**Improving Drought Stress Tolerance of Peanut Using PGPR and Orange Peel Amendment**

by

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## Abstract

Peanut provides approximately $4 billion to the US economy. Drought lowers that amount with disease and nutrition and yield loss. Due to a rising global population, less water will be available for agriculture. Inoculating peanut with *Bacillus velezensis* (*Bv*) and orange peel (OP) has not been studied as extensively as other drought control methods. Past studies have shown that *Bv* and OP increase plant growth. The objectives of this experiment were to determine if OP and *Bv* could improve drought tolerance and if any interactions could be found between the genotypes and inoculants. Four different genotypes were studied within a randomized complete block design in a greenhouse. Results indicated that *Bv* and OP helped increase drought tolerance and that interactions occurred between genotypes and inoculants. Further study can be directed towards determining AU 18's potential and how well these *Bv* and OP inoculants help these genotypes in different settings.

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| | |
|---|---|
| $\Delta^{13}C$ | Carbon Isotope Discrimination |
| %Ndfa | Percentage of Nitrogen Derived from the Atmosphere |
| µl | Microliter |
| $^{12}C$ | Carbon-12 |
| $^{13}C$ | Carbon-13 |
| $^{14}N$ | Nitrogen-14 |
| $^{15}N$ | Nitrogen-15 |
| ANOVA | Analysis of Variance |
| *Bv* | *Bacillus velezensis* |
| Ca | Calcium |
| CFU | Colony Forming Unit |
| ChlD | Chlorophyll Density |
| cm | Centimeters |
| $cm^2$ | Square Centimeters |
| $CO_2$ | Carbon Dioxide |
| DAE | Days after Emergence |
| DAP | Days after Planting |
| DW | Dry Weight |
| EPS | Exopolysaccharides |
| FW | Fresh Weight |
| g | Grams |
| Gb | Giga Base Pairs |

| | |
|---|---|
| ha | Hectare |
| $H_2O$ | Water |
| IAA | Indole-3-acetic Acid |
| K | Potassium |
| kg | Kilogram |
| LA | Leaf Area |
| mg | Milligram |
| ml | Milliliter |
| N | Nitrogen |
| NNP | Non-nodulating Peanut |
| OD | Optical Density |
| OGA | Oligogalacturonides |
| OP | Orange Peel |
| OPP | Orange Peel Powder |
| PAC | Preharvest Aflatoxin Contamination |
| PGPR | Plant Growth Promoting Rhizobacteria |
| ppb | Parts per Billion |
| PSII | Photosystem 2 |
| RCBD | Randomized Complete Block Design |
| RWC | Relative Water Content |
| SCMR | SPAD Chlorophyll Meter Reading |
| SPAD | Soil-Plant Analysis Development |
| SWC | Soil Water Content |

| | |
|---|---|
| TD | Terminal Drought |
| TE | Transpiration Efficiency |
| TW | Turgid Weight |
| WC | Water Control |
| WUE | Water Use Efficiency |
| WUEi | Intrinsic Water Use Efficiency |

# Chapter I Literature Review

## The Value of Peanut

Peanut, also known by its scientific name as *Arachis hypogaea L.*, is currently being grown on 25 million hectares (ha) worldwide, and annual production for this crop is approximately 46 million tons [1, 2]. China, India, Nigeria, and the United States (US) are the largest peanut producers [3]. Other contributing countries include Sudan and Argentina [3].

As a member of the *Fabaceae* family, the *Arachis hypogaea L.* species has two subspecies, *A. hypogaea ssp. hypogaea* and *A. hypogaea ssp. fastigiata*, which are respectively divided into two botanical varieties, *hypogaea* and *hirsuta*, and four botanical varieties, *fastigiata*, *vulgaris*, *aequatoriana*, and *peruviana* [4]. According to Zhuang et al., evidence points to *A. hypogaea L.* having been domesticated in Argentina, Bolivia, Paraguay, and Brazil. Additionally, peanut is an allotetraploid (AABB, 2n=4x=40) with a genome size of approximately 2.7 giga base pairs (Gb). Zhuang et al. also indicated that its ancestors are the diploid plants *A. duranensis*, contributing the AA genome, and *A. ipaensis*, contributing the BB genome, which hybridized to form *A. hypogaea*. With genetic recombination between these two genomes, *A. hypogaea L.* became a more domesticated polyploid crop. Studying this genome has revealed candidate genes for seed size, crop yield, peanut quality, and other agronomic traits [1, 2]. Along with this recombination, though, *A. hypogaea* also became reproductively isolated from wild species, reducing its genetic diversity [5].

The peanut crop is an annual plant and displays self-pollination and an indeterminate growth habit, meaning that the plant keeps flowering throughout its life [4, 6]. Along with these characteristics, peanut has oil that can reduce low-density lipoprotein blood cholesterol, easily

digestible protein, carbohydrates, high amounts of fiber, and other nutrients that allow it to be used in food, alcoholic beverages, shampoos, livestock feed, and other products [2, 5, 7, 8]. The most popular product in the US made from peanut, though, is peanut butter [9]. When accounting for this information, peanut is an essential resource that needs further study. The US has maintained yearly exports with an average of at least 500,000 metric tons. This total leads to over $675 million [3].

## The Relationship between Drought and Peanut

With the aforementioned effect peanut has on food systems, mentioning the effect of drought is important for understanding how to help peanut provide a more beneficial impact. Climate change is projected to exacerbate drought, particularly posing issues for agriculture and arid and semiarid locations [10, 11]. Since approximately 90% of peanut production worldwide is conducted within tropical and semi-arid lands with hot temperatures and small, sporadic amounts of rain, improving drought resistance in peanut is justified [12]. According to Kambiranda et al. [13], estimated yearly deficits in peanut production at over US$520 million were due to issues coming from drought. Anything about drought, including the intensity, length, and when it begins, can have varying effects on peanut development growth and final yield throughout its growing season [13]. All of these effects reduce the peanut yield and seed's nutritional quality [14].

For the US, an estimated 65% of peanuts have been grown in dryland areas dependent upon rain [13]. McCarty et al. revealed how exacerbated the issue of finding more land for agriculture has become by stating that the rate of available arable land has not been increasing as quickly as the global population [15]. To make matters worse, Ngumbi et al. have predicted that more than half of all crops worldwide will suffer from drought-mediated growth problems by

2050 [16]. This is compounded by a fact noted by Rubin et al. [17] that irrigation has already accounted for 70% of water used around the world.

In past studies on plant productivity, drought has negatively affected photosynthetic activity and yield in peanut crops [13]. Additionally, the change in plant lipid content has caused membranes to become more vulnerable, reducing photosynthesis [13]. Drought stressed plants have also been observed to have reduced relative water content (RWC), which indicates how much water is routed to leaf tissue and transpiration. Additionally, drought stress has reduced leghaemoglobin in peanut nodules. As this event has occurred, photosynthesis has been reduced, leading to a lack of carbohydrates in nodules and reduced $N_2$ fixation. Drought has even reduced leghaemoglobin production in peanut nodules, decreasing atmospheric nitrogen ($N_2$) fixation [13, 18].

Kambiranda et al. [13] also noted that peanut pods experienced negative consequences due to drought. Pegs elongated later than expected due to turgor reduction for pods, and they were less able to penetrate the soil if the drought had lasted long enough. Even if the pegs did successfully penetrate the soil, though, lack of water prevented many of them from becoming pods and stunted seed growth, decreasing pod yield [13].

## Drought Management Solutions

Many studies have already been conducted on drought management in peanut, and one of those methods is about improving the genetics of peanut cultivars. Dang et al. evaluated various genotypes to identify candidate genes that could enable peanuts to become more drought tolerant [19]. Chen et al. studied different genotypes to determine if germplasm like Exp8-12 could be used for later drought tolerance studies for peanut breeding [20]. More recently, Kishor et al.

indicated that transgenic peanut could aid in managing drought stress, as they have been shown to have more biomass and yield in addition to improved drought tolerance. One transgenic cultivar example, GG20, had a transcription factor named *AtDREB1A,* which improves water use efficiency (WUE) and therefore increase peanut's drought tolerance [21]. WUE is important because it indicates how efficiently water consumed by the plant can assimilate carbon for other tasks, including helping the plant make more biomass [22]. Besides these measures, Devi et al. researched molecular markers linked with WUE, which can be used to regulate transpiration rate in peanut to avoid lower yields due to drought stress [23].

Another option is nutrient management. Dinh et al. studied how different peanut genotypes performed under midseason drought in terms of nutrient uptake; more drought tolerant genotypes consumed more nutrients, which helped them with pod yield and biomass creation [14]. Htoon et al. studied peanut nutrient uptake under terminal drought (TD) conditions, which is drought during pod and seed development. The study revealed that if a peanut cultivar can maintain recommended levels of nutrient uptake and N fixation under TD then the cultivar will be more likely to avoid aflatoxin contamination [24]. As for determining the effects of a specific nutrient, Gu et al. studied the effects of calcium (Ca) fertilizer on peanut growth under drought stress. Their fertilizer applications increased pod quantity per plant and the amount of fat and protein in peanut kernels [25].

Irrigation is another research topic in the effort of drought management. Furlan et al. demonstrated that if peanut plants are rehydrated after drought stress, they can recover from symptoms that had negative impacts on photosynthesis [26]. Saudy et al., in an effort to help farmers concerned with conserving water in their arid farmlands, showed that even when watering peanut, with 25% less than what the farmers are used to, peanut continued to take in

desirable amounts of seed nutrients, like potassium (K), and produced desired biomass yield. While they stated that the traditional irrigation rate produced larger weights for seed biomass yield per hectare, they also revealed that differences between the 100% and 75% irrigation rates were negligible [27].

One other source of stress management comes from utilizing plant growth promoting rhizobacteria (PGPR). A seed coating of a specific kind of PGPR, *Bacillus subtilis*, is already applied to peanut for protection against soil borne pathogens from *Rhizoctonia, Fusarium,* and other genera [28]. PGPR have also been used as a source of support for crops like shoot growth promotion in corn, protection from soil borne pathogens in soybean, fungal disease control for peanut, and growth promotion in potato [16, 17, 28, 29]. One reason for this support being successful, as stated by Ngumbi et al., is that PGPR provide their innate stress tolerance to their hosts by expanding root systems, maintaining shoot growth in spite of drought stress, controlling transpiration in leaves, improving osmotic adjustment to help plants tolerate drought at a cellular level, and other physiological features [16]. They have performed these matters by producing exopolysaccharides (EPS), plant hormones, and performing up and down regulation of stress responsive genes [30]. Kloepper et al. also discovered a higher likelihood for increased crop yields when a relationship was formed between the PGPR and its host plant [29].

Various PGPR have already been studied as a source of drought tolerance in peanut. Sudhakar et al. applied different strains of *Pseudomonas fluorescens* to peanut in a greenhouse experiment to determine their effect on drought tolerance and yield. Plants inoculated with *P. fluorescens* strain IFT-30 displayed pod yields 10.7% higher than the control plants [31]. As for other PGPR, Cesari et al. inoculated peanut with *Bradyrhizobium* sp. and *Bradyrhizobium-Azospirillum brasilense* to determine how drought would influence interactions between plants

and microbes. When water was restricted, the microbes were able to reverse the effects of drought stress during early growth stages in peanut [32]. In this study, drought stress caused peanut roots to exude different molecules in an attempt to attract bacteria to perform the actions seen in this experiment. Cesari et al. also mentioned that these exudates influence plant-microbe interactions, which can control yield [32].

## *Bacillus velezensis* and Pectin

There is one possible method for peanut drought tolerance that has not been discussed before in the literature, and that is inoculating the crop with *Bacillus velezensis* (*Bv*) and exogenous pectin. *Bv* is a gram-positive, endophytic, aerobic bacterium that forms endospores [33, 34]. A gram-positive bacterium keeps its peptidoglycan cell wall when stained with crystal violet dye and turns blue when it's viewed with a microscope [35]. Endophytic means that the bacterium can be found inside of a plant and doesn't cause any disease symptoms when it infects its host [36]. An aerobic bacterium requires oxygen to grow [37]. An endospore is a type of spore that can withstand heat exposure and be formed into a powder with a long shelf life. This powder can then be used for agricultural products like RhizoVital (ABiTEP GmbH – Berlin, Germany), which can control soil-borne diseases [33].

*Bacillus velezensis* (*Bv*) has aided plant growth in legumes, corn, and other crops [38-40]. The complete sequencing of the genome for one *Bv* strain alone revealed that the bacteria possesses operons capable of creating secondary metabolites for potential agricultural uses, such as indole-3-acetic acid (IAA) and polyketide synthetases [41]. Some of these uses included antifungal properties, such as when one *Bv* strain produced compounds, like iturin, that prevented the spread of *Fusarium* head blight [42]. For certain *Bv* strains to carry out these functions, Hossain et al. demonstrated the *Bv* was able to use pectin as a source of carbon (C), a

trait found in many *Bv* strains [43]. With this C, *Bv* can then colonize crop roots and promote their growth more easily [38].

The other ingredient for this possible inoculation, pectin, is found in certain citrus fruits and apple, it can replace sugar in various food products, and be used in medicine to lower blood cholesterol. It has also aided in a variety of plant functions, including growth, cell expansion, keeping a seed hydrated, and producing oligogalacturonides (OGA) for plant defense purposes [44, 45]. Willats et al. stated that this compound is also a fundamental aspect of the primary cell walls of every land plant [46].

Pectin can be used for agricultural research, too. To elaborate on those applications, root border cells in plants, which have pectin polysaccharides, can interact with PGPR that colonize roots to enhance growth promotion in plants [47-49]. Exogenous pectin, or pectin from a source outside of the plant, has also helped with biocontrol of *Ralstonia solanacearum,* a pathogenic enemy of tobacco plants, via biofilm creation with *B. amyloliquefaciens* [50]. More recently, in greenhouse studies, soybean inoculated with pectin and *Bv* strain AP193 produced longer soybean shoots, higher biomass accumulation, and larger quantities of nodules created by *Bradyrhizobium japonicum* inoculant. Orange peel was used as the pectin source because orange peel has been proven to have more pectin than roots, it is cheap, and the exogenous source is what increased *Bv* activity in these greenhouse studies [38, 51-53].

In terms of dry matter, orange peel is 30% pectin [54]. As a byproduct of orange juice production, orange peel is a byproduct that can cause water pollution through ingredients such as pectin. This is because aerobic bacteria can degrade these materials, which creates the pollutants such as carbon dioxide and sulfates in the water [55]. To exacerbate this issue, the USDA predicts that orange production around the world for the 2018/2019 year will rise to 54.3 million

metric tons, which is 6.3 million more than the previous year. However, since markets around the world demand over 30,000 tons of pectin annually for purposes such as food additives, anything that can make orange peel more useful can make it less likely to stay as a waste product [55, 56]. To supplement this information, Treuer et al. used orange peels and pulp to triple the amount of biomass in a three-hectare, nutrient-poor area in a Costa Rican forest [53]. With these details in mind, peanut drought tolerance from orange peel pectin and *Bv* inoculation should be attempted to determine how the crops would then respond to drought stress.

## Evaluation of Drought Tolerance

Evaluation of the phenotypic response of crop genotypes to drought stress is very challenging due to drought tolerance is complex trait. Chlorophyll meter readings (SCMR) using a soil-plant analysis development (SPAD) reader, can provide indicators of plant health of the plant's photosystem, which links between reduced chlorophyll count and environmental stressors along with the rate of gas exchange, according to Netto et al. [57]. SPAD meters offer a less destructive and more efficient choice for aiding in analyzing chlorophyll over methods using organic solvents. Govindje [58] wrote that these solvents can remove pigment, which was supposed to be measured with help from those materials. SPAD meters, on the other hand, disregard solvents, are portable, and provide data more quickly [57].

To obtain more data from extracted leaves, a spectrophotometer is required to understand the variation in concentrations of carotenoids and chlorophylls *a* and *b*. Chlorophyll *b* is a chlorophyll that helps build up light-harvesting complexes, which helps maintain proper photosynthesis in plants, and carotenoids function as additional pigments [59, 60]. As determined by Netto et al., 480, 649, and 665 nm were used as settings to study these variables [57]. Wellburn revealed that solvents were necessary to accurately measure these concentrations.

When comparing some of the solvents used, dimethylformamide was mentioned as best for handling most known plant tissues in the study. Additionally, equations were created for each available solvent to learn which one would provide the best chlorophyll measurements at different resolutions from the spectrophotometer [61].

Midday photosynthesis and $CO_2$ may also be considered when studying drought, as curves visualizing those two variables from Demmig-Adams et al. have shown that photosynthetic $CO_2$ uptake is reduced during the middle of the day under drought conditions. In an observation from Netto et al., excitation energy intended for photochemical purposes in plants was used less efficiently when a SPAD meter received readings of at least 40. More plainly, the plant did not use photosynthesis as well when given more than enough light energy. Since these variables are linked to PSII, drought affects midday photosynthesis and SPAD meters can measure PSII, SPAD meters can then study trends in midday photosynthesis related to drought. These variables all come back to the relationship between chlorophyll and carotenoids, which are both affected by drought [57, 58, 62].

WUE is another parameter used to measure how crops adapt to drought. To reinforce the need to study WUE, three other variables SCMR, carbon isotope composition ($\delta^{13}C$) or discrimination ($\Delta^{13}C$), and leaf N levels, have all been shown to be associated with one another when one of them is affected [63]. WUE can also be measured with a scale, then calculating the amount of water used, and finally measuring $\Delta^{13}C$ from biomass [64]. In models accounting for crop yields, yield has required WUE as a variable. To acquire WUE measurements more quickly, $\Delta^{13}C$ has been used as a substitute variable to help determine how genetically variable WUE was within various groups of crops, peanut included. Studying $\Delta^{13}C$ is expensive, though, so specific leaf area (SLA) can be measured as a substitute variable due to the proven positive relationship

between SLA and $\Delta^{13}C$. Since SLA and WUE also have an inverse correlation between one another, SLA can estimate that genetic variability discussed earlier, instead of $\Delta^{13}C$, among peanut cultivars. Finally, WUE and leaf chlorophyll concentration are positively correlated with one another, so a SPAD meter can measure SCMR values and determine plenty of information about all of these variables in an efficient manner [63].

One machine that can aid in acquiring SLA is the LI-3100 Area Meter. While SLA is not directly recorded from the device itself, it can be used to quickly get leaf area (LA), measured in squared centimeters ($cm^2$), which then can be used as a denominator to dry weight of the leaf area to calculate SLA [65]. Girdthai et al. used it to help select for peanut genotypes that were more likely to accumulate lower amounts of preharvest aflatoxin contamination (PAC) [66]. Rao et al. utilized it to help test the efficiency of SPAD meters in measuring SLA and leaf N in peanut [67]. With this device, SLA can be measured more efficiently.

For other measurements concerning gas exchange, including $CO_2$ and $H_2O$, the LI-6400 Portable Photosynthesis System can be utilized [68]. Some of these variables include photosynthetic factors, stomatal conductance, and measurements involving transpiration. Scientists have been able to use this tool to gain more data for comparing and contrasting variables in their experiments. Banjara et al. were able to do just that when finding out which of their peanut genotypes expressed gene *AtNHX1,* which improved salt tolerance. The genotypes with this gene produced larger rates of photosynthesis under salt stress than the knock out cultivars [69]. Qin et al. used this machine for a similar purpose, and they saw improvements in photosynthesis in plants expressing isopentenyltransferase (IPT). Plants expressing this enzyme transpired at higher rates and experienced more stomatal conductance and higher rates of photosynthesis [70]. To reinforce the utility of the LI-6400, it was used more recently to help

reveal that peanut plants overexpressing the gene *AVP1* performed better than their wild-type counterparts. The LI-6400 recorded higher rates of photosynthesis in the plants with this particular gene, and higher yields were observed, too [71].

Relative water content (RWC) is another important variable to measure for a drought experiment, as it can account for the fresh, turgid, and dry weight of leaves. If these values are affected by drought, RWC will be affected. Bennett et al. has demonstrated this observation, as events such as a decrease in leaf turgor potential have caused a decrease in RWC when given enough time and an intense enough drought [72].

Documenting the effects of pairing variables with days after planting (DAP), a frame of reference for time passed, is also essential for drought studies. DAP can be used to learn when drought begins to affect plants the most. Chen et al. determined when drought began to harm peanut between the crops' flowering and pegging stages, and DAP helped them achieve that goal [20]. When this variable is properly applied in an experiment, researchers like Dang et al. have even analyzed the consequences of drought at a genetic and molecular level [19].

Simulating drought in experiments may vary by methods, but one thing remains the same: water must be somehow withheld to record differences in plant performance amongst treatments. Vadez et al. reviewed studies that utilized lysimetric methods, or weighing containers holding the plants, to determine how much water a plant used along with how much biomass it accumulated. Plus, this review specified that it had been used on peanut before [64]. Hamidou et al. created water stress by not irrigating some of the groundnut crops until they reached a certain leaf wilting point [12]. Another method for documenting plant performance linked to the amount of water received, according to McCarty et al., was to see what happened when fields were not irrigated at all [15]. Disregarding irrigation altogether, on the other hand, Carter took advantage

of a rainout shelter to stop rain from touching the crops or surrounding soil, simulating a mid-season drought. For results afterwards, Carter asserted that a peanut cultivar's yield can be affected by said drought, so creating an ideal drought treatment is important [73].

Last, but not least, using a visual rating scale, or looking at plants with the naked eye, can act as a supplement to materials and methods intended to analyze components of a drought experiment. While researchers such as Nutter et al. and Sullivan et al. have stated that visual ratings will provide results that are less accurate than other methods, like reflectance measurements, Carter noted that visual ratings can still provide a correlation with crop yield. To counter that statement, though, only vegetation from peanut can be observed visually through its life cycle since the rest of the crop grows underground. Additionally, the pods may perform well while the foliage suffers, as all three of these sources have noted, so visual ratings can be deceiving [73-75].

## Objectives of Research

Orange peel powder amendments and *Bacillus velezensis* (*Bv*) will be tested for their capacity to increase drought tolerance in different cultivars of peanut. Physiological measurements will also be studied to determine how well they can assess chlorophyll stability of genotypes inoculated with those amendments.

## Research Hypotheses

Orange peel powder amendments and *Bv* may enhance drought tolerance in peanut. In addition, interactions may occur between the peanut genotypes and inoculants.

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## Chapter II Improving Drought Stress Tolerance of Peanut Using PGPR and Orange Peel Amendment


### Abstract

Peanut provides approximately $4 billion to the US economy. Drought lowers that amount with disease and nutrition and yield loss. Due to a rising global population, less water will be available for agriculture. Inoculating peanut with *Bacillus velezensis* (*Bv*) and orange peel (OP) has not been studied as extensively as other drought control methods. Past studies have shown that *Bv* and OP increase plant growth. The objectives of this experiment were to determine if OP and *Bv* could improve drought tolerance and if any interactions could be found between the genotypes and inoculants. Four different genotypes were studied within a randomized complete block design in a greenhouse. Results indicated that *Bv* and OP helped increase drought tolerance and that interactions occurred between genotypes and inoculation treatments. Further study can be directed towards determining AU 18's potential and how well these *Bv* and OP inoculants help these genotypes in different settings.


### Introduction

Drought stress is a major problem for peanut farming. Yearly losses due to this issue are estimated to be at least US$520 million which is a significant loss in yield [1]. Climate change is expected to make this matter worse, and 90% of peanuts are already grown in tropical and semi-arid locations with inconsistent rainfall [2, 3]. Drought causes peanuts to lose nutritional value and reduces photosynthesis and $N_2$ fixation, leading to lower yields [1, 4]. Globally, annual yield losses have been recorded at over six million tons due to drought alone [5].

Various drought management options have been or are currently being researched to assess different problems caused by drought. For peanut breeding and genetics, Dang et al. identified candidate genes that could increase drought tolerance in peanut [6]. Chen et al. evaluated genotypes to decide if germplasm from these cultivars could enhance future drought tolerance research [7]. Nutrient management has been another option, where Gu et al. tested calcium (Ca) fertilizer and produced peanuts with higher fat and protein content [8]. For irrigation, Saudy et al. experimented with differing irrigation rates to help farmers who worried about having enough water in arid farmlands; they found that a 75% irrigation rate could produce results that were similar to the traditional 100% rate that farmers in their area of study were used to [9]. Plant growth promoting rhizobacteria (PGPR) have also been researched as possible solutions to drought stress. Examples include *Pseudomonas fluorescens*, which was used in a greenhouse study to raise pod yields in inoculated plants by 10.7%, and *Bradyrhizobium* species, which have been shown to reduce drought stress effects in peanut during early growth [10, 11]. Additionally, Yuttavanichakul et al. have shown that PGPR can be used to control *Aspergillus niger* and promote nitrogen fixation in peanut [12].

Orange peel powder (OPP) pectin combined with a *Bacillus velezensis* (*Bv*) strain have been used as inoculation treatments to increase soybean root weight and nodulation [13]. Research is lacking, though, on the drought tolerance effects of inoculating peanut with *Bacillus velezensis* (*Bv*) and orange peel powder (OPP). *Bv*, an endophytic bacterial species that can use legumes and corn as a host, has been marketed as RhizoVital to control soil-borne disease, has enhanced soybean growth-promotion, and has antifungal properties demonstrated by controlling fusarium head blight [13-18]. Orange peel (OP) has pectin, which *Bv* can use as a carbon source to enhance its growth promotion abilities [13, 19, 20]. Pectin itself can also be used as a plant

defense aid like controlling tobacco pathogens [21, 22]. With these facts in mind, a greenhouse experiment will be conducted to determine how effective *Bv* and OP can be for enhancing drought tolerance in peanut.

OPP amendments and *Bv* PGPR strain AP203 were tested for their capacity to increase drought tolerance in peanut. Also, five different peanut genotypes were utilized to determine if there were any genotype-environment-*Bv* interactions between them and the inoculation treatments. The hypotheses were as follows: OPP amendments and *Bv* may enhance drought tolerance in peanut, and genotype-environment interactions may occur between the genotypes and inoculation treatments. The first objective was to study any effects of OP amendment on the growth of peanut genotypes inoculated with *Bv* strain AP203 during drought stress. The second was to determine how well physiological measurements could assess the chlorophyll stability of peanut genotypes inoculated with *Bv* strain AP203 and OP amendment while under drought stress.

## Materials and Methods

Greenhouse Experiment

*Bv* PGPR strain AP203 was grown on spore preparation media, which consisted of 3.3 grams (g) of peptone, 1 g from beef extract, 1 g of KCl, 2 g of $K_2HPO_4$, 5 g of NaCl, 0.25 g of $MgSO_4$ combined with 7 parts $H_2O$, 5 g of lactose, 0.01 g of $MnSO_4$, and then 18 g of agar for 1 liter, for one week [23]. The bacteria were then harvested and heated for 15 minutes at 80$^{o}$C, and the optical density (OD) was adjusted for $1.0 \times 10^6$ colony forming unit (CFU) spores/milliliter (mL). 500 milligrams (mg) of OPP (Citrus Extracts – Fort Pierce, FL, USA) were then inserted into 10 ml of sterilized water (10 mg of OPP/200 microliters (µL)). One

hundred eighty nine 3.9-gallon pots were filled with soil mixed according to the ratio of 4:3 (4 scoops of non-pasteurized sandy loam field soil collected from E. V. Smith Experiment Station with previous history of peanut planting and 3 scoops of potting mix). Before each pot was filled with soil, the pots were filled with mesh to prevent soil loss. When the drought period started, every hole on the bottom of every pot was sealed with duct tape to prevent water from leaking out. This experiment was conducted within the Auburn University Plant Science Research Greenhouses (Auburn, AL, USA).

Five breeding peanut genotypes, AU 18-33, AU 18-53, AU 18-57, AU 18-58, and a non-nodulating peanut (NNP) variety known as AG55x9, were used for a greenhouse test. The NNP was included as a control for measuring $N_2$ fixation in the pots. Four peanut seeds were planted into 2 centimeter (cm) deep soil to ensure proper germination. The PGPR spore suspension mixed with sterilized water mentioned earlier was applied to the peanut seed surface, which was then covered with 100 g of soil. At 14 days after planting (DAP), the seedlings were thinned to two plants from each pot to allow for the remaining plants to grow more effectively.

A Randomized Complete Block Design (RCBD) created with ARM software (Gylling Data Management, Inc., Brookings, SD, USA) was used to randomize the treatment factorials. Regarding water treatments, we used 80% soil water content (SWC) as the well-watered treatment, and 20% SWC as the drought-stressed treatment. Each treatment was blocked in one half of the greenhouse. To test *Bv* and orange peel effects on the peanuts, we designed four treatments: 100 µl of orange peel (OP) (10 mg OPP/200 µl) liquid suspension; 100 µl of PGPR AP203 (1.0 x 106 CFU spores); 100 µl of OP (10 mg OPP/100 µl) liquid suspension combined with 100 µl of PGPR AP203; and 100 µl of sterilized water as a negative control. All the treatments were applied to the four genotypes of AU 18-33, AU 18-53, AU 18-57, and AU 18-58

with 10 replications, so we will have 2 water treatments x 4 inoculation treatments x 4 genotypes x 10 replicates. For the NNP line, it has 3 replications only.

Measuring Soil Moisture

During the drought treatment (60-100 DAP) the pots were watered twice a week to maintain their weight for their required water treatment standards of 80% and 20% of soil water content respectively. One reference used to determine proper soil water content for this experiment came from Bhatnagar-Mathur et al., particular for 80% field capacity [24]. The starting day for DAP was derived from a study by Carter concerning middle season drought stress, where it is stated that drought stress during fruiting stages can reduce yield more than other times for drought stress [25]. The amount of water used for each pot was documented. While watering them, the pots were weighed with a scale (OHAUS Corporation, Parsippany, NJ, USA) to ensure they were properly watered. Before and after watering, weights were obtained for water use efficiency (WUE) data analysis. The amount of water used per pot for the whole growing season was documented to account for water used and WUE, which involves this equation: WUE = total dry biomass/water used (Table 1).

Leaf Relative Water Content and Specific Leaf Area

During 30, 60, and 90 days after emergence (DAE), the second fully expanded leaf from the top of the main stem of 3 plants from each treatment were obtained to measure leaf fresh weight (FW), leaf turgid weight (TW), and leaf dry weight (DW). FW was measured after obtaining the leaf, while TW was measured from the leaf sample that has been immersed in distilled water at room temperature under dark conditions for 8 hours. Then, for 48 hours, the

leaf sample is oven dried to later obtain the DW. Finally, this formula was used to calculate relative water content (RWC) [26]:

$$\text{RWC} = \frac{FW-DW}{TW-DW}\ x\ 100$$

During the drought stress period, once a week for four weeks, three plants from each treatment were sampled randomly in the morning, with the sampled portion being the second fully expanded leaf from the top. Fresh collected leaves were placed into plastic bags and put on ice in coolers. Each leaf was placed into an individual petri dish fully submerged in deionized water. Then they were placed under white light for 2 hours to ensure tissues were completely turgid. Afterwards, leaves were blotted dry, and leaf area (LA) was immediately measured with a LI-3100 Area Meter (LI-COR Biosciences, Lincoln, NE, USA). Leaves were then placed into a 65 degrees Celsius oven for 48 hours to ensure complete dryness and subsequently weighed to obtain the leaf dry mass. Finally, specific leaf area (SLA) was calculated as the ratio of leaf area to leaf dry mass (LA/DW) for each leaf measured.

SPAD Chlorophyll Meter Readings

Every 15 days after 45 days after planting (DAP), soil-plant analysis development (SPAD) measurements were taken to estimate chlorophyll content, also known as the greenness of the leaves, using Minolta SPAD-502 meter (Konica Minolta, Tokyo, Japan). In addition, five plants from each treatment were randomly sampled in the morning for extracting the second fully expanded leaf from the top. This helped to obtain chlorophyll concentration per unit leaf area (ChlD). Leaf discs, each 1 square centimeter ($cm^2$), were soaked in 5 ml of N, N-dimethylformamide and kept in darkness for 24 hours before determining chlorophyll content, or the quantity of chlorophylls *a* and *b*, using light absorption techniques with a spectrophotometer.

A 3 ml aliquot was analyzed spectrophotometrically at 645 and 663 nanometers (nm) [27-29]. The concentration of chlorophyll extract ($mg/cm^2$) was calculated using a formula as follows [30]: a(OD645 nm) + b(OD663 nm), where a = optical density at 645 nm, and b = optical density at 663 nm.

Photosynthetic Measurements

15 and 25 days after starting the drought stress, or 75 and 85 DAP, mid-day photosynthesis measurements were performed by using a portable gas exchange analyzer LI-6400 (LI-COR Biosciences, Lincoln, NE, USA). Midday $CO_2$ uptake or photosynthesis provides a more detailed understanding of drought and inoculation treatment effects and assess the activity of the enzyme Rubisco at the moment of the measurement [31]. Besides photosynthesis, stomatal conductance was collected as it provides information of how open the stomata are, and its related with the transpiration of the plant [32]. Intrinsic water-use efficiency (WUEi), was calculated as the ratio of the rate of photosynthesis to the rate of transpiration, by WUEi = Photosynthesis/Stomatal Conductance [32].

Harvesting and Obtaining Peanut Pod Dry Weight

The peanut plants were harvested at 135 days after planting separating the organs in leaves, stems, pods, and roots. Roots were cleaned with water and a shive to avoid losing root biomass. Main stem length, root length, and number of peanut pods were measured for each plant. The stems, roots, and pods were all oven dried for 48 hours at 70$^{o}$C and then weighted separately. For total plant biomass, we added all weights of stems, leaves, roots, and pods.

Isotope Analysis

The isotopes of $^{13}C$ and $^{15}N$ in each plant were analyzed from the mixed ground samples of stems, roots, leaves, and pods. A Thomas-Wiley Laboratory Mill Model 4 Grinder (Thomas Scientific, Swedesboro, NJ, USA) was used to grind the plant material into particles of 2 millimeters (mm) each. Then the samples were ground further using a UDY Corporation Model 3010-030 grinder (UDY Corporation, Fort Collins, CO, USA) to refine particles to 1 mm in size.

After grinding every sample into 1 mm particles, the samples were taken to a Sartorius Microbalance (Southern Balance Calibrations, Inc., Braselton, GA, USA) to weigh out 2.8-3.2 mg samples into 5x9 mm tin capsules (Costech Analytical Technologies Inc., Valencia, CA, USA). The isotope analysis of $^{13}C$ and $^{15}N$ natural abundance was conducted at the University of California-Davis Stable Isotope Facility. At the facility, an isotope ratio mass spectrometer and elemental analyzer were used to analyze samples. Before that, $^{13}C/^{12}C$ ratio (R) found within the plant material was calculated using δ notation ($\delta^{13}C$, carbon isotope discrimination) with Vienna Pee Dee Belemnite calcium carbonate with analytical precision being 0.1%. Then the material was transformed to apparent C isotope discrimination ($\Delta^{13}C$, ‰) with this formula [33]:

$$\delta^{13}C = \frac{R_{sample}}{R_{standard}} - 1$$

The accuracy of $\delta^{13}C$ was checked with international secondary standards for $^{13}C/^{12}C$ ratios, which were USGS-40 glutamic acid, IAEA-CH6 sucrose, and IAEA-CH7 polyethylene foil (International Atomic Energy Agency, Vienna, Austria), and internal controls known for this ratio, which were leaves from peach, nylon 5, and bovine liver. This is the formula that was then used to calculate $\Delta^{13}C$ [33]:

$$\Delta^{13}C = \frac{\delta^{13}C_{atm} - \delta^{13}C_{sample}}{\delta^{13}C_{sample} + 1}$$

$\Delta^{13}C_{atm}$ is atmospheric carbon dioxide's C isotope composition, or -8%, and $\delta^{13}C_{sample}$ represents the plant sample's C isotope composition [33].

For $^{15}N$, all biomass was analyzed with an automated CN analyzer (Europa Science, Cambridge, England) and mass-spectrometer (Model 20-20, Europa Science, Crewe, Creshire, England). This formula is for percentage of $N_2$ fixation [34]:

$$\%N_2\ fixation = \frac{\delta^{15}\text{N non fixing reference} - \delta^{15}\text{N fixing legume}}{\delta^{15}\text{N non fixing reference} - \text{B}} x100$$

B, in this case, represents the $\delta^{15}N$ of legume shoots that completely depend on $N_2$ fixation, and since this experiment was conducted in a greenhouse, the B value, -0.865, was averaged from two B values, -0.7 [35] and -1.03 [36], that were used in previous studies for this same context [37].

Data Analysis

Analysis of variance (ANOVA) and Tukey Kramer analyses were performed to account for different genotypes, inoculants, water treatments. ANOVA was used to study variability within and among these groups [38]. Tukey Kramer analyses were used as pairwise post-hoc analyses to check for any differences between means for every pair that could be created from every group [39]. The analyses were done using the car, agricolae, emmeans, ggplot2, gplots, multcomp, readxl, openxlsx, rlang, and devtools packages in R with RStudio version 1.2.5042 (RStudio, Inc., Boston, MA, USA).

The car package was used for type 2 and 3 ANOVA [40]. The agricolae package was used to make finding a proper post-hoc analysis easier [41]. The emmeans package was used to obtain means from a linear model [42]. The ggplot2 package was used to create bar graphs [43].

The gplots package was used to add or improve details for visuals such as bar plots [44]. The multcomp package was used to add letters to the Tukey Kramer analysis results [45]. The readxl package was utilized to import excel files for use in R [46]. The openxlsx package allowed for making and editing .xlsx files [47]. The rlang package was used to take advantage of more tools that are considered core R features [48]. The devtools package was used just in case anything used in RStudio for this experiment was considered package development software [49].

## Results

Chlorophyll Density

ANOVA results indicated significant differences among variables of inoculation treatments and genotypes, but not for water treatments and interactions between any variables for chlorophyll density (ChlD) ($mg/cm^2$) at 30 days after emergence (DAE) as the drought treatment was still not applied (Table 2). Among inoculation treatments, Tukey Kramer analysis showed that OPAP203 has the highest mean for ChlD (21.89), with OP and AP203 showing lower values and WC showing the lowest mean (8.04) (Table 3). For genotypes, AU 18-33 has the highest ChlD (17.01) and AU 18-58 has the lowest (13.13) (Table 4).

For 60 DAE, significant differences were found for the variables of inoculation, genotypes, water treatments, and interactions between inoculation and genotype and inoculation and water treatment (Table 2). Both inoculation treatments AP203 and OPAP203 produced higher ChlD (18.42 vs. 19.52) than OP and WC (9.3 vs 10.2) (Table 3). For genotypes, AU 18-33 produced the highest mean (15.12) and AU 18-57 had the lowest (13.53) (Table 4). The drought-stressed water treatment had a higher mean (15.80) than the well-watered treatment (12.94) (Table 5). For the inoculation and genotype interaction, the AP203 and AU 18-33

combination had the highest ChlD values of 19.89 (Table 6). Moving onto the inoculation and water treatment interaction, drought-stressed AP203 produced the highest mean of 20.35, followed by drought-stressed OPAP203 with 20.08. The highest well-watered inoculation treatments were also OPAP203 and AP203 (18.96 vs. 16.49) (Table 7).

Unlike 30 and 60 DAE, for 90 DAE, we didn't find significant differences among genotypes (Table 2). We did find significant differences among variables of inoculation, water treatment, and interactions between inoculation and genotype, genotype and water treatment, and all three parameters, though (Table 2). For inoculation treatments, OPAP203 produced the highest ChlD value of 7.51, followed by AP203 with 5.88, OP with 4.75, and WC with 3.94, respectively (Table 3). For water treatment, drought-stressed treatments produced higher means than well-watered treatments (6.04 vs. 5.00) (Table 4). For the inoculation and genotype interaction, all OPAP203 inoculated genotypes produced higher means than every other treatment, and WC inoculated genotypes produced lower means than every other treatment. For OPAP203 genotype combinations, AU 18-57's mean was 8.61, AU 18-58's was 7.64, AU 18-53's was 7.11, and AU 18-33's was 6.69, and for WC genotype combinations, AU 18-53's was 4.35, AU 18-33's was 4.10, AU 18-57's was 3.66, and AU 18-58's was 3.64 (Table 6). For the interaction between genotype and water treatment, well-watered AU 18-57 had the lowest mean and drought-stressed AU 18-57 had the highest mean (4.4 vs. 6.63) (Table 8). In summary, the inoculation treatment of OPAP203 made the highest effect of increasing ChlD values, even under drought stress.

Harvest Data

For harvested shoot length (cm), ANOVA results indicated that there are significant differences among the variables of inoculation, genotype, water treatment, and the interactions

between inoculation and genotype, genotype and water treatment, and all three variables (Table 2). For inoculation, OPAP203 produced a significant higher mean (57.65) than the rest of the treatments, with AP203 (53.92), OP (53.11), and WC (51.56) (Table 3). For genotype, AU 18-53 produced the highest mean for shoot length (58.33) and AU 18-58 has the lowest mean (49.745) (Table 4). For water treatment, the well-watered treatment had a higher mean (58.28) than the drought-stressed treatment (49.84) (Table 5). For the inoculation and genotype interaction, OP inoculated AU 18-53 had the highest mean (60.93) and OP inoculated AU 18-33 had the lowest mean (45.81). AP203 inoculated AU 18-53 (60.8), and OPAP203 inoculated AU 18-53 (59.85), AU 18-57 (59.1), and AU 18-33 (58.45) all produced means that were close to the OP and AU 18-53 combination (Table 6). For the interaction between genotype and water treatment, drought stressed AU 18-58 had the lowest mean (43.89) and well-watered AU 18-53 had the highest mean (62.4) (Table 8).

For harvested root length (cm), ANOVA results indicated significant differences among variables of inoculation, genotype, water treatment, and every possible interaction between the three variables (Table 2). For inoculation, OPAP203 had the highest mean (35.68) and showed higher root length than AP203. AP203 showed higher root length than WC and OP, which had the lowest values, 22.3 and 24.01 respectively (Table 3). For genotype, AU 18-57 had a significantly higher mean (30.15) than AU 18-58 (22.69) (Table 4). For water treatment, well-watered treatments had a higher root length than drought-stressed ones (29.42 vs. 25.11) (Table 5). For the inoculation and genotype interaction, the four highest means were OPAP203 inoculated AU 18-57 (38.96), AU 18-53 (35.66), AU 18-33 (35.47), and AU 18-58 (32.65), and OP inoculated AU 18-58 had the lowest mean (19.07) (Table 6). For the inoculation and water treatment interaction, the two highest root length means were well-watered and drought stressed

OPAP203 treatments (39.37 vs. 32.00 respectively), and the two lowest were well-watered and drought stressed WC treatments (22.35 vs. 22.25) (Table 7). For the interaction between genotype and water treatment, well-watered AU 18-58 had the lowest mean and well-watered AU 18-33 had the highest mean (21.84 vs. 33.19) (Table 8). Once again, OPAP203 treatments had the highest impact on increasing biomass, even under drought stress.

For the number of pods harvested, ANOVA results indicate significant differences among the variables of inoculation, genotype, and the interactions between inoculation and genotype, inoculation and water treatment, genotype and water treatment, and all three variables, but not for water treatment alone (Table 2). For inoculation, OP, OPAP203, and AP203 (10.71 vs. 10.46 vs. 10.38) had higher means than WC (6.35) (Table 3). For genotypes, AU 18-58 had the highest mean, and AU 18-33 had the lowest mean (10.93 vs. 7.06) (Table 4). For the interaction between inoculation and genotype, OPAP203 inoculated AU 18-58 had the highest mean (13.36) and WC inoculated AU 18-53 had the lowest mean (5.59) (Table 6). For the interaction between inoculation and water treatment, the drought stressed AP203 (11.75) and well-watered OPAP203 (11.68) combinations had the highest means, and the drought stressed (6.95) and well-watered (5.76) WC combinations had the lowest means (Table 7). For the interaction between genotype and water treatment, well-watered AU 18-33 had the lowest mean and well-watered AU 18-57 had the highest mean (6.7 vs. 11.32) (Table 8). For the interaction between all three variables, well-watered, OPAP203 inoculated AU 18-58 (15.1) and AU 18-57 (15) had the two highest number of pods, and well-watered, WC AU 18-53 had the lowest mean (3.38). The highest drought stressed number of pods mean was AP203 inoculated AU 18-57 (14.4), and the lowest was WC inoculated AU 18-33 (6.1) (Table 9). In summary, the three other inoculation treatments had a higher impact over water only in terms of the number of pods produced (Figure 1).

For harvested plant dry weight (g), ANOVA results indicated significant differences among the variables of inoculation, genotype, and the interactions between inoculation and water treatment and genotype and water treatment, but not for water treatment only (Table 2). For inoculation, OPAP203 had the highest mean (27.99), and WC had the lowest mean (15.45) (Table 3). For genotype, the means from highest to lowest were AU 18-53 (26.17), AU 18-57 (26.08), AU 18-58 (22.79), and AU 18-33 (21.16) (Table 4). For the interaction between inoculation and water treatment, the two highest means were the well-watered OPAP203 (28.89) and drought stressed OP combinations (28.16), and the lowest mean was the drought stressed WC combination (15.29) (Table 7). For the interaction between genotype and water treatment, drought stressed AU 18-33 had the lowest mean and drought stressed AU 18-53 had the highest mean (21.04 vs. 27.97) (Table 8). OPAP203 had the highest effect for increasing plant dry weight.

For harvested pod dry weight (g), ANOVA results revealed significant differences among only the variables of genotype and inoculation (Table 2). For genotype, AU 18-57 had the highest mean (20.49) and AU 18-33 had the lowest mean (17.42) (Table 4). For inoculation, OPAP203, OP, and AP203 had the highest mean (21.44, 20.62, and 20.62 respectively) (Table 3). The information shows that OPAP203 had the highest impact on pod dry weight (Figure 2).

Isotope Analysis

For carbon isotope discrimination ($\Delta^{13}C$) (‰), ANOVA results indicated significant differences among variables of inoculation, genotype, water treatment, and the interactions between inoculation and genotype, genotype and water treatment, and all three variables (Table 2). For inoculation, OP had the highest mean and WC had the lowest (20.36 vs. 20.15) (Table 3). For genotype, AU 18-33 had the highest mean and AU 18-53 had the lowest (20.77 vs. 19.83)

(Table 4). For water treatment, well-watered produced a higher mean than drought stressed (20.55 vs. 19.86) (Table 5). For the interaction between inoculation and genotype, AP203 inoculated AU 18-33 had the highest mean (20.93) and AP203 inoculated AU 18-53 had the lowest mean (19.68). The highest mean from an OPAP203 inoculated genotype was AU 18-33 with 20.68 (Table 6). For the interaction between genotype and water treatment, drought stressed AU 18-53 had the lowest mean and well-watered AU 18-33 had the highest mean (19.38 vs. 21.43) (Table 8). The OPAP203 inoculation treatment had a smaller impact on $\Delta^{13}C$ but having any inoculant still produced slightly higher values (Figure 3).

For the percentage of nitrogen (N) derived from the atmosphere (%Ndfa), ANOVA results indicated significant differences among the variables of inoculation, genotype, water treatment, and the interactions between inoculation and water treatment and genotype and water treatment (Table 2). For inoculation, OP had a significant effect on nitrogen fixation (46.6) and the other three inoculation treatments OPAP203, WC and AP203 have significantly lower means of 40.3, 40.3 and 38.5, respectively, without differences statistically between the three of them, indicating *Bv* PGPR strain AP203 has no effect on nitrogen fixation (Table 3). For the interaction between inoculation and water treatment, drought stressed OPAP203 had the lowest mean and well-watered OPAP203 had the highest mean (27.78 vs. 52.96) (Table 7). For the interaction between genotype and water treatment, drought stressed AU 18-33 had the lowest mean and well-watered AU 18-33 had the highest mean (37.11 vs. 49.47) (Table 8).

For total N content in plant biomass (g N per plant), ANOVA results revealed significant differences among the variables of inoculation, genotype, water treatment, and the interactions between inoculation and water treatment, and genotype and water treatment (Table 2). For inoculation, Tukey Kramer test showed three treatments of AP203 (1.25), OP (1.23), and

OPAP203 (1.21) have significant higher values of N content in plant biomass than WC (0.77) indicating that OP, *Bv* PGPR strain AP203, and the combination of the two have impacts on N content in plant biomass but there is no difference among the three inoculations (Table 3). For genotype, AU 18-58 had the highest mean and AU 18-33 had the lowest mean (1.26 vs. 1.00) and they are a significant difference (Table 4). For water treatment, well-watered had a significant higher mean than drought stressed (1.15 vs. 1.07) (Table 5). There was an interaction between inoculation and water treatment, and we found that the means of well-watered AP203 and OP are similar to the means of drought stressed AP203 and OP (Table 7). For the interaction between genotype and water treatment, drought stressed AU 18-33 had the lowest mean and well-watered AU 18-58 had the highest mean (0.98 vs. 1.38) (Table 8). Overall, AP203 had the same impact for well-watered and drought stressed conditions.

For total N derived from N fixation, calculated as %NDFA by total N constant, ANOVA results indicated significant differences among the variables of inoculation, genotype, water treatment, and the interactions between inoculation and genotype and inoculation and water treatment (Table 2). For inoculation, similar to N content in plant biomass, OP (0.58), OPAP203 (0.51), AP203 (0.49) are not significantly different from one another, but all are significantly higher than WC (0.32) (Table 3). For genotype, only AU 18-58 with the highest mean (0.56) is significant different from AU 18-53 with the lowest mean (0.39) (Table 4). For water treatment, well-watered had a significant higher mean than drought stressed plants (0.53 vs. 0.41) (Table 5). For the interaction between inoculation and genotype, OP inoculated AU 18-58 had the highest mean and WC AU 18-53 had the lowest (0.68 vs. 0.26). The highest OPAP203 inoculated mean was AU 18-57 (0.62) (Table 6). For the interaction between inoculation and water treatment, well-watered OPAP203 had the highest mean (0.70) and is significantly different from drought

stressed OPAP203 (0.31). However, AP203 is not significantly different in either well-watered or drought stressed conditions (0.52 vs 0.45) (Table 7).

Photosynthetic Measurements

For photosynthesis measurements ($A_N$) taken 75 days after planting (DAP), ANOVA results demonstrated significant differences among the variables of water treatment and the interaction between genotype and water treatment, but not for inoculation or anything else (Table 2). For water treatment, drought-stressed plants showed a photosynthetic rate that was more than twice as high as well-watered (16.50 vs. 7.20) (Table 5). For the interaction between genotype and water treatment, drought-stressed AU 18-33 had the highest mean and well-watered AU 18-33 had the lowest mean (18.97 vs. 4.17) (Table 8). For photosynthesis measurements taken 85 DAP, the well-watered treatment was significantly higher than drought stressed instead (17.29 vs. 14.49), and no interaction occurred between genotype and water treatment (Tables 1 and 4).

For stomatal conductance ($g_s$) measurements taken 75 DAP, ANOVA results indicated significant differences among variables of water treatment and the interaction between genotype and water treatment, but not for inoculation as well. This observation is the same as the photosynthetic measurements mentioned before (Table 2). For water treatment, drought-stressed had a higher mean than well-watered (0.21 vs. 0.09) (Table 5). Similar results were observed for the data collected on 85 DAP (Tables 1 and 4). Also, during 85 DAP for the interaction between inoculation and water treatment, the drought stressed AP203 had the lowest mean (0.18) and well-watered AP203 had the highest mean (0.58) (Table 7). For the interaction between genotype and water treatment for 75 DAP, well-watered AU 18-33 had the lowest mean and drought stressed AU 18-33 had the highest mean (0.05 vs. 0.3) (Table 8). As mentioned with photosynthesis, drought-stressed treatments experienced the most stomatal conductance during

this time. This observation may be due to a spider mite infestation that occurred during this experiment.

For intrinsic water use efficiency (WUEi) ($A_N/g_s$) calculated from measurements taken on 75 DAP, ANOVA results indicated significant differences among variables of inoculation and the interaction between genotype and water treatment. This time, water treatment alone didn't have significant differences (Table 2). For inoculation, the means of OP (81.72), and AP203 (81.40) were significantly lower than WC (100.11) and OPAP203 (95.55) was in between (Table 3). For the interaction between genotype and water treatment, drought-stressed AU 18-57 had the highest mean (102.00) and drought-stressed AU 18-33 had the lowest mean (74.03) (Table 8).

Relative Water Content

We have measured three time points for leaf relative water content (LRWC) (%) at 30 DAE, 60 DAE, and 90 DAE. Among all variables tested, inoculation showed consistent results across the three time points. OPAP203 and AP203 (92.7, 94.9, 95.9 / 88.2, 93.3, 93.1) always had significant higher means than OP and WC (79.4, 77.7, 88.0 / 76.0, 67.3, 78.2) (Tables 2-3), indicating *Bv* PGPR strain AP203 has a significant impact on relative water content.

SPAD Chlorophyll Meter Readings

Similar to RWC, Soil-Plant Analysis Development (SPAD) Chlorophyll Meter Readings (SCMR) were collected at 30 DAE, 60 DAE, and 90 DAE, respectively. ANOVA detected consistent results of significant differences among the variables of inoculation, genotype, and the interaction between inoculation and genotype for the three data sets (Table 2). Again, OPAP203 and AP203 (46.3, 46.2, 44.0 / 41.1, 44.1, 40.0) always had significant higher means than OP and WC (34.7, 34.9, 40.0 / 32.5, 34.4, 32.7) (Table 3), indicating *Bv* PGPR strain AP203 has a

significant impact on SCMR. Besides these findings, for the interaction between inoculation and water treatment 60 DAE, well-watered WC had the lowest mean and well-watered OPAP203 had the highest mean (31.17 vs. 46.31) (Table 7). For the interaction between genotype and water treatment during the same time period, well-watered AU 18-57 had the lowest mean and drought stressed AU 18-58 had the highest mean (37.47 vs. 41.44) (Table 8).

Specific Leaf Area

We have measured four times of specific leaf area (SLA), a ratio of leaf area to leaf dry weight [50], at one week after the start of the drought stressed treatment for four weeks, or 67, 74, 81, and 88 DAP. Like relative water content, the most consistent results across the four time points was for the variable of inoculation and all ANOVA results showed significant difference for inoculation (Table 2). Among all variables tested, inoculation showed consistent results across three time points. Tukey Kramer tests indicated OPAP203 (564.1, 606.2, 491.8, 446.6) and AP203 (404.3, 529.3, 380.0, 359.6) always had significant higher means than OP ( 323.3, 305.3, 252.7, 248.5) and WC (196.5, 230.5, 204.1, 211.2), indicating *Bv* PGPR strain AP203 has a significant impact on SLA (Table 3). Also, 88 DAP for the interaction between inoculation and water treatment, well-watered WC had the lowest mean and drought stressed OPAP203 had the highest mean (180.58 vs. 531.18) (Table 7).

Total Water Used

For total amount of water transpired in kilograms (kg), ANOVA results indicated significant differences among the variables of inoculation, water treatment, and the interactions between inoculation and genotype and inoculation and water treatment (Table 2). For inoculation, Tukey Kramer test showed OPAP203 (10.1) and AP203 (10.7) always had

significant lower water use than WC (20.9), and OP (16.8) also had a significant lower mean than WC but not like *Bv* PGPR strain AP203 having a significant larger impact on specific leaf area (Table 3). For the interaction between inoculation and genotype, the highest means were WC AU 18-57 (21.69), AU 18-53 (21.67), AU 18-58 (20.42), and AU 18-33 (20.00), and the lowest means were OPAP203 inoculated AU 18-58 (9.60) and AU 18-53 (9.18) (Table 6). For the interaction between inoculation and water treatment, well-watered OPAP203 had the lowest mean and well-watered WC had the highest mean (8.78 vs. 23.33) (Table 7).

For water use efficiency (WUE) measured as (g biomass/kg water), ANOVA results indicated significant differences among the variables of inoculation, genotype, and the interactions between inoculation and water treatment and genotype and water treatment but not for water treatment (Table 2). For inoculation, OPAP203 (5.45), AP203 (4.68), OP (2.93) and WC (1.49) were significantly different from each other (Table 3). It seems the effects from WC, to OP, AP203 and OPAP203 were gradually increasing like additive effects so OPAP203 treatments had the highest effect on WUE. For the interaction between inoculation and water treatment, well-watered WC had the lowest mean and well-watered OPAP203 had the highest mean (1.31 vs. 6.19) (Table 7). For the interaction between genotype and water treatment, well-watered AU 18-33 had the lowest mean and well-watered AU 18-57 had the highest mean (3.09 vs. 4.31) (Table 8).

## Discussion

Previous research have revealed a significant, positive relationship between ChlD and SCMR, and that the amount of chlorophyll is related to drought tolerance [29]. The 30 DAE results of SCMR and ChlD showed similarities, indicating a strong relationship between SCMR and ChlD, and the correlation coefficient was 0.77. For 60 DAE, in the inoculation and water

treatment interactions, drought stressed AP203 produced the highest ChlD mean (Table 5). For 90 DAE in the interaction between inoculation, water treatment, and genotype, though, a drought-stressed OPAP203 treatment produced the highest mean, showing the inoculation treatment of OPAP203 made the highest effect of increasing ChlD values under drought stress that has been mentioned by Arunyanark et al. (Table 9) [29].

Among our measured agronomic characteristics including shoot length, root length, number of pods per plant, plant dry weight, and pod dry weight, the yield related traits of number of pods per plant and pod dry weight are most interesting. The results of this study revealed that any inoculant can improve the pod number per plant and pod dry weight when compared to the control treatment (WC). For inoculation, results for shoot length and plant and pod dry weight were similar to findings reported by Hassan et al. in which they indicated that soybean seeds inoculated with a *Bv* strain and orange peel pectin had significantly increased dry weight and shoot length [13]. For quantity of pods, though, some drought-stressed treatments produced higher yields when only inoculated with AP203, which was different from Ding et al's results [51]. Additionally, for the interaction between inoculation and water treatment for this same variable, since AP203 inoculated drought stressed plants produced the highest mean for number of pods, this finding is similar to a meta-analysis conducted by Rubin et al. [52], in which they broadly stated that PGPR improved plant performance with a greater effect on yield, especially under drought stress. Legumes were also considered in this meta-analysis [52]. Observing plant dry weight and pod dry weight separately revealed again that any inoculant was better than only water, but the interaction between inoculation and water treatment for plant dry weight showed well-watered OPAP203 (28.89) and drought stressed OP combinations (28.16) as having the highest plant dry weight (Table 7). While the highest drought stressed value didn't include

PGPR, the well-watered OPAP203 value is similar to a finding stated by Mondani et al. in which PGPR was shown to improve soybean total above ground dry weight at all tested irrigation levels [53].

Isotope analysis was needed for this study because peanut requires N and carbon (C) as essential nutrients, and this technique can be used to determine WUE, photosynthetic activity and N fixation [4, 34, 54-56]. Variables encompassed by this analysis included carbon isotope discrimination ($\Delta^{13}C$), which was important for its relationships with SCMR, chlorophyll concentration, and %Ndfa, which gives an idea of the amount of N that is derived from atmospheric nitrogen fixed through $N_2$ fixation [37]. This was the first study on how *Bv* PGPR strain AP203 affects $\Delta^{13}C$ in peanut crops. The results showed *Bv* PGPR strain AP203 decrease $\Delta^{13}C$, and $\Delta^{13}C$ has been shown to have a negative correlation with water use efficiency (WUE) so lower $\Delta^{13}C$ would mean higher WUE, indicating higher drought tolerance (Table 3) [57]. This can be demonstrated with the WUE (g biomass/kg water) as the inoculation with AP203 and OPAP203 increased WUE (Table 3).

The endorhizosphere of the plant has plenty of nutrients, due to an abundance of root exudates, which release important materials like carbon compounds that attract beneficial bacteria, and some compounds released can repel malicious bacteria. In this area, plant roots can also communicate with rhizobacteria to acquire resources. They do this by sending out organic compounds to attract PGPR [58]. In exchange, PGPR help with tasks such as assimilating N and releasing phytohormones to stimulate plant growth [59]. Past studies have revealed a relationship between higher root growth and higher amounts of N accumulated, one of which was a study conducted by Cortivo et al. concerning common wheat [60]. This finding can be seen to a lesser extent in a study by Olivera et al. [61] concerning effects of added phosphorus on growth and N

fixation in common bean. In this study, though, the applications increased the amount and weight of nodules more than root dry weight [61]. Referring back to inoculation results for our study, OPAP203 produced the highest mean for root length while OP produced the highest mean for the amount of N accumulated by N fixation (Table 3). As for the percentage of nitrogen derived from the atmosphere, while a study concerning inoculating grass and rice with PGPR stated that one of the varieties studied had over half of its nitrogen derived from the atmosphere (%Ndfa) due to being inoculated with a PGPR strain, OP inoculated plants from our study produced a significantly higher %Ndfa than either AP203 or OPAP203. Even then, our results were not near the 70% found by Malik et al. [62].

Drought stress has been shown to have an adverse effect on photosynthesis, stomatal conductance, and intrinsic water use efficiency (WUEi), so measuring these variables under this study's parameters may help determine new strategies for drought tolerance [63]. However, for this study, the results coming from the LI-6400 measurements indicated that no significant differences were found for inoculation treatments for photosynthesis and stomatal conductance, but we did find significant differences for WUEi from measurements taken 75 DAP (Table 2). This finding may be due to the spider mite infestation that occurred during the experiment damaging leaves and decreasing photosynthetic potential of well-watered treatment plants. Even when considering the infestation, more measurements should be taken for another study.

RWC was important since it demonstrates a balance between the amount of water in a leaf and its rate of transpiration [64]. Just like in Sudhakar et al., inoculating peanut with PGPR had a positive effect on its RWC. This was seen in all three days of measurement in this study. When referring to the same study for SLA, though, while comparison of results between peanuts inoculated with *Pseudomonas fluorescens* and just water were somewhat similar, our study

revealed mostly higher results for peanut inoculated with OPAP203 or AP203 throughout all four weeks when measurements were taken (Table 3) [10]. In a studies by Wright et al. [57] and Craufurd et al. [65], both about peanut WUE during drought conditions, higher SLA was linked with lower WUE. These results differ from our observations, though, because when looking at results for inoculation, plants with higher SLA also had higher WUE instead.

When observing the interactions between inoculation and genotype for total water transpired in kg, adding either AP203 or OPAP203 helped make all genotypes transpire less. Basu et al. has indicated that less transpiration during drought can improve WUE, which this study has been able to repeat through these figures (Table 6) [66]. Also, when looking through literature concerning peanut WUE during drought, such as a study conducted by Songsri et al., peanut cultivated under field capacity usually has higher WUE than drought stressed peanut [67]. Our results, though, when referring to the interaction between genotype and water treatment, show the opposite, with some drought stressed genotypes having higher WUE than well-watered counterparts. Unfortunately, this may be due to the spider mite infestation, too (Table 8).

Although there were some inconsistencies in this study, it may partially be due to spider mites and white flies. This study is one of the first to illustrate if *B. velezensis* can improve drought tolerance in peanuts and the orange peel amendment's effect on the growth of peanut genotypes inoculated with *B. velezensis* strain AP203 during drought stress. Overall, though, AP203 and OPP amendments enhanced drought tolerance in peanut, and interactions did occur between genotypes and inoculation treatments for the following variables: ChlD 60 and 90 DAE, shoot and root length, number of pods, carbon isotope discrimination, N accumulated from N fixation, total water transpired in kg, RWC 30 DAE, SCMR 30, 60, and 90 DAE, and SLA during 67 and 88 DAP. To further determine how well these materials can work in other settings,

later studies should be directed towards larger scale environments like rainout shelters or a field trial. This information can be used to help peanut farmers struggling with mid-season drought stress.

## Tables

Table 1: Table for soil water content.

| Percentage | Water Weight (kg) |
|---|---|
| 10 | 0.275 |
| 20 | 0.55 |
| 30 | 0.825 |
| 40 | 1.1 |
| 50 | 1.375 |
| 60 | 1.65 |
| 70 | 1.925 |
| 80 | 2.2 |
| 90 | 2.475 |
| 100 | 2.75 |

Table 2: ANOVA table for all data.

| | Inoculation (I) | Genotype (G) | Water Treatment (W) | I:G | I:W | G:W | I:G:W |
|---|---|---|---|---|---|---|---|
| Df | 3 | 3 | 1 | 9 | 3 | 3 | 9 |
| | Pr(>F) | Pr(>F) | Pr(>F) | Pr(>F) | Pr(>F) | Pr(>F) | Pr(>F) |
| ChlD ($mg/cm^2$) 30 DAE | **P ≤ 0.001** | **P ≤ 0.001** | 0.37492 | 0.14487 | 0.5508 | 0.99707 | 1 |
| ChlD (mg/cm2) 60 DAE | **P ≤ 0.001** | **0.02345** | **P ≤ 0.001** | **P ≤ 0.001** | **0.04599** | 0.83572 | 0.54925 |
| ChlD (mg/cm2) 90 DAE | **P ≤ 0.001** | 0.40964 | **P ≤ 0.001** | **P ≤ 0.001** | 0.29498 | **P ≤ 0.001** | **0.04224** |
| Shoot Length (cm) | **P ≤ 0.001** | **P ≤ 0.001** | **P ≤ 0.001** | **0.00105** | 0.11341 | **0.00226** | **0.00837** |
| Root Length (cm) | **P ≤ 0.001** | **P ≤ 0.001** | **P ≤ 0.001** | **P ≤ 0.001** | **P ≤ 0.001** | **P ≤ 0.001** | **0.00618** |
| Number of Pods | **P ≤ 0.001** | **P ≤ 0.001** | 0.27394 | **0.00143** | **P ≤ 0.001** | **0.00758** | **P ≤ 0.001** |
| Plant Dry Weight (g) | **P ≤ 0.001** | **P ≤ 0.001** | 0.23804 | 0.14844 | **0.02872** | **0.00632** | 0.11923 |
| Pod Dry Weight (g) | **P ≤ 0.001** | **P ≤ 0.001** | 0.7864 | 0.70498 | 0.08027 | 0.34909 | 0.11035 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $\Delta^{13}C$ | **0.02606** | **P ≤ 0.001** | **P ≤ 0.001** | **0.00577** | 0.06763 | **P ≤ 0.001** | **P ≤ 0.001** |
| %Ndfa | **P ≤ 0.001** | **P ≤ 0.001** | **0.01154** | 0.05797 | **P ≤ 0.001** | **0.00223** | 0.14897 |
| Total N Content in Plant Biomass (g N per Plant) | **P ≤ 0.001** | **P ≤ 0.001** | **0.01508** | 0.22212 | **0.02093** | **0.00239** | 0.63648 |
| Amount of N Derived from N Fixation (non percent decimal number) | **P ≤ 0.001** | **P ≤ 0.001** | **P ≤ 0.001** | **0.03734** | **P ≤ 0.001** | 0.55393 | 0.26102 |
| Photosynthesis ($A_N$) 75 DAP | 0.85629 | 0.52016 | **P ≤ 0.001** | 0.96267 | 0.60073 | **0.00424** | 0.54773 |
| Stomatal Conductance ($g_s$) 75 DAP | 0.18595 | 0.51826 | **P ≤ 0.001** | 0.79804 | 0.19039 | **P ≤ 0.001** | 0.42297 |
| WUEi ($A_N/g_s$) 75 DAP | **0.0092** | 0.13035 | 0.27811 | 0.30439 | 0.17611 | **0.0194** | 0.34769 |
| Photosynthesis (AN) 85 DAP | 0.58742 | 0.06898 | **0.01309** | 0.33693 | 0.1407 | 0.53224 | 0.83768 |
| Stomatal Conductance (gs) 85 DAP | 0.72037 | 0.13412 | **P ≤ 0.001** | 0.75917 | **0.01493** | 0.71425 | 0.65129 |
| WUEi (AN/gs) 85 DAP | 0.85386 | 0.70441 | **P ≤ 0.001** | 0.68996 | 0.25608 | 0.20811 | 0.71268 |
| RWC (%) 30 DAE | **P ≤ 0.001** | 0.30251 | 0.67979 | **P ≤ 0.001** | 0.07326 | 0.477 | 0.39117 |
| RWC (%) 60 DAE | **P ≤ 0.001** | 0.27654 | 0.30783 | 0.84006 | 0.1881 | 0.18719 | 0.9629 |
| RWC (%) 90 DAE | **P ≤ 0.001** | **0.00123** | **0.04047** | 0.06589 | 0.12793 | 0.46766 | 0.06177 |
| SCMR 30 DAE | **P ≤ 0.001** | **P ≤ 0.001** | 0.85725 | **0.00444** | 0.78557 | 0.99244 | 0.98857 |
| SCMR 60 DAE | **P ≤ 0.001** | **0.01916** | 0.45768 | **P ≤ 0.001** | **P ≤ 0.001** | **0.0061** | **P ≤ 0.001** |
| SCMR 90 DAE | **P ≤ 0.001** | **P ≤ 0.001** | **P ≤ 0.001** | **P ≤ 0.001** | 0.65207 | 0.88842 | 0.14823 |
| SLA 67 DAP | **P ≤ 0.001** | **0.04102** | 0.48733 | **0.02929** | 0.53694 | 0.50837 | 0.83437 |
| SLA 74 DAP | **P ≤ 0.001** | 0.14219 | 0.25826 | 0.33763 | 0.14691 | 0.36227 | 0.05643 |
| SLA 81 DAP | **P ≤ 0.001** | 0.50943 | 0.38934 | 0.19685 | 0.06014 | 0.08331 | 0.86739 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SLA 88 DAP | **P ≤ 0.001** | **0.00788** | **P ≤ 0.001** | **P ≤ 0.001** | **0.03761** | 0.06923 | **P ≤ 0.001** |
| Total Water Transpired (kg) | **P ≤ 0.001** | 0.36483 | **0.00455** | **0.00734** | **P ≤ 0.001** | 0.08808 | 0.0755 |
| WUE (g Biomass/kg Water) | **P ≤ 0.001** | **P ≤ 0.001** | 0.18434 | 0.3877 | **P ≤ 0.001** | **0.04179** | 0.07858 |

Table 3: Tukey Kramer table for inoculation results.

| Inoculation | WC | OP | AP203 | OPAP203 |
|---|---|---|---|---|
| ChlD (mg/cm$^2$) 30 DAE | 8.04a | 13.29b | 18.57c | 21.89d |
| ChlD (mg/cm$^2$) 60 DAE | 10.2a | 9.34a | 18.42b | 19.52b |
| ChlD (mg/cm$^2$) 90 DAE | 3.94a | 4.75b | 5.88c | 7.51d |
| Root Length (cm) | 22.3a | 24.01a | 27.06b | 35.68c |
| Shoot Length (cm) | 51.56a | 53.11a | 53.92a | 57.65b |
| Number of Pods | 6.35a | 10.71b | 10.38b | 10.46b |
| Plant Dry Weight (g) | 15.45a | 27.16bc | 25.61b | 27.99c |
| Pod Dry Weight (g) | 14.19a | 20.62b | 20.62b | 21.44b |
| $\Delta^{13}C$ (‰) | 20.15a | 20.36b | 20.16ab | 20.15ab |
| %Ndfa | 40.35a | 46.63b | 38.52a | 40.37a |
| Total N Content in Biomass (g N per Plant) | 0.77a | 1.23b | 1.25b | 1.21b |
| Amount of N Derived from N Fixation (non percent decimal number) | 0.32a | 0.58b | 0.49b | 0.51b |
| WUEi ($A_N/g_s$) 75 DAP | 1b | 0.82a | 0.81a | 0.96ab |
| RWC (%) 30 DAE | 76.1a | 79.38a | 88.23b | 92.69b |
| RWC (%) 60 DAE | 67.33a | 77.74b | 93.33c | 94.87c |
| RWC (%) 90 DAE | 78.3a | 87.98b | 93.07c | 95.87c |
| SCMR 30 DAE | 32.53a | 34.72b | 41.09c | 46.34d |
| SCMR 60 DAE | 34.44a | 34.89a | 44.09b | 46.2c |
| SCMR 90 DAE | 32.75a | 40b | 39.78b | 43.96c |
| SLA 67 DAP | 196.47a | 323.29b | 404.26b | 564.1c |
| SLA 74 DAP | 230.49a | 305.35a | 529.32b | 606.18b |
| SLA 81 DAP | 204.08a | 252.72a | 380.07b | 491.79c |
| SLA 88 DAP | 211.21a | 248.46a | 359.57b | 446.62c |
| Total Water Transpired (kg) | 20.95c | 16.8b | 10.71a | 10.12a |
| WUE (g Biomass/kg Water) | 1.49a | 2.94b | 4.68c | 5.45d |

Table 4: Tukey Kramer table for genotype results.

| Genotype | AU 18-33 | AU 18-53 | AU 18-57 | AU 18-58 |
|---|---|---|---|---|
| ChlD ($mg/cm^2$) 30 DAE | 17.01c | 16.23bc | 15.42b | 13.13a |
| ChlD ($mg/cm^2$) 60 DAE | 15.12b | 14.58ab | 13.53a | 14.25ab |
| Root Length (cm) | 28.1b | 28.1b | 30.15b | 22.69a |
| Shoot Length (cm) | 52.51ab | 58.33c | 55.65bc | 49.75a |
| Number of Pods | 7.06a | 9.26b | 10.66bc | 10.93c |
| Plant Dry Weight (g) | 21.16a | 26.17b | 26.08b | 22.79a |
| Pod Dry Weight (g) | 17.43a | 18.76ab | 20.49b | 20.19b |
| Δ13C (‰) | 20.77c | 19.83a | 19.84a | 20.38b |
| Total N Content in Biomass (g N per Plant) | 1a | 1.06ab | 1.15bc | 1.26c |
| Amount of N Derived from N Fixation (non percent decimal number) | 0.44ab | 0.39a | 0.49bc | 0.56c |

Table 5: Tukey Kramer table for water treatment results.

| Water Treatment | Drought Stressed | Well-Watered |
|---|---|---|
| ChlD ($mg/cm^2$) 60 DAE | 15.8b | 12.94a |
| ChlD ($mg/cm^2$) 90 DAE | 6.04b | 5a |
| Root Length (cm) | 25.11a | 29.42b |
| Shoot Length (cm) | 49.84a | 58.28b |
| $\Delta^{13}C$ (‰) | 19.86a | 20.55b |
| Total N Content in Biomass (g N per Plant) | 1.07a | 1.15b |
| Amount of N Derived from N Fixation (non percent decimal number) | 0.41a | 0.53b |

| | | |
|---|---|---|
| Photosynthesis ($A_N$) 75 DAP | 16.5b | 7.2a |
| Stomatal Conductance ($g_s$) 75 DAP | 0.21b | 0.09a |
| Photosynthesis ($A_N$) 85 DAP | 14.49a | 17.29b |
| Stomatal Conductance ($g_s$) 85 DAP | 0.24a | 0.5b |

Table 6: Tukey Kramer table for the interaction between inoculation and genotype.

| Inoculation | Genotype | ChlD ($mg/cm^2$) 60 DAE | ChlD ($mg/cm^2$) 90 DAE | Root Length (cm) | Shoot Length (cm) | Number of Pods | $\Delta^{13}C$ (‰) | Amount of N Derived from N Fixation (non percent decimal number) | Total Water Transpired (kg) |
|---|---|---|---|---|---|---|---|---|---|
| WC | AU 18-33 | 11.73b | 4.1ab | 22.44abc | 53.15abcd | 6.3a | 20.6bcd | 0.31ab | 20cde |
| WC | AU 18-53 | 11.57ab | 4.35abc | 20.51ab | 51.73abc | 5.59a | 19.71a | 0.26a | 21.67e |
| WC | AU 18-57 | 8.56ab | 3.66a | 26.45bcd | 53.85abcd | 6.73ab | 19.69a | 0.33ab | 21.69e |
| WC | AU 18-58 | 8.95ab | 3.64a | 19.8a | 47.5ab | 6.8ab | 20.6bcd | 0.38abc | 20.42de |
| OP | AU 18-33 | 9.18ab | 4.85abcd | 22.35abc | 45.81a | 7.72abc | 20.89d | 0.51abcd | 17.13bc |
| OP | AU 18-53 | 7.82a | 4.55abc | 26.55bcd | 60.93d | 10.29bcd | 20.03ab | 0.6cd | 17.84bcd |
| OP | AU 18-57 | 8.52ab | 4.65abc | 28.05cd | 54.85bcd | 12.21d | 19.89a | 0.52abcd | 15.45b |
| OP | AU 18-58 | 11.86b | 4.97abcd | 19.07a | 50.83abc | 12.62d | 20.64cd | 0.68d | 16.77b |
| AP203 | AU 18-33 | 19.89c | 5.65cdef | 32.15de | 52.64abcd | 6.85ab | 20.93d | 0.45abcd | 12.2a |
| AP203 | AU 18-53 | 19.87c | 6.47efg | 29.7de | 60.8d | 10.75cd | 19.68a | 0.32ab | 9.98a |
| AP203 | AU 18-57 | 17.23c | 5.14bcde | 27.14cd | 54.8bcd | 13d | 19.81a | 0.51abcd | 10.18a |
| AP203 | AU 18-58 | 16.69c | 6.26defg | 19.25a | 47.45ab | 10.94cd | 20.24abc | 0.66d | 10.51a |
| OPAP203 | AU 18-33 | 19.69c | 6.69fg | 35.47ef | 58.45cd | 7.36abc | 20.68cd | 0.5abcd | 10.98a |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| OPAP203 | AU 18-53 | 19.08c | 7.11g | 35.66ef | 59.85cd | 10.4bcd | 19.89a | 0.38abc | 9.18a |
| OPAP203 | AU 18-57 | 19.79c | 8.61h | 38.96f | 59.1cd | 10.71cd | 19.99a | 0.62cd | 10.73a |
| OPAP203 | AU 18-58 | 19.51c | 7.64gh | 32.65def | 53.2abcd | 13.36d | 20.06ab | 0.54bcd | 9.6a |

Table 7: Tukey Kramer table for the interaction between inoculation and water treatment.

| Inoculation | WC | WC | OP | OP | AP203 | AP203 | OPAP203 | OPAP203 |
|---|---|---|---|---|---|---|---|---|
| Water Treatment | DS | WW | DS | WW | DS | WW | DS | WW |
| ChlD ($mg/cm^2$) 60 DAE | 12.06b | 8.35a | 10.73b | 7.96a | 20.35d | 16.49c | 20.08d | 18.96d |
| Root Length (cm) | 22.25a | 22.35a | 22.51a | 25.5a | 23.67a | 30.44b | 32b | 39.37c |
| Number of Pods | 6.95ab | 5.76a | 10.82cd | 10.6cd | 11.75d | 9.02bc | 9.24bc | 11.68d |
| Plant Dry Weight (g) | 15.29a | 15.61a | 28.16c | 26.16bc | 27.16bc | 24.05b | 27.09bc | 28.89c |
| %Ndfa | 50.01de | 30.69ab | 43.71cd | 49.56de | 37.25bc | 39.78c | 27.78a | 52.96e |
| Total N Content in Plant Biomass (g N per plant) | 0.77a | 0.78a | 1.15bc | 1.31bc | 1.27bc | 1.22bc | 1.11b | 1.31c |
| Amount of N Derived from N Fixation (non percent decimal number) | 0.38abc | 0.27a | 0.5cd | 0.65de | 0.45bc | 0.52cd | 0.31ab | 0.7e |
| Stomatal Conductance ($g_s$) 85 DAP | 0.23a | 0.42ab | 0.2a | 0.56b | 0.18a | 0.58b | 0.36ab | 0.42ab |
| SCMR 60 DAE | 37.71b | 31.17a | 31.89a | 37.9b | 44.63c | 43.54c | 46.1c | 46.31c |
| SLA 88 DAP | 241.85ab | 180.58a | 240.54ab | 256.39ab | 402.56cd | 316.58abc | 531.18d | 362.06bc |
| Total Water Transpired in kg | 18.56d | 23.33e | 15.6c | 17.99d | 11.17b | 10.26ab | 11.47b | 8.78a |
| WUE (g biomass/kg water) | 1.68a | 1.31a | 3.14b | 2.73b | 4.65c | 4.71c | 4.72c | 6.19d |

DS – Drought Stressed, WW – Well-Watered

Table 8: Tukey Kramer table for the interaction between genotype and water treatment.

| Genotype | AU 18-33 | AU 18-33 | AU 18-53 | AU 18-53 | AU 18-57 | AU 18-57 | AU 18-58 | AU 18-58 |
|---|---|---|---|---|---|---|---|---|
| Water Treatment | DS | WW | DS | WW | DS | WW | DS | WW |
| ChlD ($mg/cm^2$) 90 DAE | 5.11ab | 5.54bc | 6.17cd | 5.08ab | 6.63d | 4.4a | 6.26cd | 5ab |
| Shoot Length (cm) | 51.12bc | 53.91bc | 54.26bc | 62.4d | 50.1b | 61.2d | 43.89a | 55.6c |
| Root Length (cm) | 23.02ab | 33.19e | 26.48bcd | 29.73de | 27.39cd | 32.91e | 23.54abc | 21.84a |
| Number of Pods | 7.42a | 6.7a | 10.45c | 8.06ab | 10.01bc | 11.32c | 10.88c | 10.98c |
| Plant Dry Weight (g) | 21.04a | 21.28ab | 27.97d | 24.38abcd | 27.05cd | 25.11bcd | 21.65ab | 23.94abc |
| $\Delta^{13}C$ (‰) | 20.12b | 21.43c | 19.38a | 20.28b | 19.57a | 20.12b | 20.39b | 20.37b |
| %Ndfa | 37.11a | 49.47c | 37.95ab | 38.42ab | 37.47a | 40.49ab | 46.22bc | 44.62abc |
| Total N Content in Plant Biomass (g N per plant) | 0.98a | 1.02ab | 1.1ab | 1.01ab | 1.09ab | 1.2bc | 1.13ab | 1.38c |
| Photosynthesis ($A_N$) 75 DAP | 18.97c | 4.17a | 16.51c | 7.35a | 17.13c | 8.7ab | 13.41bc | 8.57ab |
| Stomatal Conductance ($g_s$) 75 DAP | 0.3c | 0.05a | 0.2bc | 0.08a | 0.19bc | 0.11ab | 0.14ab | 0.13ab |
| WUEi (AN/gs) 75 DAP | 74.03a | 88.37a | 93.54a | 98.51a | 102a | 85.24a | 99.5a | 76.38a |
| SCMR 60 DAE | 39.09ab | 41.02b | 40.01ab | 40.61b | 39.78ab | 37.47a | 41.44b | 39.82ab |
| WUE (g biomass/kg water) | 3.1ab | 3.09a | 3.94bc | 3.6abc | 3.95bc | 4.31c | 3.2ab | 3.94abc |

DS – Drought Stressed, WW – Well-Watered

Table 9: Tukey Kramer table for the interaction between inoculation, genotype, and water treatment.

| Inoculation | Genotype | Water Treatment | ChlD ($mg/cm^2$) 90 DAE | Shoot Length (cm) | Number of Pods | $\Delta^{13}C$ (‰) |
|---|---|---|---|---|---|---|
| WC | AU 18-33 | DS | 4.16abcde | 52.3abcdef | 6.1abc | 20.44fghij |

| | | | | | | |
|---|---|---|---|---|---|---|
| WC | AU 18-33 | WW | 4.05abcde | 54abcdef | 6.5abcd | 20.76ijk |
| WC | AU 18-53 | DS | 4.97bcdefghi | 51.67abcdef | 7.8abcdefgh | 19.33ab |
| WC | AU 18-53 | WW | 3.74abcd | 51.8abcdef | 3.38a | 20.1abcdefghij |
| WC | AU 18-57 | DS | 4.78bcdefgh | 47.3abcd | 6.9abcdef | 19.51abcde |
| WC | AU 18-57 | WW | 2.53a | 60.4defg | 6.56abcdef | 19.88abcdefgh |
| WC | AU 18-58 | DS | 4.42abcdef | 43.2ab | 7abcdef | 20.3defghij |
| WC | AU 18-58 | WW | 2.87ab | 51.8abcdef | 6.6abcde | 20.89jkl |
| OP | AU 18-33 | DS | 4.6abcdefgh | 48abcde | 6.88abcdefg | 20.02abcdefghi |
| OP | AU 18-33 | WW | 5.11cdefghij | 43.63abc | 8.57abcdefghi | 21.75m |
| OP | AU 18-53 | DS | 4.47abcdef | 52.86abcdef | 12.2efghij | 19.37abc |
| OP | AU 18-53 | WW | 4.62abcdefg | 69g | 8.37abcdefgh | 20.69hijk |
| OP | AU 18-57 | DS | 5.71cdefghij | 53.6abcdef | 12.3fghij | 19.58abcdef |
| OP | AU 18-57 | WW | 3.59abc | 56.1bcdefg | 12.13defghij | 20.19cdefghij |
| OP | AU 18-58 | DS | 5.49cdefghij | 44.17abc | 11.9defghij | 20.66ghijk |
| OP | AU 18-58 | WW | 4.45abcdef | 57.5cdefg | 13.33hij | 20.61hij |
| AP203 | AU 18-33 | DS | 5.12cdefghij | 51.29abcdef | 8.1abcdefgh | 20.21cdefghij |
| AP203 | AU 18-33 | WW | 6.18efghijk | 54abcdef | 5.6ab | 21.65lm |
| AP203 | AU 18-53 | DS | 7.98kl | 56.8cdefg | 11.5cdefghij | 19.3a |
| AP203 | AU 18-53 | WW | 4.96bcdefghi | 64.8fg | 10bcdefghij | 20.06abcdefghij |
| AP203 | AU 18-57 | DS | 6.05defghijk | 46.4abc | 14.4ij | 19.4abcd |
| AP203 | AU 18-57 | WW | 4.23abcde | 63.2fg | 11.6cdefghij | 20.21cdefghij |
| AP203 | AU 18-58 | DS | 6.96hijk | 42.6a | 13ghij | 20.24defghij |
| AP203 | AU 18-58 | WW | 5.56cdefghij | 52.3abcdef | 8.87abcdefghi | 20.23bcdefghij |
| OPAP203 | AU 18-33 | DS | 6.55fghijk | 52.9abcdef | 8.6abcdefgh | 19.81abcdefgh |
| OPAP203 | AU 18-33 | WW | 6.83ghijk | 64fg | 6.12abcd | 21.55klm |
| OPAP203 | AU 18-53 | DS | 7.24jk | 55.7abcdefg | 10.3bcdefghij | 19.51abcde |
| OPAP203 | AU 18-53 | WW | 6.98ijk | 64fg | 10.5bcdefghij | 20.26efghij |
| OPAP203 | AU 18-57 | DS | 9.97l | 53.1abcdef | 6.43abcdef | 19.79abcdefgh |
| OPAP203 | AU 18-57 | WW | 7.24ijk | 65.1fg | 15j | 20.19cdefghij |
| OPAP203 | AU 18-58 | DS | 8.18kl | 45.6abc | 11.63cdefghij | 20.36efghij |
| OPAP203 | AU 18-58 | WW | 7.11ijk | 60.8efg | 15.1j | 19.77abcdefg |

DS – Drought Stressed, WW – Well-Watered

# Figures

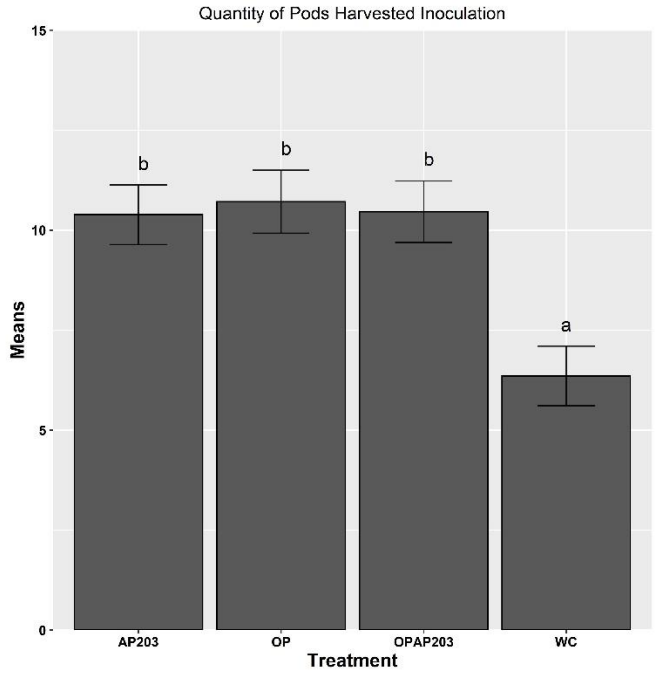


Figure 1: Number of pods harvested for inoculation treatment.

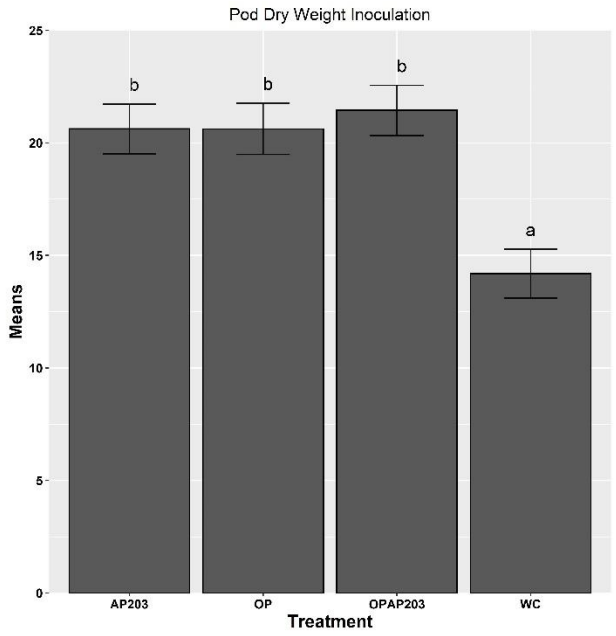


Figure 2: Pod dry weight for inoculation treatment.

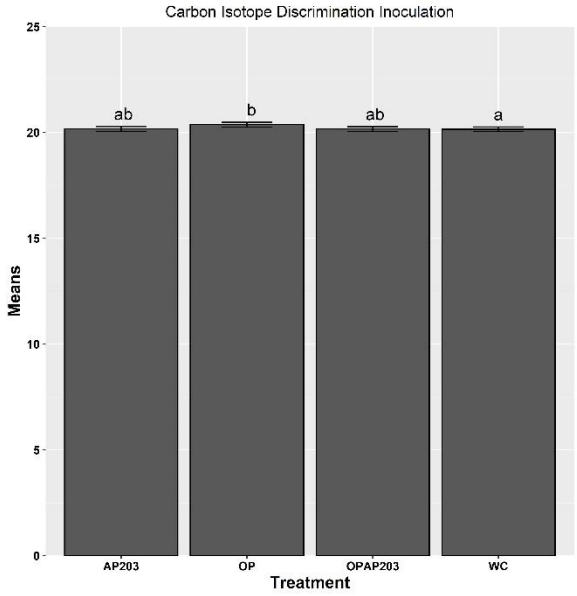


Figure 3: Carbon isotope discrimination for inoculation treatment.

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