the rhizobacteria-treated plants lost water at a significantly slower rate. Fig. 15B displays the state of the plants after 1 week of water withholding, whereby the rhizobacteria-treated plants look desiccated but still maintain

their morphological integrity, whereas the untreated control does not. Mechanistically, this could be accomplished due to several reasons. An example is the production of the enzyme, 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase), which lowers the stress ethelyene levels by converting its precursor, 1-aminocyclopropane-1-carboxylate and convert it into oxobutanoate and ammonia (Glick et al. 2007).

The stimulation of abscisic acid, a phytohormone, could play a significant role in stomatal conductance, further affirming the water accumulation found in the bacteria-treated plants in Fig. 13 and being a possible mechanism for priming plants for drought stress. Exogenous application of abscisic acid have shown to induce stomatal closure (Jones & Mansfield 1970). *B. japonicum* is known to stimulate production of abscisic acid (Boiero et al. 2007), which can attribute to the closure of its stomata in stressful conditions losing moisture at a much slower rate than the untreated control. *B. amyloliquefaciens* have been known to stimulate the production of abscisic acid, in terms of biocontrol capability against *Rhizoctonia solani* of Chinese cabbage (Kang et al. 2015). This could attribute to the water retention of plants treated by *B. amyloliquefaciens*.

5. FUTURE DIRECTIONS

In the ever-changing world we exist in, climate change adaptability is of importance particularly for resources we are dependent on. This project focused on identifying possible roles of rhizobacteria in priming plants for drought stress tolerance. Beneficial microbes have shown to confer plants with several capabilities that they cannot solely accomplish, or at the very least are enhanced with these interactions.

The tools used today to identify the possibilities presented with plant-microbe interactions are omnipresent, but their application and thorough mechanistic elucidation lacking. Tools such as proteomic profiling of the promotional effect of some PGPR have been very recently studied in hopes to pinpoint the molecular basis of their growth promoting and abiotic stress tolerance capability. Additionally, interactions yield metabolites that are often minute but have grandiose effects and would require analyses using sophisticated equipment such as LC-MS/MS. Such analyses make proposed postulations concrete. Notably, the study of these interactions often is dismantled from the lab settings, promoting the need for studies to be done in gnotobiotic systems. In order to employ ideologies into applied innovations, proper understandings of these mechanisms are necessary.

This study looked at the priming for drought tolerance, however, a next step would be to study at what point can the PGPR-treated plants post-drought, come back to life due to rehydration. Additionally, further affirming the upregulation of PGPR-induced stress-related genes can provide a crisper and precise picture in regard to what occurs. Another interesting perspective and future approach would be to study rhizobacteria that coevolved with plants in arid environments to study their effect on priming non-arid

plants for drought stress tolerance. Plant-microbe interactions are an environmentally friendly approach to ameliorating anthropogenically driven detriment that ought to be more thoroughly sought after, particularly in our environmentally tumultuous world.

TABLES

Code	Species	Germination Rate	Seedling root length (cm)	Seedling biomass (ug)
312	*Bacillus coagulans*	3.2	0.43	2.76
313	*Bacillus thuringiensis*	4	0.54	2.48
314	*Bacillus circulans*	3.5	0.39	2.07
315	*Bacillus polymyxa*	2	0.69	1.98
316	*Bacillus licheniformis*	1.8	0.75	2.95
317	*Bacillus firmus*	3	0.70	2.84
318	*Bacillus lentus*	3.4	1.2	1.89
319	*Bacillus pumilus*	3	0.67	1.20
322	*Streptomyces hygroscopicus*	2.8	0.58	2.62
323	*Streptomyces rimosus*	3.7	0.56	2.36
324	*Streptomyces fasciculatus*	4.1	0.47	1.43
327	*Pseudomonas aeruginosa*	3.1	1.04	2.87
329	*Pseudomonas putida*	4.2	0.74	1.77
332	*Pseudomonas fluorescens*	4.6	0.46	2.00
338	*Azospirillum lipoferum*	4	1.02	2.75
405	*Bacillus amyloliquefaciens*	2.75	1.1	3.02
407	*Bradyrhizobium japonicum*	2.75	1.44	3.98

Table 1: Preliminary screening the effects of the PGPR library on *Arabidopsis thaliana*.

Bacteria Species	Code Number	Gram Result	Endospore-forming
Bacillus amyloliquefaciens	405	Positive	Yes
Bradyrhizobium japonicum IRAT FA3	407	Negative	No

Table 2: Summary of rhizobacteria species studied.

Phytohormone	Functions	References
Auxins	Cell division, cell differentiation, cell expansion stimulation, seed dormancy, lateral root formation and emergence, leaf production and development, primary root development	Kapulnik et al. 1985; Turner & Backman 1991; Bartel 1997; Antoun et al. 1998; Trehin et al. 1998; Trewavas 2000; Casimiro et al. 2003; Hay et al. 2006; Boiero et al. 2007; Spaepen et al. 2007; Delker et al. 2008; Remans et al. 2008; Swarup et al. 2008; Guo et al. 2010; Mashiguchi et al. 2011Perrot-Rechenmann 2010; ; Vanneste & Friml 2012; Novikova et al. 2013; Lavenus et al. 2013; Vacheron et al. 2013; Talboys et al. 2014; Shao et al. 2015
Cytokinins	Reproductive development, delayed senescence, leaf size expansion, *de novo* bud release and formation, seed germination promotion, cell division, shoot meristem formation, cell proliferation, cell differentiation, flowering induction, trichome initiation, senescence	Turner & Backman 1991; Mok 1994; Antoun et al. 1998; Timmusk et al. 1999; Fletcher & Meyerowitz 2000; Mok & Mok 2001; Reva et al. 2004; Boiero et al. 2007; Novikova et al. 2013; Talboys et al. 2014; Kefela et al. 2015; Matias-Hernandez et al. 2016
Gibberellic Acids (GA)	Meristem to shoot growth process, Vegetative to reproduction process, juvenile to adult leaf stages, germination stimulation, sexual expression determination, cell differentiation, flowering induction, trichome initiation, senescence	Turner & Backman 1991; Antoun et al. 1998; Gutierrez-Manero et al. 2001; Mok & Mok 2001; Reva et al. 2004; Busov et al. 2006; Boiero et al. 2007; Guo et al. 2010; Talboys et al. 2014; Gupta & Chakrabarty 2013; Matias-Hernandez et al. 2016
Abscisc acid	Induction of stomatal closure, seed dormancy with auxin-mediated signaling,	Jones & Mansfield 1970; Liu et al. 2013

Table 3: Summary of phytohormones discussed with functional relevance to the study

FIGURES

Figure 1: Drought is a Global Issue: The Global Drought Portal Data Map, displaying global drought intensity. The darker the red, the higher the drought intensity (http://www.drought.gov/gdm/current-conditions). (In Supplement)

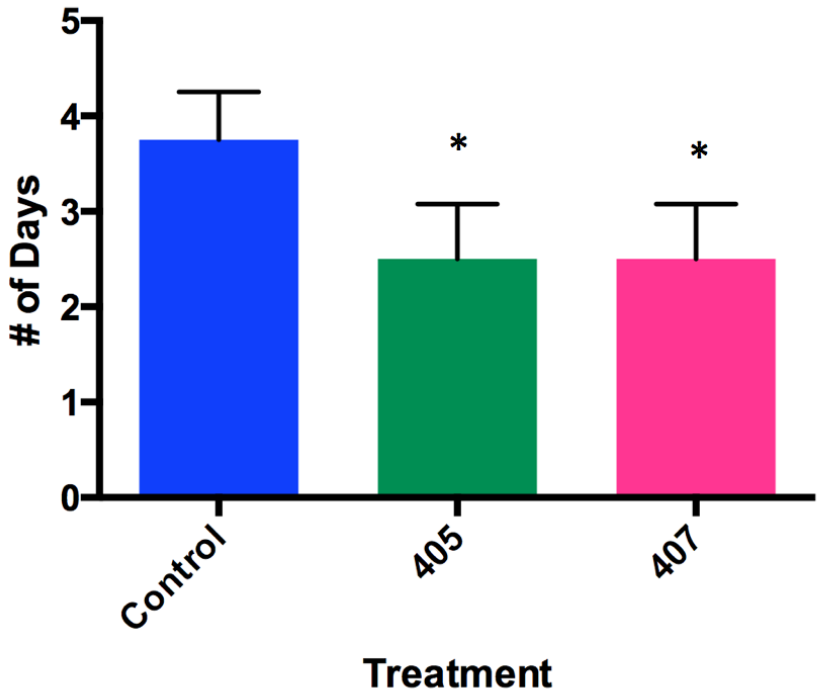


Figure 2: Both rhizobacteria significantly promote faster germination rates in *A. thaliana* in comparison to the untreated control. One-way ANOVA analysis yielded a p value of <0.05. Unpaired t-tests with Welch's Correction, displayed that both treatments against the untreated control yielded values of $p<0.05$ as denoted by asterisks (*).

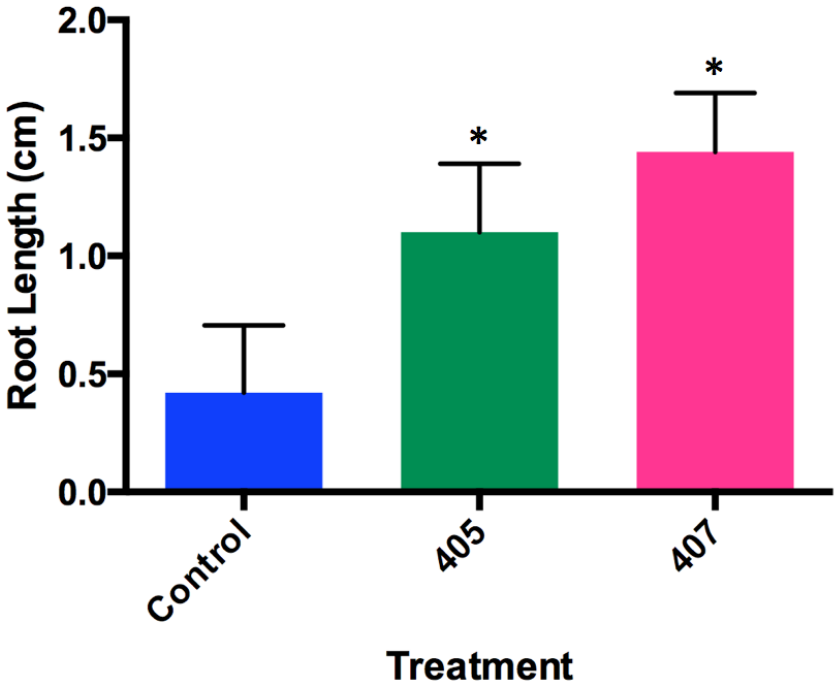


Figure 3: PGPR significantly promote root length development in *A. thaliana* seedlings in comparison to the untreated control. One-way ANOVA analysis yielded a p value of <0.05. Unpaired t-tests with Welch's Correction, displayed that both treatments against the untreated control yielded values of $p<0.05$ as denoted by asterisks (*).

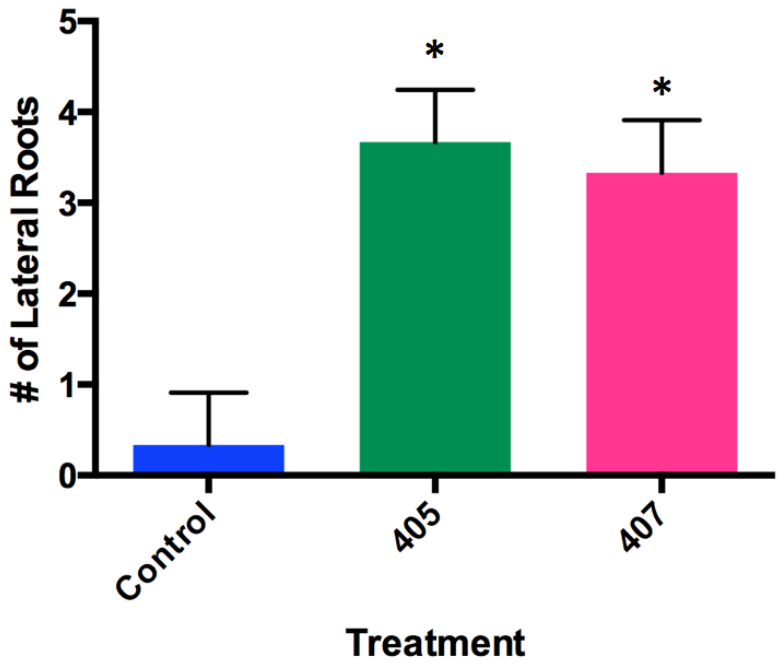


Figure 4: PGPR significantly promote lateral root development in *A. thaliana* seedlings in comparison to the untreated control. Unpaired t-tests with Welch's Correction, displayed that both treatments against the untreated control yielded values of $p<0.05$ as denoted by asterisks (*). One-way ANOVA analysis yielded a p value of <0.05.

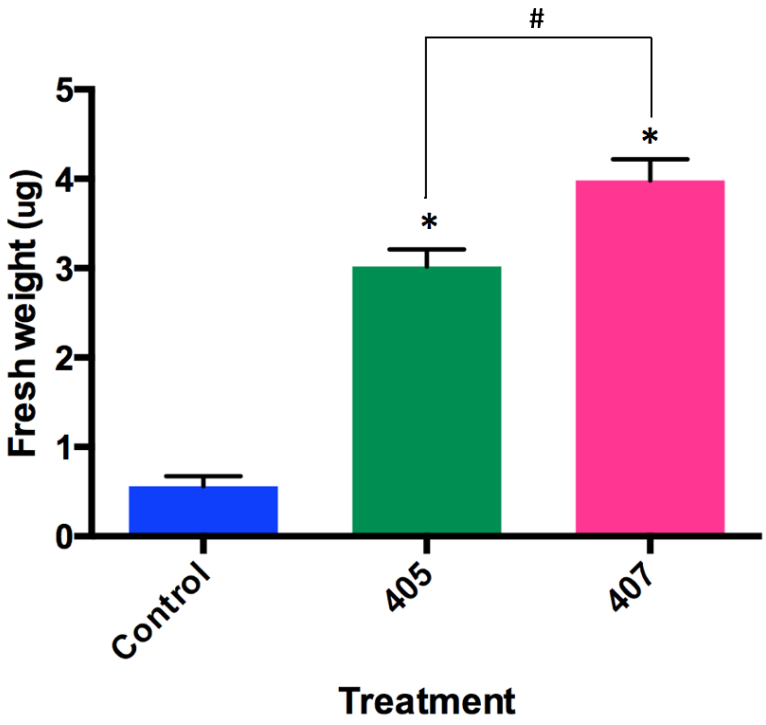


Figure 5: PGPR significantly promote biomass increase in *A. thaliana* seedlings in comparison to the untreated control. Unpaired t-tests with Welch's Correction, displayed that both treatments against the untreated control yielded values of $p<0.05$ as denoted by asterisks (*). Unpaired t-tests with Welch's Correction comparing both treatment groups, yielded $p<0.05$ as denoted by the octothorp (#). One-way ANOVA analysis yielded a p value of <0.05.

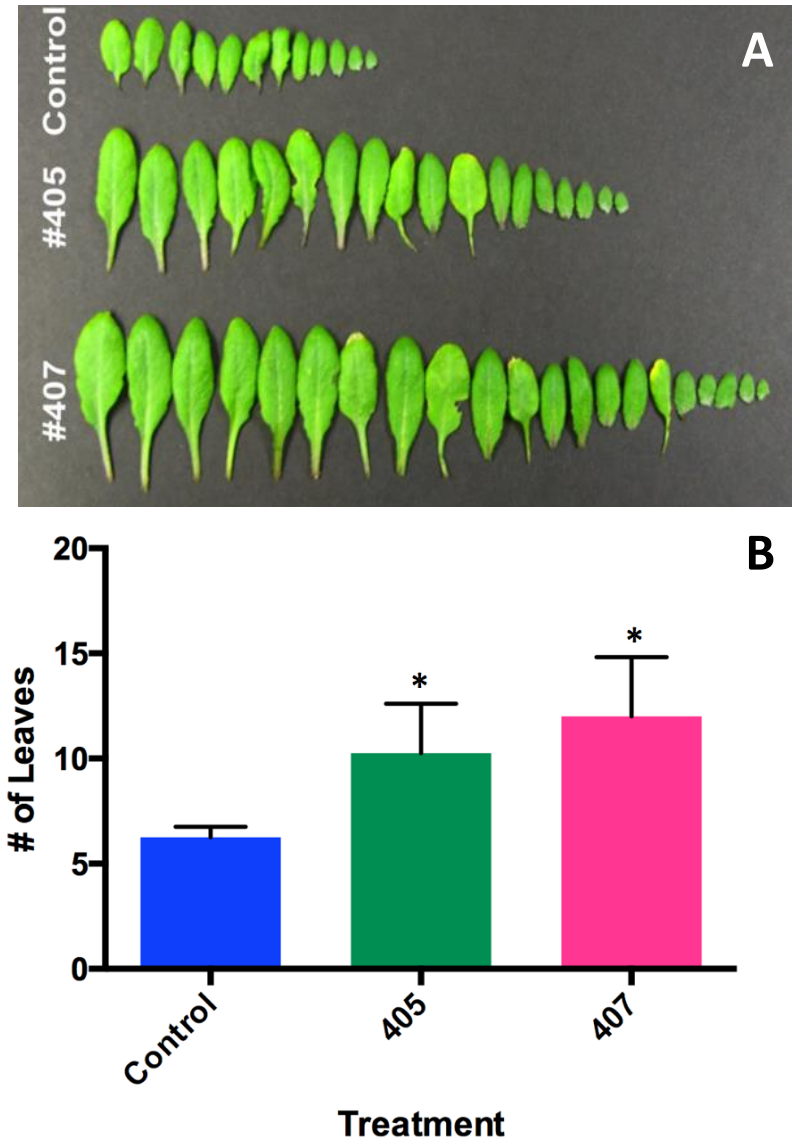


Figure 6: PGPR significantly promotes faster leaf development in *A. thaliana* in comparison to the untreated control at reproduction. **A:** A photographic image displaying the leaf profile of both treated plants, as well as the untreated control. **B:** Unpaired t-tests with Welch's Correction, displayed that both treatments against the untreated control yielded values of $p<0.05$ as denoted by asterisks (*). One-way ANOVA analysis yielded a p value of <0.05.

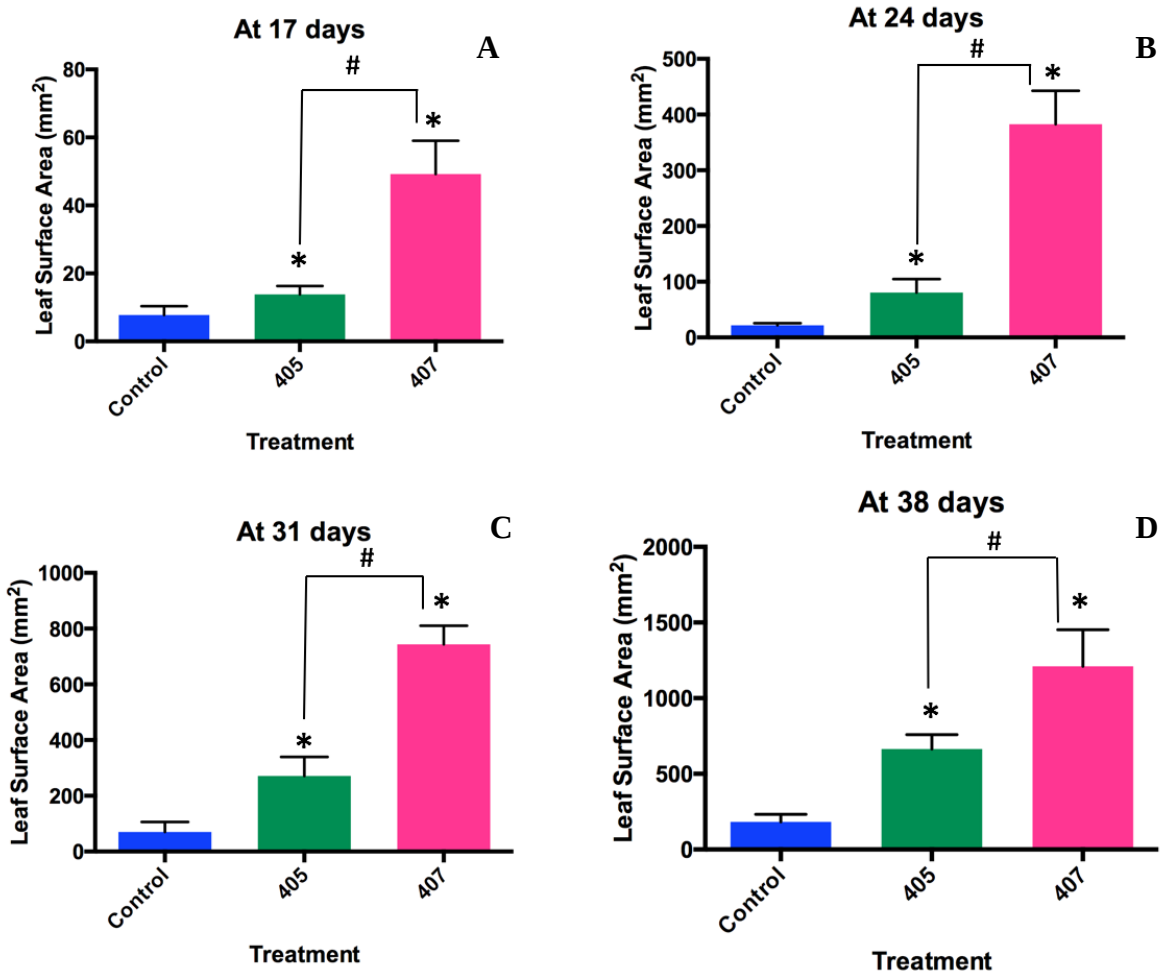


Figure 7: PGPR significantly increase the leaf surface area of *A. thaliana* in comparison to the untreated control. Unpaired t-tests with Welch's Correction, displayed that both treatments against the untreated control yielded values of $p<0.05$ as denoted by asterisks (*). Unpaired t-tests with Welch's Correction comparing both treatment groups, at all time points, yielded $p<0.05$ as denoted by the octothorp (#). One-way ANOVA analyses for all the different sets yielded a p value of <0.05.

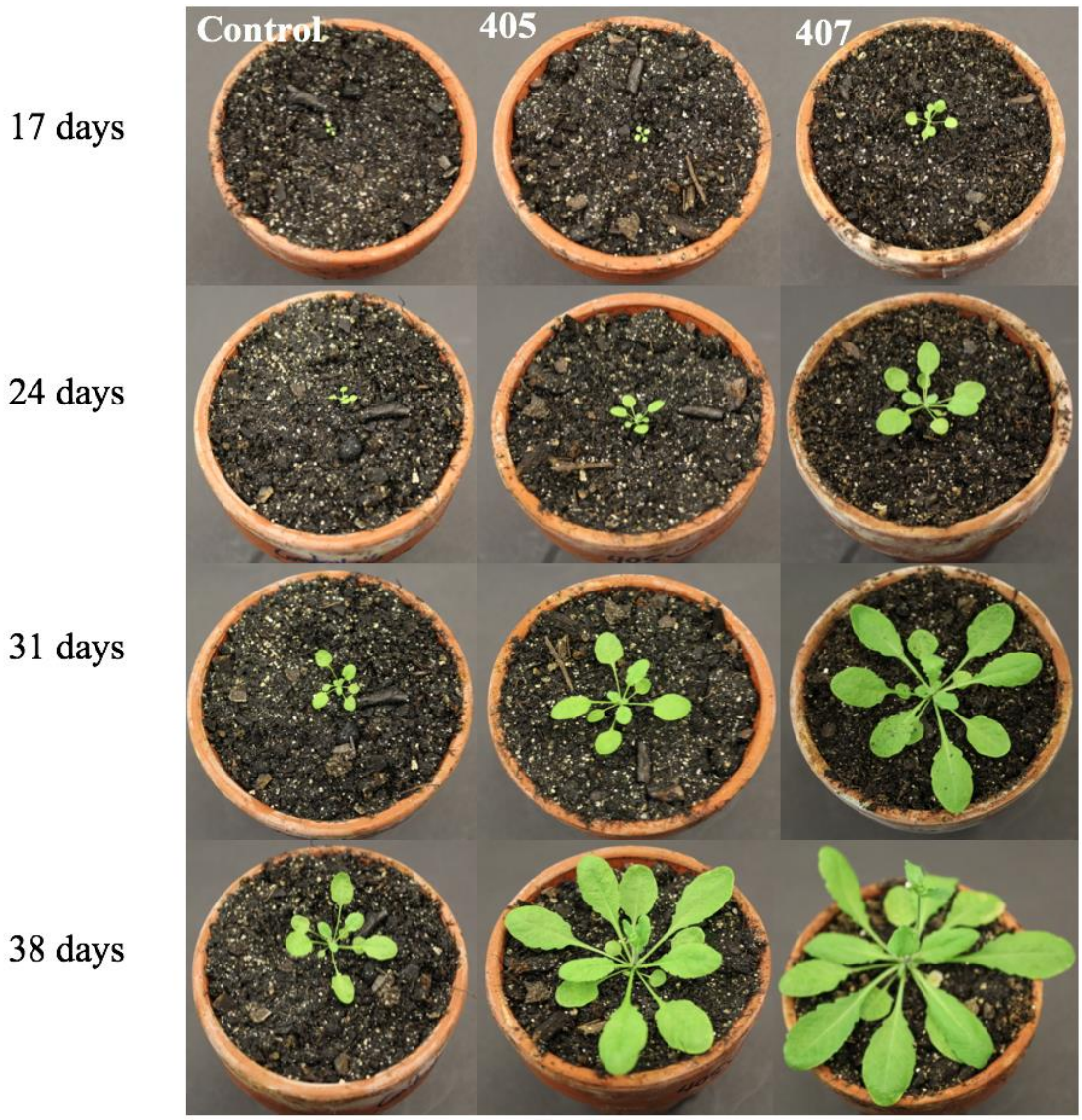


Figure 8: Rhizobacteria significantly increase the vegetative biomass of *A. thaliana* over time.

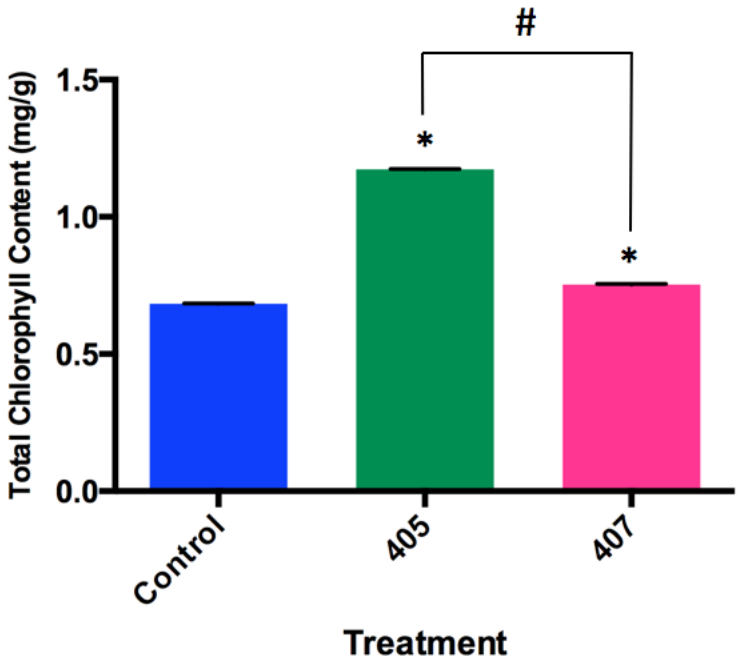


Figure 9: PGPR significantly increase the total chlorophyll content in *A. thaliana* in comparison to the untreated control at 31 days. One-way ANOVA statistical analysis yielded a P value of <0.05. Unpaired t-tests with Welch's Correction, displayed that both treatments against the untreated control yielded values of $p<0.05$ as denoted by asterisks (*). Unpaired t-tests with Welch's Correction comparing both treatment groups yielded $p<0.05$ as denoted by the octothorp (#).

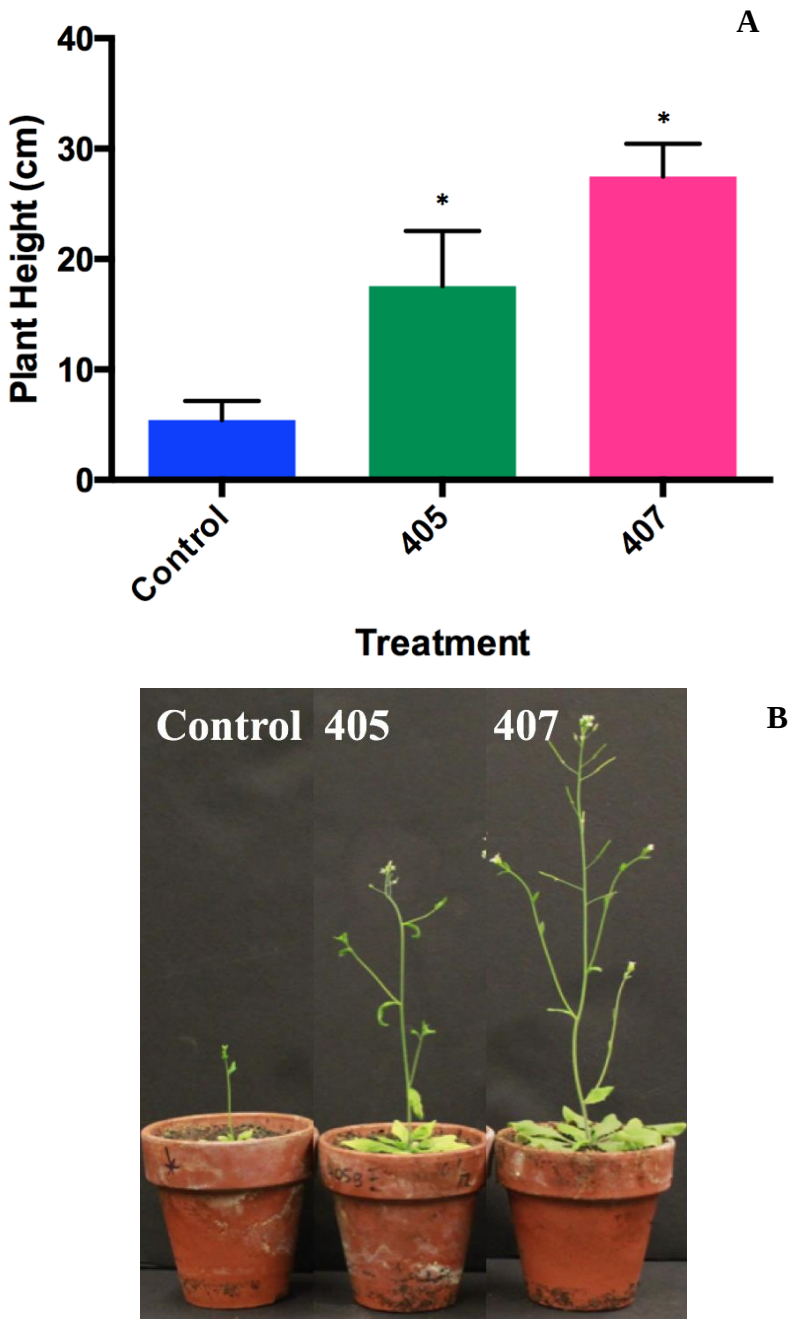


Figure 10: PGPR significantly increase plant height of *A. thaliana* in comparison to the untreated control at 46 days. One-way ANOVA statistical analysis yielded P value of <0.05. Unpaired t-tests with Welch's Correction, displayed that both treatments against the untreated control yielded values of $p<0.05$ as denoted by asterisks (*).

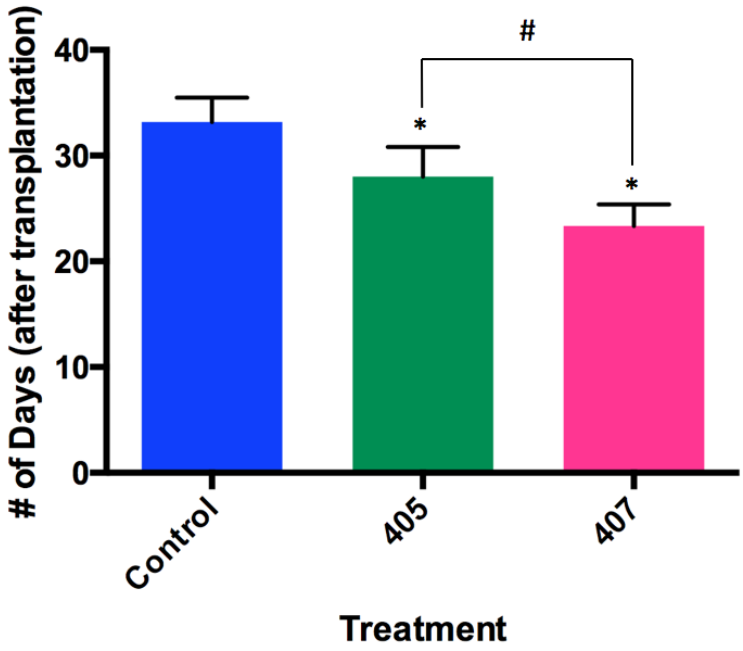


Figure 11: PGPR significantly promotes earlier flowering time in *A. thaliana* in comparison to the untreated control at reproduction. One-way ANOVA statistical analysis yielded a P value of <0.05. Unpaired t-tests with Welch's Correction, displayed that both treatments against the untreated control yielded values of $p<0.05$ as denoted by asterisks (*). Unpaired t-tests with Welch's Correction comparing both treatment groups, at all time points, yielded $p<0.05$ as denoted by the octothorp (#).

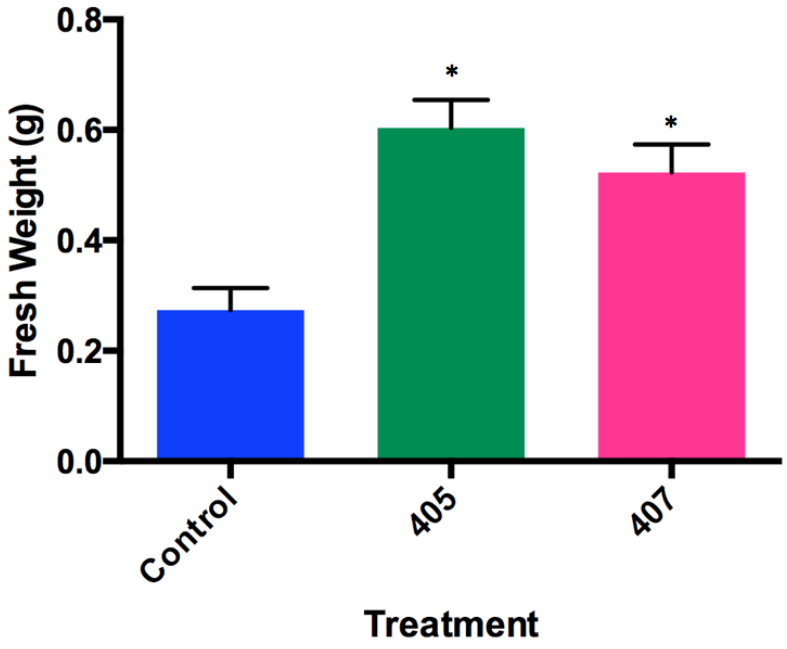


Figure 12: PGPR significantly promotes fresh weight biomass accumulation in *A. thaliana* in comparison to the untreated control at 33 days. One-way ANOVA statistical analysis yielded a P value <0.05. Unpaired t-tests with Welch's Correction, displayed that both treatments against the untreated control yielded values of $p<0.05$ as denoted by asterisks (*).

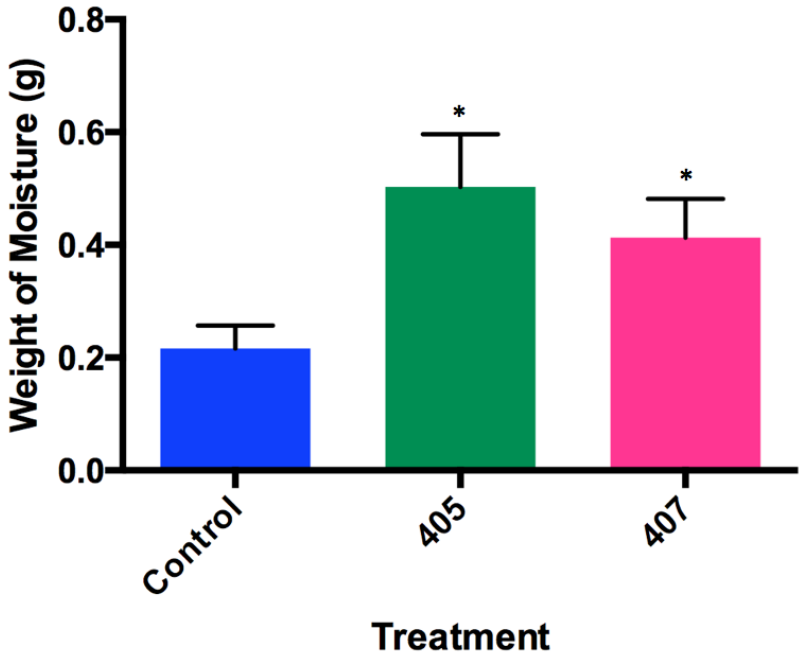


Figure 13: PGPR significantly promote the weight of moisture in *A. thaliana* in comparison to the untreated control at reproduction. One-way ANOVA statistical analysis yielded a P value <0.05. Unpaired t-tests with Welch's Correction, displayed that both treatments against the untreated control yielded values of p<0.05 as denoted by asterisks (*).

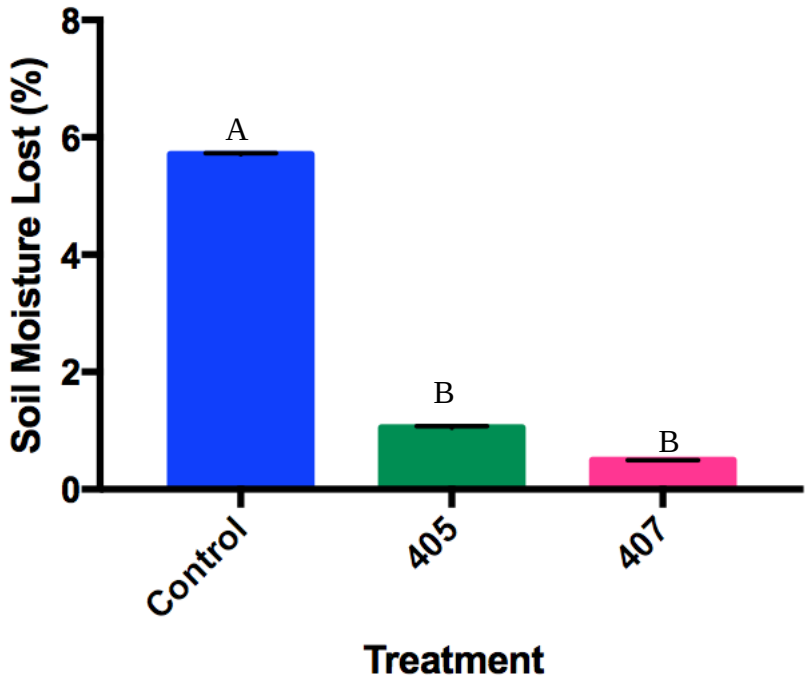
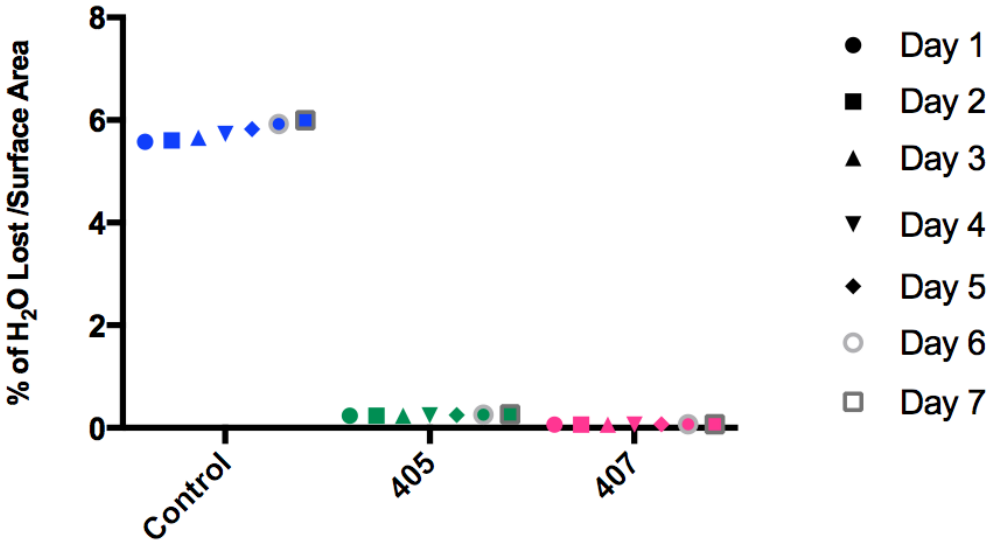


Figure 14: PGPR increases the soil moisture retention in comparison to the untreated control at reproduction. One-way ANOVA statistical analysis yielded a P value of <0.05. Unpaired t-tests with Welch's Correction, displayed that both treatments against the untreated control yielded values of $p<0.05$ as denoted by different lettering pairs.

A



B

Figure 15: PGPR decreases the amount of water lost/surface area *A. thaliana* in comparison to the untreated control at 1-week of drought stress. 2nd-way ANOVA statistical analysis yielded a $p < 0.05$ (A). B: *A. thaliana* subjected to 1 week of drought stress.

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PUBLICATION LIST

Relevant publications to the thesis

1. Kefela T, Gachomo EW, Kotchoni SO (2015) *Paenibacillus polymyxa, Bacillus licheniformis* and *Bradyrhizobium japonicum* IRAT FA3 promote faster seed germination rate, growth and disease resistance under pathogenic pressure. Journal of Plant Biochemistry and Physiology, 3(1):1-5.
2. Gachomo EW, Kefela T, Houngnandan P, Baba-Moussa L & Kotchoni SO (2014) *Bradyrhizobium japonicum* IRAT FA3 increases biomass, yield and drought tolerance in plants. Journal of Biology & Nature, 1(1): 12-23

Research Accomplishments

1. Sika KC, Adoukonou-Sagbadja H, Ahoton L, Saidou A, Kefela T, Gachomo EW, Baba-Moussa L, Kotchoni SO (2015) Genetic characterization of Cashew (*Anacardium occidentale* L.) cultivars from Benin. Journal of Horticulture, 2:3.
2. Kefela T, Gachomo EW, Kotchoni SO (2015) *Paenibacillus polymyxa, Bacillus licheniformis* and *Bradyrhizobium japonicum* IRAT FA3 promote faster seed germination rate, growth and disease resistance under pathogenic pressure. Journal of Plant Biochemistry and Physiology, 3(1):1-5.
3. Sika KC, Kefela T, Adoukonou-Sagbadja H, Ahoton L, Saidou A, Baba-Moussa L, Jno Baptiste L, Kotchoni SO, Gachomo EW (2015) A simple and efficient genomic DNA extraction protocol for large scale genetic analyses of plant biological systems. Plant Gene, 1: 43-45.

4. Gachomo EW, Kefela T, Houngnandan P, Baba-Moussa L & Kotchoni SO (2014) *Bradyrhizobium japonicum* IRAT FA3 increases biomass, yield and drought tolerance in plants. Journal of Biology & Nature, 1(1): 12-23.
5. Gachomo EW, Jno Baptiste L, Kefela T, Saidel WM & Kotchoni SO (2014) The *Arabidopsis CURVY1 (CVY1)* gene encoding a novel receptor-like protein kinase regulates cell morphogenesis, flowering time and seed production. BMC Plant Biology, 14:221.

Achievements

1. Dean's First Year Award for Academic Excellence, Rutgers University, Camden Faculty of Arts and Sciences (2014-2015)
2. Graduate Fellowship, LEAP STEAM Academy Fabrication Lab (2014-2016)
3. Civic Engagement Graduate Fellowship, Office of Civic Engagement, Rutgers University-Camden (Fall 2014-Spring 2015)
4. Certificate of Recognition for outstanding commitment and exemplary service for the benefit of children and families of the City of Camden, New Jersey, Community Leadership Center, Rutgers University-Camden (2014-2015; 2015-2016)
5. LEAP STEAM Academy Chemistry Teaching Fellowship (2015-2016)
6. Inaugural National Social Justice Challenge National Track Award Recipient, Rutgers Newark School of Public Affairs and Administration (2015-2016)

Kefela T, Gachomo EW, Kotchoni SO (2015) *Paenibacillus polymyxa, Bacillus licheniformis* and *Bradyrhizobium japonicum* IRAT FA3 promote faster seed germination rate, growth and disease resistance under pathogenic pressure. Journal of Plant Biochemistry and Physiology, 3(1):1-5.

(In Supplementary)

Gachomo EW, Kefela T, Houngnandan P, Baba-Moussa L & Kotchoni SO (2014) *Bradyrhizobium japonicum* IRAT FA3 increases biomass, yield and drought tolerance in plants. Journal of Biology & Nature, 1(1): 12-23

(In Supplementary)