

Thesis for the Degree of Master of Agriculture

**Dual Application of *Brevundimonas vesicularis*
and *Pseudomonas koreensis* Confers Drought
Stress Tolerance via Regulation of Gene
Expression, Phytohormone Synthesis and
Antioxidant Systems in *Arabidopsis thaliana***

Department of Food Security and Agricultural Development
The Graduate School

EDDY CERVANTES ZAVALA

December 2022

**The Graduate School
Kyungpook National University**

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December 2022

Chairman _____

**The Graduate School Council
Kyungpook National University**

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ABSTRACT

Climate change is increasing drought events around the world and mainly affects the availability of water, necessary to maintain agricultural production. In this research, we highlight the role of plant growth promoter rhizobacteria (PGPR) of *Brevundimonas vesicularis* strain S1T13 and *Pseudomonas koreensis* strain S4T10, which were applied and investigated in *A. thaliana*, genotypes Col-0 and *Atnced3* ABA pathway-related gene, *AtNCED3*-deficient mutant. The recovery capacity after drought, chlorophyll content, electrolyte leakage, the ability to reduce the reactive oxygen species (ROS), antioxidant system, as well the transcriptional factor of gene expression of drought-related genes such *AtDREB1*, *AtDREB2*, and ABA-related genes *AtA03*, *AtABA3*, were evaluated.

The effect of dual application of S1T13 and S4T10 on Col-0 and *Atnced3*, the recovery of plants after a drought process is very favorable due to the effect of PGPR, restoring physiological functions optimally. The total chlorophyll content was determined with SPAD, and it was also contrasted with the photosynthetic pigments of chlorophyll *a* and *b*, showing healthiness in plants inoculated with S1T13+S4T10, under drought conditions was positively helped by maintaining optimal levels of chlorophyll, to increase the photosynthetic capacity, consequently improving the phenotypic characteristics of the plant. The damage caused the by oxidation of reactive oxygen species (ROS) such as hydrogen peroxide H₂O₂ and superoxide anion -O₂ was reduced, also the levels of electrolyte leakage with K⁺ efflux were reduced. Antioxidant levels were increased by the effect of accumulation of (ROS), with the immediate response to remove these oxidative compounds by the defense system of superoxide dismutase that catalyzes the -

O_2 in H_2O and O_2 , peroxidase catalyzes the H_2O_2 in H_2O , glutathione protects the membrane and prevents oxidative damage, and polyphenol oxidase, which actively increased their antioxidant capacity system, releasing enzymes that control oxidation damage and symbiosis between the roots and (PGPRs). The protein-coding genes related to the ABA pathway such as *AtAO3*, and *AtABA* which had a lower expression that means the healthiness of the plant was improved, and the ABA stress hormone is low, contributing directly to the control of opening and closing of stomata, in leaves to maintain the turgidity and physiologic functions to protect plants. A clear effect on the expression level drought-related element binding genes of *AtDREB2A* and *AtDREB2B* of Col-0 and *atnced3*, by dual application of S1T13 and S4T10, was highly expressed that activate the defense system of antioxidants, to induce a quick response to drought events and tolerate these effects in the nature.

1. INTRODUCTION

1.1. Drought Effect.

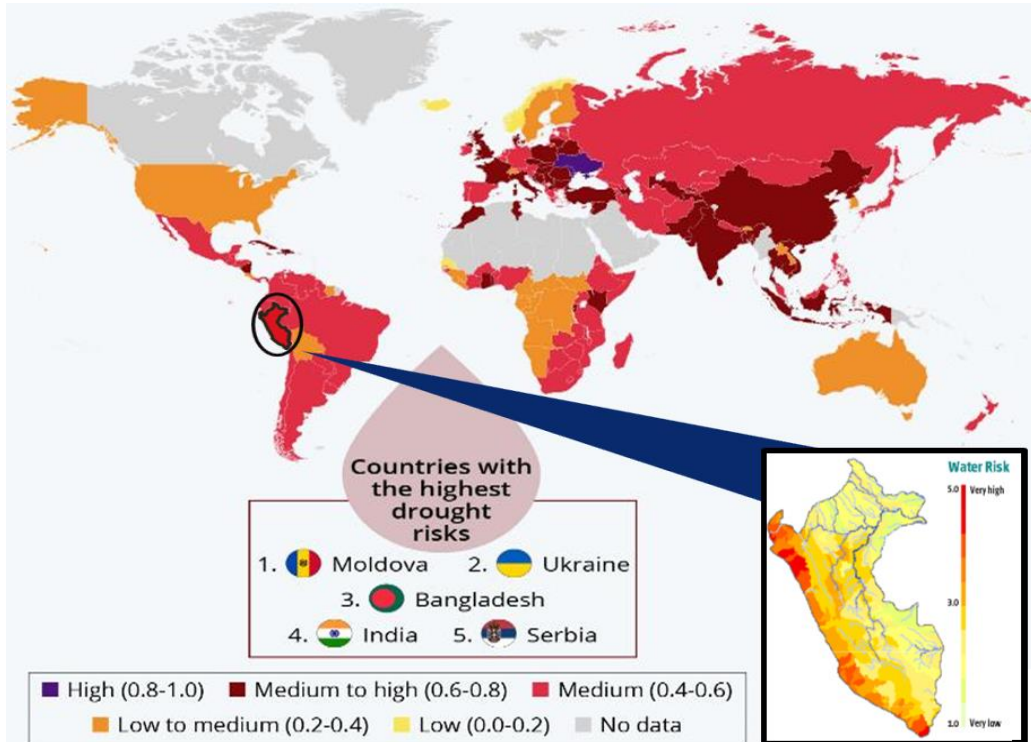
Drought is one of the most representative and relevant physical effects that limits the availability of water, which affects the yields and growth of the crops, necessary for people's daily alimentation. The world is facing water and land limitations, which force us to improve agriculture production practices, to increase the yields of crops (Santaniello et al., 2017). (Lu et al., 2019), drought is aggravating future climatic scenarios such as temperature and rainfall necessary for the population's water availability, (Mpelasoka et al., 2018), **Figure 1**, shows the climacteric map situation.

Peruvian territory is considered to have a medium risk of drought (20-40%) in the world susceptible to scarce water (MINAM, 2016), Peru had a direct dependence on water coming from the glaciers and aquifers in the last years dramatically decreasing, anomalies in rainy seasons and high relative temperatures consequently will affect the availability of water in the Andean region, which feeds the largest agriculture productive zones of the Peruvian territory, (Lasage et al., 2015). Several climatic events in Peru, are directly linked to “El Niño” or “La Niña” a global climatic event, which modifies the presence or absence of rain (Imfeld et al., 2019).

Lately, these environmental changes induce to improve the traditional productive system, to develop drought resistance plants (Rani et al., 2020). In nature, plants have naturally developed several physiological mechanisms at different levels, like creating synergism and symbiosis with other organisms and also changes at the cellular and molecular stages to reduce the stress caused by drought (Mahmood et al., 2019).

Figure 1

Major drought causes in the world environment



Taken from: (Buchholz, 2021)

In nature, plants have naturally developed several physiological mechanisms at different levels, like creating synergism and symbiosis with other organisms and also changes at the cellular and molecular stages to reduce the stress caused by drought (Mahmood et al., 2019).

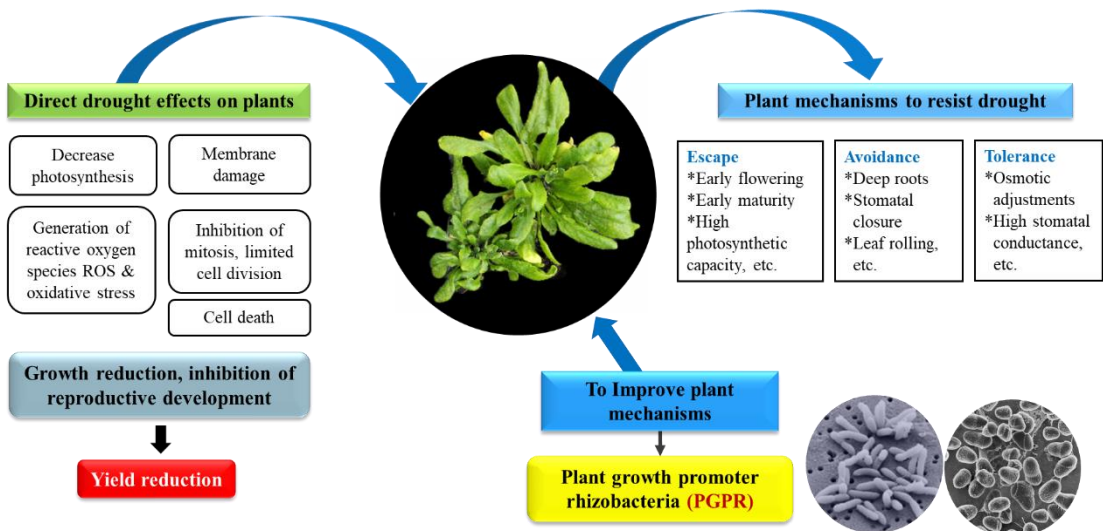
1.2. Plant Stress Response.

Plants have mechanisms that protect them from abiotic stress, but this defense system is activated at expense of plant growth (Bechtold & Field, 2018). These physiological changes can reduce crop yields by approximately 70%. To improve these

external factors, new technologies have been developed, and one of these is biotechnological processes, to understand drought at the physiological and molecular level that occurs in plants with genes tolerant to abiotic stress, which are being developed to adapt to drought stress, (Gosal et al., 2009). According to the severity of abiotic stress occurrences, this leads to the production or overproduction of reactive oxygen species (ROS), this reaction in plants is highly toxic and reactive, generating oxidative stress and damage to the DNA, protein, lipids, and carbohydrates (Gill & Tuteja, 2010).

Figure 2

Schematic view plant mechanism against drought



Taken from: (Badigannavar et al., 2018).

1.3. Role of PGPR *Brevundimonas vesicularis* and *Pseudomonas koreensis* in Biotic and Abiotic Stress.

The microbes have evidence of a drought tolerance role, (Vaishnav & Choudhary, 2019). In the plant transduction pathway, several bacterial colonies play a role to protect against drought effects, through morphological and architectural functions, (Shafique et

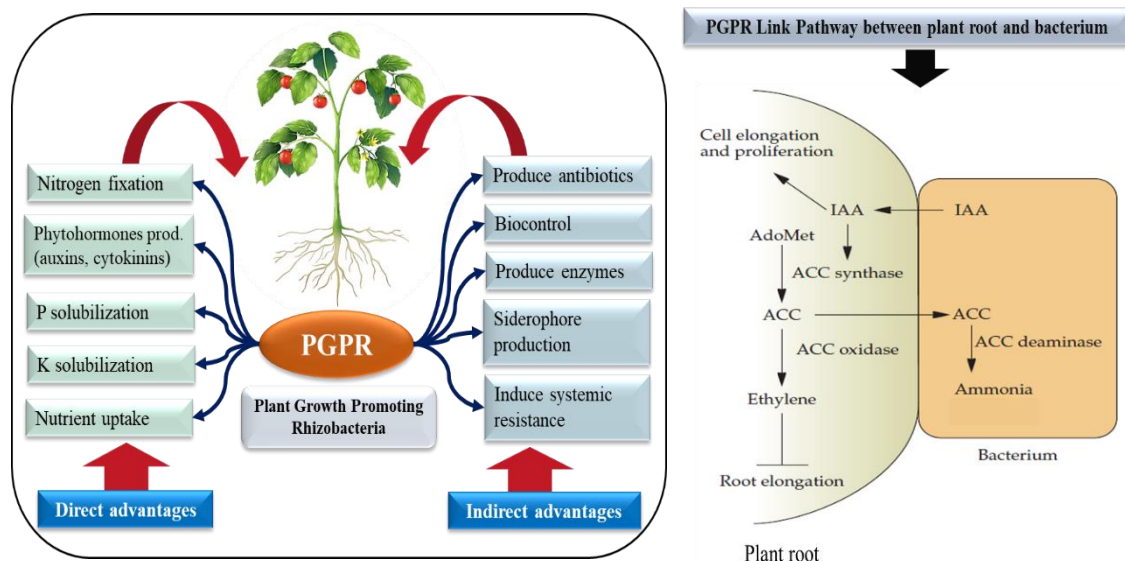
al., 2022). In nature when the plant growth decrease is because the levels of abscisic acid, reactive oxygen species, and ethylene have been increased. To fight against drought the plants can increase the phenolic compounds production, soluble sugar, and proline, (Macleod et al., 2015). Plant growth promoter rhizobacteria (PGPR), are widely studied, in this case, *Brevundimonas vesicularis* and *Pseudomonas koreensis*, can improve the water holding around the soil and between the root system, in this symbiotic environment, (Moon & Ali, 2022).

The importance lies in the fact that stressed plants for their survival require antioxidants to participate in redox reduction, which would provide better protection and regeneration of plants (Dumanović et al., 2021). Knowledge about enzymes is still limited, regarding gene expression patterns (Filiz et al., 2019).

The role and functionality of bacteria observed, the phylogenetic composition and nutrient-releasing population of *Pseudomonas* under long drought periods, to see external changes, the composition of the plants are seasonal effects, and partly concordance between metabolic activities and 16S Phylogenecy, (Breitkreuz et al., 2021). For growth in plants with multiple traits, based on the 16s rRNA gene sequence which was compared with *Brevundimonas vesicularis* strain (S1T13) and *Pseudomonas koreensis* strain (S4T10), they offer positive aspects in the promotion of plant growth, under normal conditions and abiotic stress, (Moon & Ali, 2022). When soil moisture decreases the response is high. (Breitkreuz et al., 2021).

Figure 3

Mode of action of PGPR in the environment and root system



Taken from: (Glick et al., 2010).

1.4. Antioxidants and Reactive Oxygen Species (ROS), Reaction.

One of the most significant effects of abiotic stress is the disruption of the balance between ROS production and antioxidant defense mechanisms, (Hasanuzzaman et al., 2020). Plants generate aerobic metabolism like (ROS), which permanently occurs under physiological conditions in plant metabolism, under this process and biotic or abiotic stress, the production of ROS exceeds the scavenging capacity of the plant, which generates significant damage to the protein and DNA membrane, (Impa et al., 2012).

When stress conditions occur, electrolyte leakage is detected, and K⁺ efflux is the main cause for this including reaction. Reactive oxygen species (ROS), hydrogen peroxide (H₂O₂), and superoxide anion (-O₂), are frequently present in the electrolyte leaks caused by stress, and all these reactions are interconnected, (Demidchik et al.,

2014). The majority of the time, plant cells can produce defenses against ROS, including the creation of phytoalexins, the manufacture of pathogenesis-related proteins, and the regulation of photosynthetic function via programmed cell death., (YH, 2015). H₂O₂ is essential for the oxidative burst because it signals the targeted death cells and acts as a diffuse signal to activate cellular protection genes in nearby healthy cells and tissues, (Fan et al., 2017).

Being very difficult to control and prevent the fluxes of O₂ in a ROS reaction, like superoxide (-O₂), when ROS concentrations reach a certain level, it triggers programmed cell death, (Jambunathan, 2010). Additionally, some hormones like ethylene (ET) and abscisic acid (ABA), influence reactions through interaction with ROS and improve stress tolerance, (Huang et al., 2018).

1.5. Phytohormones and Antioxidant System to Eliminate ROS.

Phytohormones, or plant growth regulators, modulate plant growth and development. Among them, are auxins, cytokinins, gibberellins, ethylene, abscisic acid, salicylic acid, etc. Abscisic acid (ABA) regulates maturation and seed dormancy and also acts in response to drought, accumulating in plant cells under water stress, promoting stomatal closure, and regulating the expression of a large number of genes. Instead, ethylene intervenes as a regulator of aging, fruit ripening, and response to pathogens, causing premature aging and the abscission of plant leaves. This is produced naturally, due to its antagonistic character in plant development: ethylene promotes senescence and ABA defends against drought, interacting physiologically in plant growth and development, (Salvador et al., 2012).

Superoxide dismutase (SOD) is the enzyme that acts first to detoxify the plants' cells and is the most effective (Ighodaro & Akinloye, 2018). These are universal enzymes present in all living things that need oxygen to survive (Y. Wang et al., 2018). In the first lines of defense against reactive oxygen species (ROS) and reactive nitrogen species

(RNS) is an endogenous antioxidant enzyme, by accelerating the dismutation of two molecules of superoxide anion (-O_2) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), (Ighodaro & Akinloye, 2018).

To control several metabolic processes, a potent nonenzymatic antioxidant known as glutathione is released to protect the membranes; this substance is a tiny molecule, due to all these factors, has recently shifted to the ability to tolerate abiotic stress, (Hasanuzzaman et al., 2017).

Reactive oxygen species (ROS), are oxidized by peroxidases in stages, as organs, tissues, cells, and subcellular levels all operate differently according to the types of peroxidases involved. To comprehend overexpression, malfunction, and its deficiency in various occurrences caused by oxidative stress, various processes have been postulated that are based on different bacterial types. (Khan et al., 2014). The importance of glutathione peroxidases (GPUs), which catalyze the conversion of organic hydroperoxides or H_2O_2 to water, (Bela et al., 2022).

To link the plant root system with microbes that will help the defense system against abiotic stress, primarily polyphenols that plants produce are phenolic acids. These substances serve a variety of purposes and are crucial for symbiosis between plants and PGPRs. Phenolic substances can serve as plant defense agents, announcing the beginning of rhizobial symbioses, and influencing the expression of genes in the *A. thaliana* plant, (Santi M. Mandal et al., 2010).

1.6. Gene Expression.

Currently, in *Arabidopsis thaliana* around 2000 genes sensitive to drought were identified, (D. Huang et al., 2008), in some experiments after drought-induced plants later recover, the expression was restored and normalized to the original levels. In addition, abscisic acid (ABA) has been shown to induce various changes in gene expression, and

several analyzes suggest that the promoters of the remaining genes are affected by ABA demarcation in response to drought.

To study molecularly the genes expression, several strategies were analyzed, and relevant genes are identified, which have a potential role in drought tolerance, particularly the importance of the areas of metabolism of abundant proteins and sugars. Studies that control gene expression in plants have enormous agronomic importance, (Ingram & Bartels, 1996).

The dehydration-responsive element binding (DREBs) proteins are plant transcription factors that control a variety of stress responses and plant development processes. To evaluate the degree of transcription factor expression, the DREBs gene was investigated for drought resistance, (Rustamova et al., 2021). DREBs family protein can help plants survive under abiotic stress and modifies downstream metabolism, and delays development, (Feng et al., 2019). When DREBs are overexpressed in *Arabidopsis thaliana*, proline and soluble sugar levels are only mildly elevated when normal growth circumstances are present, (Feng et al., 2019).

1.7. Problem statement and justification

To alleviate the effects of climate change, which causes subsequent effects such as drought, which directly affects crops, various methods of adaptation to drought are being studied, and one of them is the study through biotechnology induced by plant growth promoter bacteria (PGPR).

In nature, PGRRs abound in a large number of species, various studies have determined that microorganisms are natural promoters of growth, promote defense against certain biotic and abiotic factors, and intervene in the expression of genes in plants, when entering into symbiosis as a synergism with plants, providing benefits in the biochemistry of the plant, as well as being sustainable and friendly to the environment.

The current study can be adapted to South America, and contribute to developing new research in these areas by isolating new PGPRs in the Amazon mountains, Andeans, or coast, potentially diverse. Being aware that the Peruvian territory is vulnerable to the effects of climate change.

1.8. Objectives

- Investigate the action of Brevundimonas vesicularis strain (S1T13) and Pseudomonas koreensis strain (S4T10) in the mitigation of drought effect, in *Arabidopsis thaliana*.
- Evaluate the biochemical, physiological, and molecular role of *A. thaliana* under drought stress.

2. MATERIALS AND METHODS

2.1. Sequence of the experimental setup.

To initiate the experiment, we set up the sequence of the experiment, by considering the genotype of Col-0 Wild type with full genome sequence and *Atnced3*, ABA pathway-related gene, *AtNCED3*-deficient mutant. The sequence is described in **Table 1**, to compare the effect of plant growth-promoting rhizobacteria as *Brevundimonas vesicularis* strain S1T13, and *Pseudomonas koreensis* strain S4T10.

Table 1

Experiment Sequence

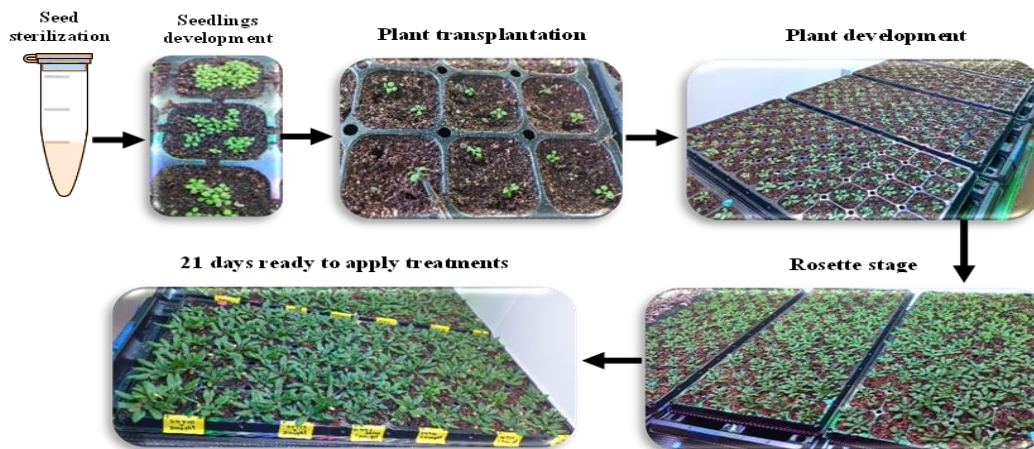
Plant material	Treatments
Col-0	- Control
	- S1T13+ S4T10
	- Drought
	- Drought+S1T13+ S4T10
Atnced3	- Control
	- S1T13+ S4T10
	- Drought
	- Drought S1T13+ S4T10

2.2. Plant Materials and Growing Conditions.

For the experiment *Arabidopsis thaliana* were used to study, genotypes of Col-0 wild type (WT) and, *Atnced3* mutant type, deficient in ABA, the seeds of these plants were gotten from Kyungpook National University, Department of Agriculture and Life Science (Functional Genomic Lab). The sequence of plant growth is in **Figure 4**, we set a tray with Skoog (MS) medium or soil and 1/2 Murashige, under controlled conditions of $23 \pm 2^\circ\text{C}$, long day conditions (16 h light and 8 h dark). The *Arabidopsis* genotypes were grown for 14th days after seedling, and then transplanted in trays of 50 pots, and grow until see the rosette stage (4 weeks) to start the application of treatments, (Murtaza, 2018).

Figure 4

Schematic View of Growth Materials and Conditions



Note: The Figure represents the sequence of vegetative growth of Arabidopsis, for the drought stress experiment.

2.3. Isolation and Identification of Plant Grow Promoter Rhizobacteria Strains.

The sampling, isolation, screening, solubilization capability, and molecular identification of the rhizobacteria were described, (Moon & Ali, 2022).

Table 2

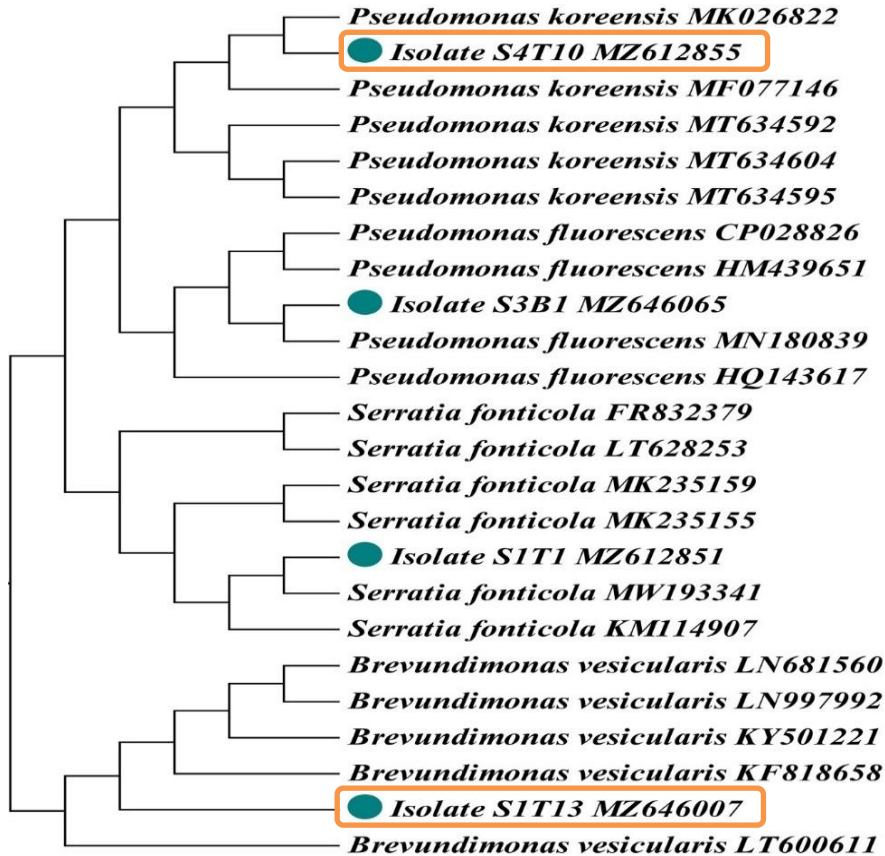
Rhizobacterial Functionality of SIT13 and S4T10

Sample Code	Siderophore Production	Phosphate Solubilization	IAA Production	ACC Deaminase	
	Positive/ Negative	Positive/ Negative	Positive/ Negative Without L. Tryptophan	Positive/ Negative With L. Tryptophan	Positive/ Negative
S1T13	++	++	+	+++	++
S4T10	++	++	+	+++	+++

Note. Rhizobacteria isolate functionality, qualitative and diversity values of siderophore production, IAA, phosphate, and ACC deaminase solubilization, isolated from rhizosphere soil. Taken from, (Moon & Ali, 2022).

Figure 5

Phylogenetic Analysis



Note: The Phylogenetic analysis of multi-trait (PGPR) was isolated in the Pohang seashore, from various plants around the root rhizosphere. The BLAST search tool was used to compare the nucleotide sequence. All of the rhizobacteria-type tree strains were developed using the Molecular Evolutionary Genetics Analysis (MEGA 6.0) program, (Tamura et al., 2013) from related rhizobacteria strains, most similar 16S rRNA regions, were obtained from NCBI, (Moon & Ali, 2022).

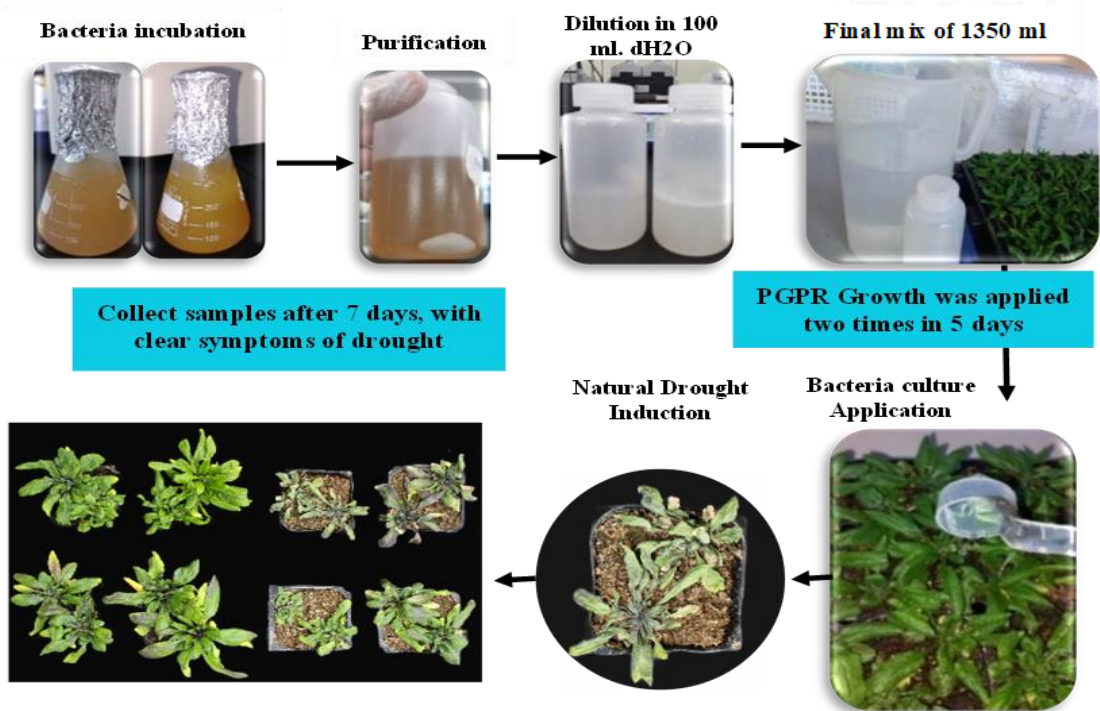
2.4. *Brevundimonas vesicularis* Strain S1T13 and *Pseudomonas koreensis* Strain S4T10 Culture and Inoculation.

We grow these two strains of bacteria of PGPR *Brevundimonas vesicularis* strain S1T13 and *Pseudomonas koreensis* strain S4T10 used for this study and provided by (Moon & Ali, 2022), and gotten through Ph.D. Murtaza Khan.

Primarily, both bacterial strains were cultured in 50 ml., falcon tubes, by adding 10 ml., containing LB (Luria-Bertani-agar media), previously sterilized. Then the tubes were incubated at 28°C and shaken at 180 rpm continuously overnight for 24 hrs., following, a single colony was transferred into 500 ml conical flasks of the LB broth, and incubated at 28°C with continuous shaking at 180 rpm overnight for 24 hrs. The Bacteria culture measured their optical density (OD), by electro photometer, to check the colony ability of each bacteria strain. The bacteria were collected by centrifugation at 5000 rpm for 6 minutes, once we got the bacteria colony and discarded the over natant to get the pellet of the single bacteria, we diluted by adding 100 ml of distilled water and re-suspended in 1350 ml of water. The plants were inoculated at the age of 4 weeks, by two times applications between 5 days, by adding 20 ml to each cell in a tray containing 50 cells per tray (two trays), in the following proportion (10 ml were inoculated of S1T13 and 10 ml of S4T10). Plants under control were applied only with water at the same amount (20 ml., per cell in a tray). Plants were regularly noticed for drought symptoms, (Murtaza, 2018), the sequence is illustrated in **Figure 6**.

Figure 6

PGPR Culture and Inoculation



Note: The graph represents the sequence of strains S1T13 and S4T10 inoculation in *Arabidopsis*, for the drought stress experiment.

2.5. RNA Synthesis for *Arabidopsis thaliana* for Drought Experiment, and Gene Expression.

The total amount of RNA was synthesized and extracted from the leaf samples, from plants inoculated with the PGPRs (S1T13 and S4T10), and plants from the control groups, by using Trizol® (Invitrogen USA) as the established lab protocol of Kyungpook National University, (Functional Genomic Lab). optimized for *Arabidopsis thaliana*, and the detailed protocol:

Grind the leaf sample in LN2 well, and add 1ml Trizol, before thawing, (Trizol solution should not exceed 10% sample). Keep the sample at room temperature for 5-10 min., mix gently the solution to homogenize the sample and Trizol and centrifuge at (13,000 RPM / 10 min., 4°C). The supernatant was transferred to a fresh tube. (Use pipet and transfer 800 ul of liquid). Add 200 ul Chloroform (per 1ml., Trizol), invert mixing by hand for 15 sec., and keep the samples at room temperature for 2-3 min. Centrifuge at (13,000 RPM / 15 min., 4°C). Transfer supernatant (450 ul) to a fresh E-tube. Add 500 ul Isopropanol and invert to mix gently, keep the sample at room temperature for approximately 10 min. Centrifuge at (13,000 RPM / 10 min., 4°C). Remove the supernatant carefully. (Pellet = RNA). Add 1ml., of 75% DEPC EtOH (Ethanol). Centrifuge at (13,000 RPM / 5 Min, at room temperature). Remove the supernatant, and dry completely at room temperature. (Use the pipets to remove the supernatant liquid, and place it in the laminar flow hood for 20 minutes). Dissolve the pellet by adding 30 ul of RNase-Free water, to E-tubes. Make mini centrifugation for a couple of seconds, the final solution. Keep samples refrigerated at -23°C.

2.6. Complementary DNA (cDNA) Synthesis for *A. thaliana* for Drought Experiment and Gene Expression.

Complementary DNA (cDNA) was synthesized with BioFACT™ RT-Kit (M-MLV, RNase H), according to the manufacturer guide, also mentioned by, (Murtaza, 2018), and the lab protocol of Kyungpook National University, (Functional Genomic Lab), a short illustration is in

Figure 7.

Add Oligo-DT (Primer) = 1 ul to each tube. Add the template in each PCR tube (RNA Concentration in volume ul), according to the lecture made in (nano Q, nanodrop machine). Incubate the samples for 5 mins in the Gene Touch PCR system.

Master Mix

- a) 5X RT Buffer = 4 ul x n° samples
- b) 8mM DTT = 1 ul x n° samples
- c) RTase =1 ul x n° samples, were used at the end (sensitive), centrifuged, and keep in ice.

Add 5.5 ul of the master mix, to each PCR tube. Add RNase-free water, according to the amount calculated from the concentration, and centrifuge it. Run the samples in the Gene Touch PCR system, for about 1 hour, and finally keep them in the fridge at -20°C.

2.7. Quantitative Real-Time PCR (qRT-PCR) Analysis, for Drought Experiment and Gene Expression.

Quantitative real-time PCR (qRT-PCR) analysis was performed following the procedures briefly mentioned below. (Murtaza, 2018). To synthesize cDNA, Eco Illumina Real-time PCR System was used, to review plant drought defense, we use “marker genes”, such as *AtDREB1*, *AtDREB2*, Actin, *AtAAO3*, and *AtABA3*, these primers are marker genes pathways, involved in drought stress defense. The marker detail sequence list in **Table 3**, mentioned in PCR (qRT-PCR) was synthesized by using Eco Illumina Real-time PCR System, by adding 1 ul template of cDNA product, and added to each sample qPCR master mix of 10ul, Master Mix including SYBR® Green I BioFACT™, 1 ul of FP (forward primer), and 1 ul of RP (reverse primer), of each gene markers mentioned above, complete with 7ml of RNase free water, to make a final working solution of 20 ul volume, for a (no template control) was filled as a negative control replaced with distilled water in a place of cDNA. PCR reactions were performed in two-step for 40 cycles according to the next conditions: activation of the polymerase at 95°C for 15 minutes, denaturation at 95°C for 15 seconds, and annealing in an

extension of 60°C for 30 seconds. The melting was assessed at 60-95°C for amplicon specificity verification for each primer pair, actin gene was used as a reference.

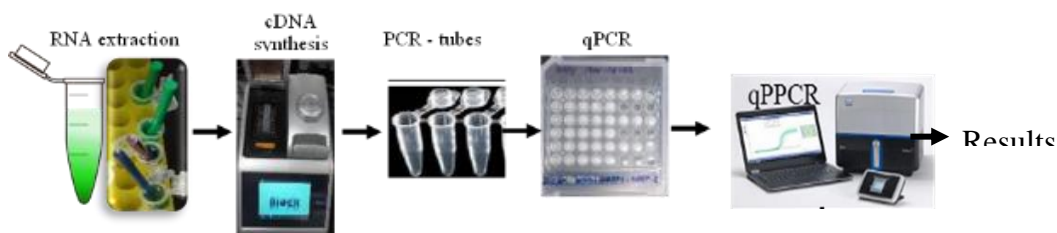
Table 3

List of Gene Primer Markers

No.	Name	Forward primer (5'-3')	Reverse primer (5'-3')
1	<i>AtAAO3</i>	TGGTAGAGGAGGTCTCGGAA	GGTTCACCCACTGGATGGTA
2	<i>AtABA3</i>	CGTCGTCAGTGGAAGGTTTC	AATTCACCCGGTCAGACCCT
3	<i>AtDREB1</i>	GACTCATTGCCTCGCCCA	TTTCACCCAGTTCCTCCG
4	<i>AtDREB2</i>	TGCATTCTGCTGCCTACGATGC	TCGGCATTGTCCAACCTGGTAT
5	<i>Actin</i>	GCTGGACGTGACCTTACTGA	CCATCTCCTGCTCGTAGTCA

Figure 7

Schematic View of cDNA and qPCR Materials and Methods for Genotyping



2.8. Electrolyte Leakage (EL) Measurement for Cell Death Quantification and Chlorophyll Content.

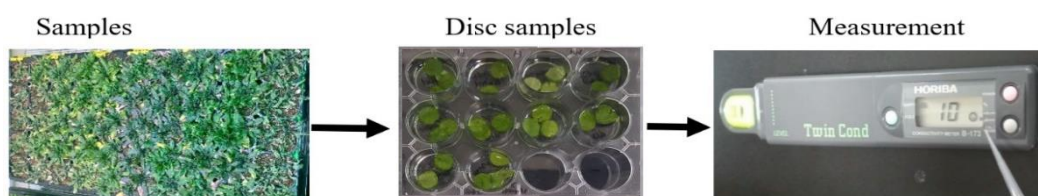
Electrolyte leakage (EL) is an “ion” that was measured as reported by (Murtaza, 2018), and viewed in **Figure 8**. Samples (leaves) were taken from plants under drought stress, for control and experimental groups, at 0 and 5 days, after drought treatment, the collection of samples was by collecting discs from leaves of 1 cm diameter by three repetitions from different plants and leaves, to remove any external material in leaf discs, after that was washed and rinsed with deionized (DI) water. The disc samples were transferred to a glass tube containing 5 ml (DI) water. Sample tubes were gently shaken at room temperature, and kept in continuous shaking for 24 hours, for the final value of electrolyte leakage, was measured from each sample by using “HURIBA Twin Cond B-173, Japan” (portable conductivity meter), called electrolyte leakage 1 (EL1), after that

the samples were autoclaved at 120°C for 20 minutes, and kept at room temperature to cooled down. Each sample was measured again (EL2). The EL % was measured according to the next equation.

$$EL(\%) = \left(\frac{EL1}{EL2} \right) \times 100$$

Figure 8

Electrolyte Leakage Measuring



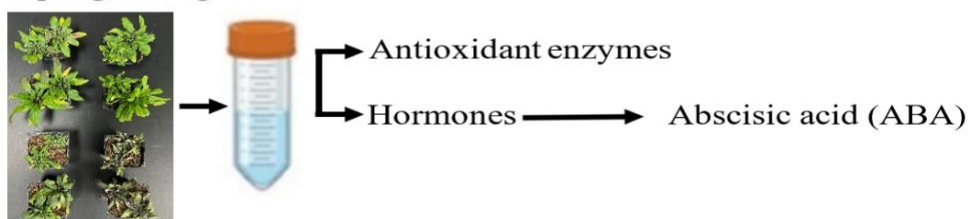
2.9. Chemical Analysis for ABA Content and Other Hormones.

This parameter analysis was made by following the method of (Murtaza, 2018), and illustrated in **Figure 9**. To analyze this parameter, the samples were collected, from all treatments and control groups, by taking a whole plant (3 or 4 plants) from each group and freezing immediately and subsequence dried to grind until getting a very fine powder. Store in a falcon tube under freezing conditions of -80°C, the extraction was made from this powder by chromatography standardized for ABA analysis. Therefore, methyl diazomethane was applied to the sample portion and finally, GCMS (6890N network gas chromatograph) was used for the measurement and identification of ABA.

Figure 9

Chemical Analysis

Sample grinding



2.10. Chlorophyll Content Measurement (SPAD) in Plants Under Drought Stress Experiment.

During the drought effects, several metabolic alterations occur in the normal functionality of the plant, the photosynthetic pigment absorbs light as a source of energy, and transfers to a photosynthetic laboratory center inside the plant, due to the stress conditions, the photosynthetic reaction in the leaves was analyzed. To measure the chlorophyll content Chorofylmeter SPAD (Soil Plant Analysis Development) was used. This parameter was analyzed on intact leaves during the drought stress and after the recovery of experimental groups, (Murtaza, 2018). For chlorophyll a and b determination, 0.1 g leaf tissue is ground into a powder with liquid nitrogen, and then homogenized with 1 ml 100% dimethylformamide DMF. Centrifuge homogenized samples at 10,000 rpm for 10 min, and then gather the supernatant, and measure the optical density (OD) of the supernatant at 664 nm for chlorophyll and 647 nm for b chlorophyll respectively, (Abdelaal et al., 2020).

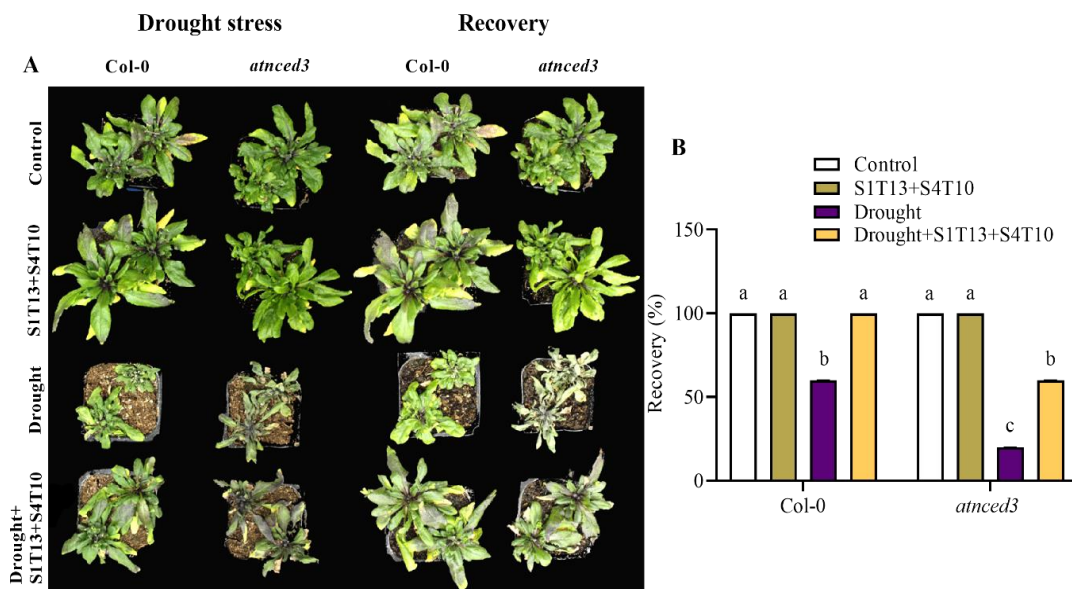
3. RESULTS

3.1. Recovery Phase for Drought Stressed Plants, Under Drought Effect and PGPRs Inoculation

After drought stress induced plants, by water deficit conditions, it was evaluated for five days, and irrigated with water to measure drought parameters. Plants were taken pictures at the beginning just as drought **Figure 10 A**, after the first evaluation to review the optimal recovery of the plants was monitored and pictured after 24 hours, **Figure 10 B**, the recovery phase in the experimental group shows for Col-0 (drought+S1T13+S4T10) 100% of recovery and (drought) 60%, in case of *Atnced3* (drought+S1T13+S4T10) 60% of recovery and (drought) 20%. The control group does not have any phenotypical changes showing the 100% of vitality.

Figure 10

Drought Recovery Appearance in Genotypes of A. thaliana



Note: (A and B), Phenotypic evaluation and recovery percentage

3.2. Chlorophyll Content

The results for chlorophyll content were measured with SPAD **Figure 11 A** and correlated with the measurement of chlorophyll "*a* and *b*" **Figure 11 B** and **C** by quantification in (mg/g FW), by statistical analysis of ANOVA one way, set for Col-0 and *Atnced3*.

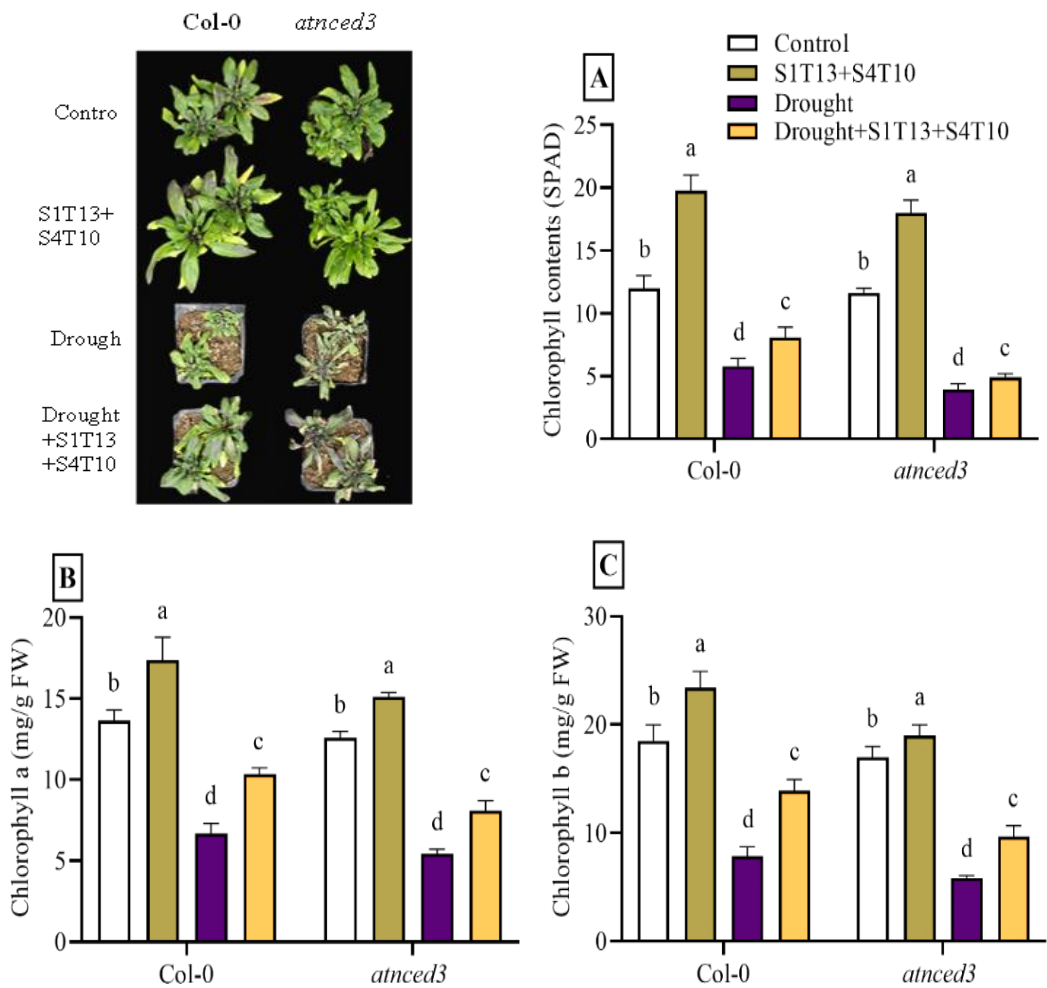
For Col-0, chlorophyll content shows a significant difference for SPAD and was correlated with (a) S1T13+S4T10 of 19.7%, followed by (b) control of 12%. The experimental group has a difference of (c) S1T13+S4T10+drought with 7.5%, followed by (d) drought with 5%. In general, the photosynthetic functions in plants were improved by the effect of PGRRs. For *Atnced3* genotype shows a significant difference in chlorophyll content, for (a) S1T13+S4T10 is 19%, followed by (b) drought at 11.5%, in the experimental group, the difference of (c) drought+S1T13+S4T10 with 5% is followed by (d) drought with 4%. Some limitations of deficiency are for the mutant genotype deficient in ABA (*ATNCED3* pathway gene), which influences a decrease in the photosynthetic functions of plants. Regarding the ratio of chlorophyll, *a* and *b* in **Figure 11 B, C**, the concentration in *a* is lower than chlorophyll *b*.

For the Col-0 genotype, chlorophyll "*a* and *b*" in the control group, (a) S1T13+S4T10 and (b) control, show a favorable increasing content of both types in the case of the experimental group, in the Col-0 genotype the chlorophyll content in *a* and *b*, by (c) drought+S1T13+S4T10, shows a significant difference in chlorophyll content for both types "*a* and *b*", compared to plants subjected to drought conditions (d), without any inoculation of PGRRs. For the *Atnced3* genotype, chlorophyll "*a* and *b*" in the control groups, (a) S1T13+S4T10 and (b) control, shows a favorable increase in the content of both types "*a* and *b*". In the case of the experimental group, in the *Atnced3* genotype by S1T13+S4T10+drought (c), it shows a significant difference in chlorophyll content for both types "*a* and *b*", compared to plants subjected to (d) drought, without any application of PGRRs. Under a similar condition due to the absence of abscisic acid (ABA), the role

of S1T13+S4T10 is significant to increase the photosynthates. ABA could show higher efficiency on the stomatal activity, in contrast, the plants with only water, the chlorophyll content is much lower.

Figure 11

Chlorophyll Content



Note: (A) Chlorophyll content in (SPAD), and (B) and (C) Chlorophyll type “a and b”

3.3. Antioxidative Capacity by Electrolyte Leakage, Hydrogen Peroxide (H₂O₂), and Superoxide Anion (-O₂)

The electrolyte leakage signal in **Figure 12 A**, shows for Col-0 for (a) drought is 80 uS/cm² and (b) drought+S1T13+S4T10 is 55 uS/cm². In the case of *Atnced3*, (a) drought is 95 uS/cm², (b) drought+S1T13+S4T10 is 70 uS/cm², and (c) is on normal conditions. Under drought conditions without the effect of PGPRs, shows the highest amount of electrolyte leakage. In the case of the effect of S1T13+S4T10 on plants, the level of antioxidants was increased trying to resist the reactive oxygen species (ROS) effect, and the bacteria are the main reason the cells have a better response to control the K⁺ Efflux.

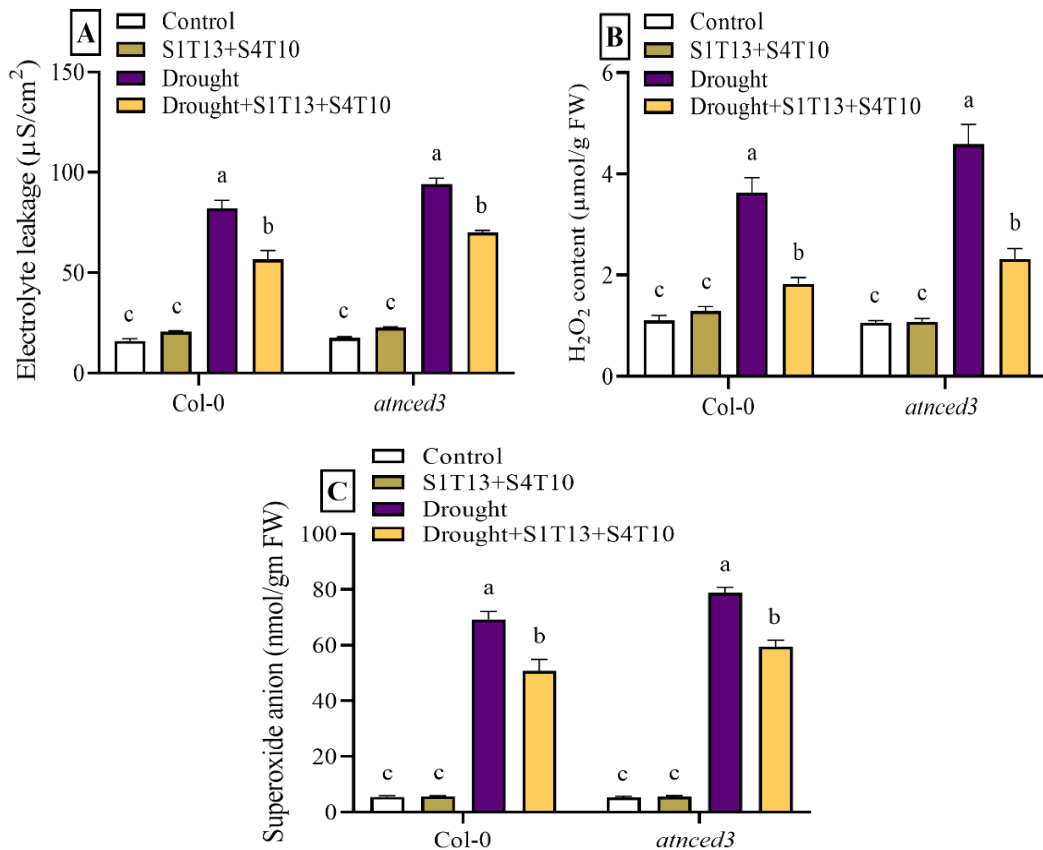
Hydrogen Peroxide H₂O₂ levels, in high quantities, can deactivate enzymes and photosystems by oxidizing proteins, lipids, and nucleic acids. Our experiment was reported in **Figure 12 B**. As a result, the experimental group of Col-0 (a) drought is 3.8 μ mol/g FW compared with (b) drought+S1T13+S4T10 is 1.9 μ mol/g FW. In *Atnced3*, (a) drought is 4.5 μ mol/g FW compared with (b) drought+S1T13+S4T10 is 2.5 μ mol/g FW, the control group of (c) for both genotypes does not have any significant changes. The excessive production of ROS under drought stress is clear, also the ABA deficiency in this mutant, plays a role in the H₂O₂ overproduction, compared with the plants by dual application of S1T13+S4T10, diminishing the oxidative damage of the cells in plants, and maintaining H₂O₂ at low levels.

In addition, the results for superoxide anion (-O₂), are shown in **Figure 12 C**, and have a significant difference for Col-0, for the experimental group (a) drought is 68 nmol/gm FW, and (b) drought+S1T13+S4T10 is 50 nmol/gm FW. In the case of *Atnced3* (a) drought is 78 nmol/gm FW, and (b) drought+S1T13+S4T10 is 58 nmol/gm FW. The levels in the control group under normal conditions (-O₂) for both genotypes are in normal conditions without any apparent changes (c). The production of (-O₂) was

reduced in (b) by the effect of the dual application of S1T13+S4T10, this bacterium plays an important role by increasing the antioxidant levels that control the stress in plants.

Figure 12

Electrolyte Leakage, H₂O₂ and -O₂ Content



Note: (A) Electrolyte leakage, (B) hydrogen peroxide, and (C) superoxide anion

3.4. Enzymatic Scavenger Antioxidants System of Superoxide Dismutase (SOD), Peroxidase (POD), Glutathione, and Polyphenol oxidase

The first line of defense and the most effective antioxidant enzyme is, Superoxide dismutase (SOD) **Figure 13 A**, in the experimental group, shows more significance.

In the case of Col-0, (a) drought+S1T13+S4T10, is 70 U/mg protein, the high amount is because the wild-type genotype can produce ABA faster in this group of this stress hormone to fight against the drought, (b) drought is 40 U/mg protein. The next high concentration is for *Atnced3* mutant, (a) drought+ S1T13+S4T10 is 56 U/mg protein, and (b) drought is 27 U/mg protein, even though the PGPRs play a clear role in antioxidant production by improving the PGPRs the production of ABA in the genotype mutant. The levels in the control group under normal conditions are the same for both genotypes and without any enzymatic releasing (c).

To compare the following experimental group under drought conditions without PGPRs effect (b), the concentration of Superoxide dismutase (SOD) was low, but in (a), the effect is clear in antioxidant production, to control the accumulation of ROS effects in plants.

A tiny intracellular molecule but potent nonenzymatic antioxidant is known as glutathione, **Figure 13 B**. The experimental groups of Col-0, (a) drought+S1T13+S4T10, show a concentration of 40 U/mg protein, the high amount is because of the wild type genotype, antioxidants production is high, in case of (b) drought is 28 U/mg protein. The following genotype of *Atnced3* (a) drought+S1T13+S4T10 is 25 U/mg protein, and (b) drought is 18 U/mg protein. The control group under normal conditions does not have significance between (c) and (d). The PGPRs play a specific role in antioxidant production, even under ABA deficiency of *Atnced3*, but still is lower than Col-0, the bacterium helps to produce antioxidants such as glutathione, and reduce the oxidative effects.

Peroxidases **Figure 13 C**, actively oxidize reactive oxygen species (ROS). The experimental groups of Col-0, (a) drought+S1T13+S4T10, have a concentration of 70 U/mg protein. The high amount is because in the wild-type genotype the production of the antioxidants, in case of (b) drought is 40 U/mg protein. For the *Atnced3* (a) drought+S1T13+S4T10 has a concentration of 50 U/mg protein, and for (b) drought is 38

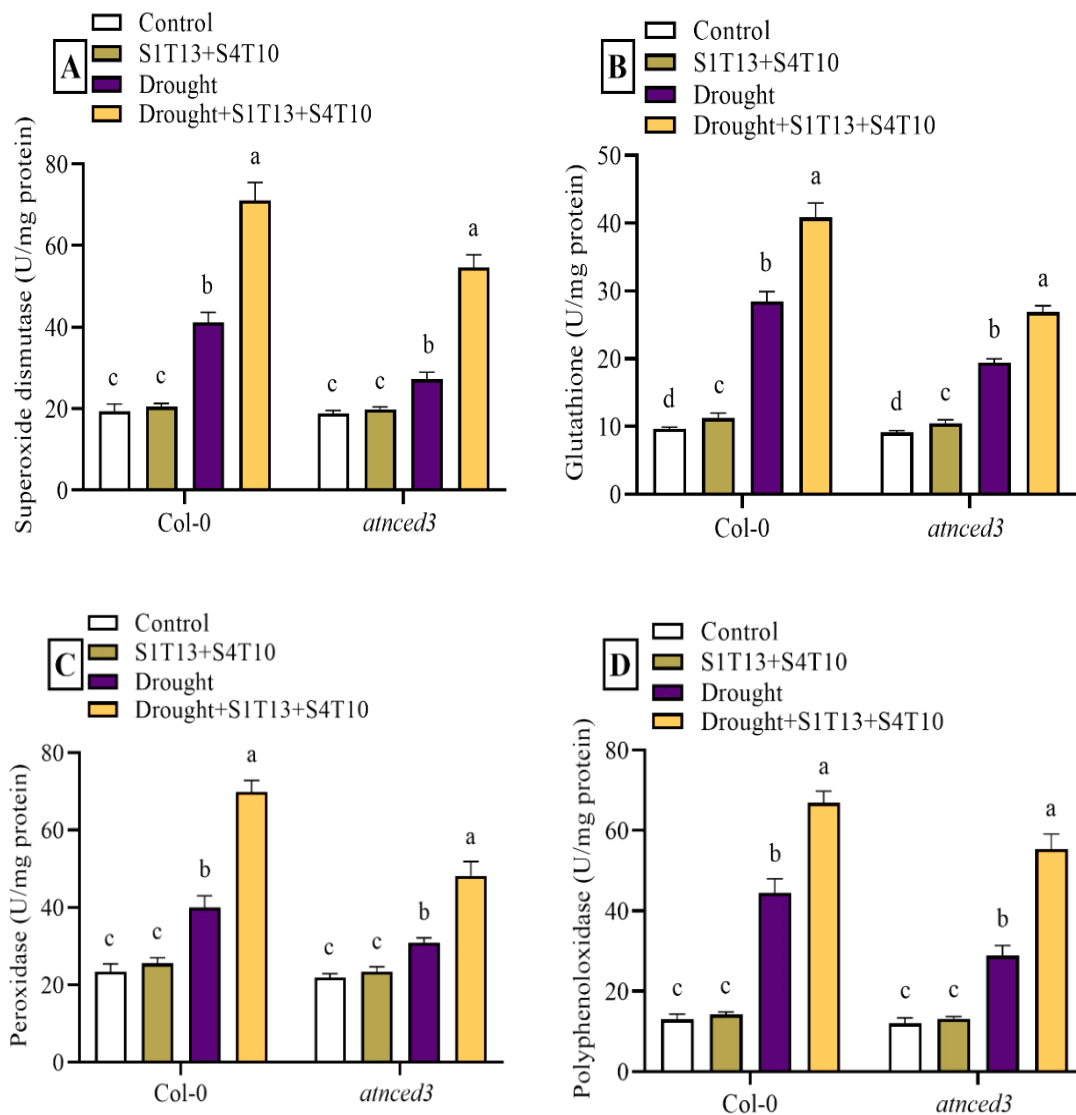
U/mg protein. The control group in normal conditions does not have significance between their water and bacterial inoculation (c).

The dual application of PGPRs plays a specific role to oxidize the oxidative compounds and maintain antioxidant production under stress conditions.

Polyphenol oxidase substances serve a variety of purposes and are crucial for symbiosis between plants and PGPRs, as shown in **Figure 13 D**, in the experimental group of Col-0, (a) drought+S1T13+S4T10, shows a concentration of 65 U/mg protein, and (b) drought 42 U/mg protein. In the case of *Atnced3* (a), drought+SIT13+S4T10 is 55 U/mg protein, and (b) drought is 30 U/mg protein. The control group under normal conditions does not have significant changes between them, (c). The PGPRs play a specific role in microbial symbiosis to protect the plant from external factors and improve the IAA transfer into the plant root. In the case of *Atnced3*, the symbiosis in this genotype is strong with S1T13+S4T10, fundamental antioxidant machinery functions.

Figure 13

Antioxidant System



Note Antioxidants (A, B, C, and D) superoxide dismutase, glutathione, peroxidase, and polyphenol oxidase.

3.5. Molecular Characterization of *AtAO3* and *AtABA3*, and ABA Content to Induced Gene Expression Under Drought Stress

The molecular characterization ABA pathway *AtBRED* of *A. thaliana* in Col-0 and *Atnced3* genotypes under dual application of PGPR were analyzed in this experiment.

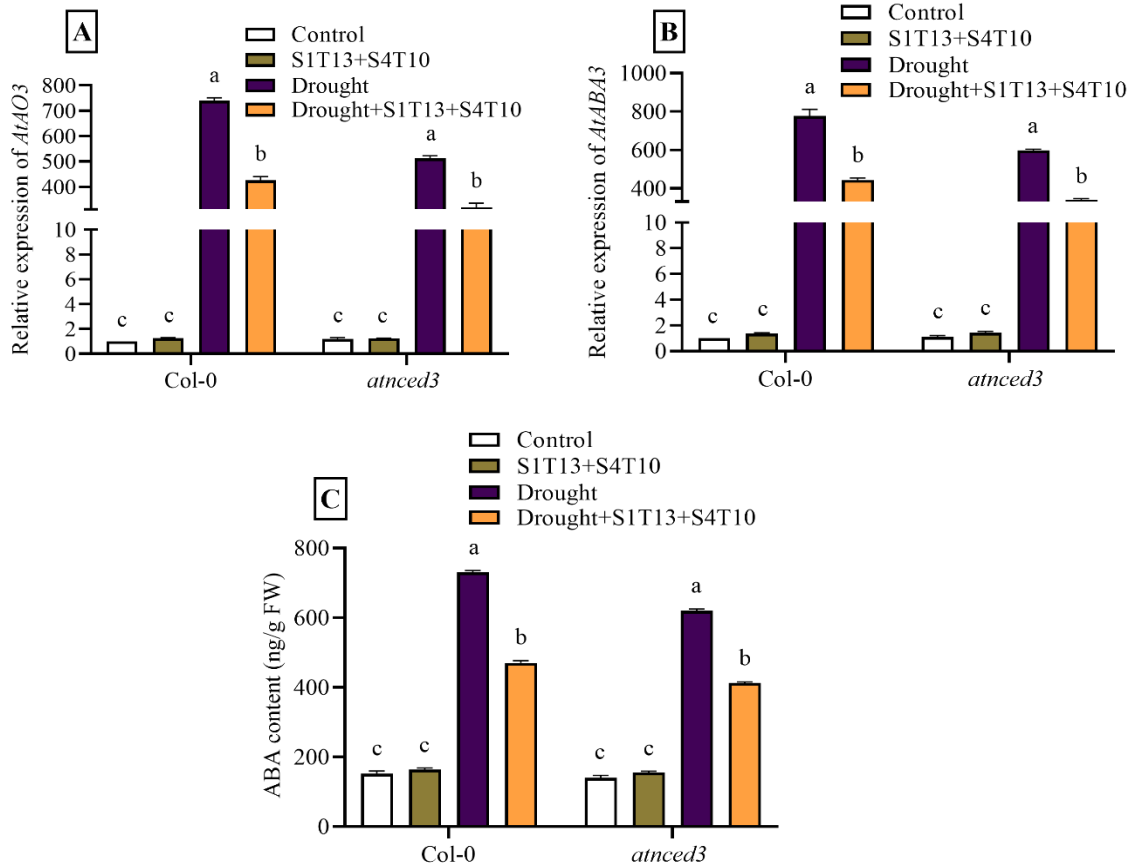
Protein coding genes for *AtAO3* **Figure 14 A**, were expressed differentially in the genotypes under study, for Col-0, under (a) drought without any bacterial inoculation, has a higher *AtAO3* expression, followed by (b) drought+S1T13+S4T10. In the case of *Atnced3* under (a) drought, without any bacterial inoculation, has a higher expression, followed by (b) drought+S1T13 +S4T10. In the case of (c) control for both genotypes, the *AO* gene pathway was not expressed. The PGPRs help to diminish gene overexpression and make less ABA gene pathway expression, and it is a positive effect.

The *AtABA3* pathway expression in **Figure 14 B**, the experimental groups of Col-0, under (a) drought, this treatment is higher compared with (b) drought+S1T13+S4T10, the expression is lower than the previous one. For *Atnced3*, under (a) drought, without any application of PGPRs is high, compared to (b) drought+S1T13+S4T10. The control group has not any ABA gene expression in both genotypes. The ABA-related genes were improved in (b) for both genotypes, by maintaining the stress hormone in optimal conditions, the overexpression shows higher stress, and the effect of S1T13+S4T10 plays a key role to control the gene overexpression, as a positive effect.

For abscisic acid (ABA) hormone, **Figure 14 C**, in the experimental group under (a) drought for Col-0 is high at 700 ng/g FW, followed by (b) drought+S1T13+S4T10, is 450 ng/g FW. *Atnced3* (a) drought is 600 ng/g FW, (b) drought+S1T13+S4T10, is 400 ng/g FW. In contrast, in plants without the application of rhizobacteria, (c) there is no significant hormone production. In case (a) both genotypes were overproduced and the plants are unable to control the stomatal closing, but in (b) this function was improved by maintaining the stomas closed and keeping the photosynthetic activity, even under stress conditions, helped by the dual application of S1T13+S4T10.

Figure 14

Relative Expression of *AtAO3*, *AtABA3* and ABA Content



Note: Relative expression of *AtAO3*, *AtABA3* (A and B), and ABA synthesis (C)

3.6. Molecular Characterization of *AtDREB2A* and *AtDREB2B*, Expression Induced by Drought Stress

The molecular characterization of drought-related element bindings *DREB*, of *A. thaliana* in Col-0 and *Atnced3* genotypes under PGPRs application under drought stress.

The results for *AtDREB2A* **Figure 15 A**, were expressed differentially in the genotypes under study. In the experimental group, the marker gene that expresses a high

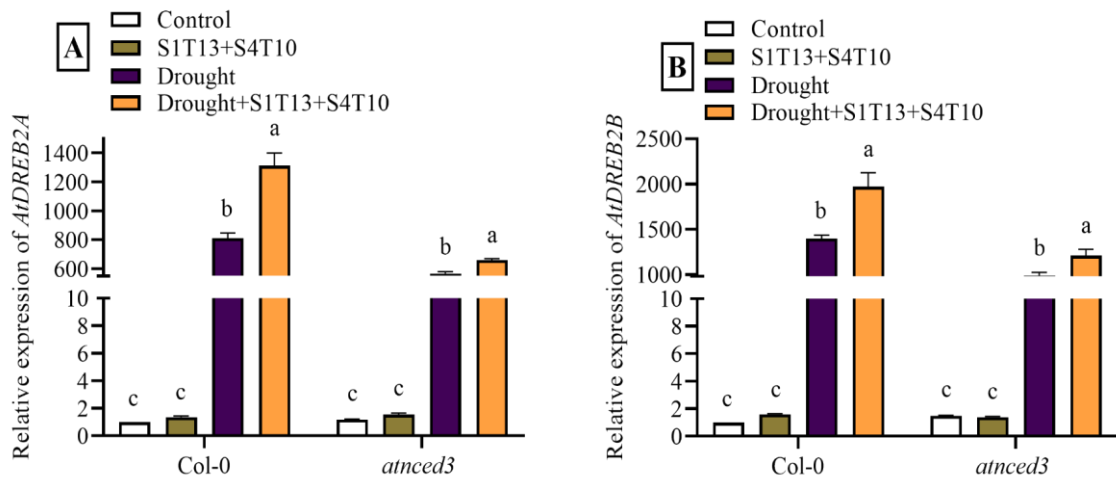
transcript accumulation was for Col-0 (a) drought+S1T13+S4T10, followed by (b) drought without any bacterial inoculation. In the case of *Atnced3*, the treatment of (a) drought+S1T13 +S4T10, is slightly superior, followed by (b) drought. The control group in both genotypes does not show any gene expression with the related marker.

The results for *AtDREB2B* expression **Figure 15 B**, for the experimental group, shows differential expression in Col-0 (a) drought+S1T13+S4T10, followed by (b) drought. The following group is for *Atnced3*, (a) drought+S1T13+S4T10, which is slightly higher than (b) drought. According to the evaluation in the control group does not appear any gene significant expression with the related marker.

To correlate the DREB family drought-related genes, both A and B, are directly related to drought stress, and the dual application of S1T13+S4T10, helps to reduce the overexpression of these related genes, which is a clear effect on (a) of both gene markers, and for *atnced3* the *ATNCED3* pathway gene is directly involved in the drought response and the expression is not too high compared to (b).

Figure 15

Relative Molecular Characterization of AtDREB2A and AtDREB2B



Note: Relative expression of AtDREB2A and AtDREB2A

4. DISCUSSION

Drought is the main climatological anomaly that limits the availability of water and is considered an increasingly frequent and widespread natural event throughout the world, (Mpelasoka et al., 2018). The biodiversity of crops in Peruvian territory has a medium risk vulnerability in the world for drought effect (MINAM, 2016) due to direct dependence on water coming from the Andean glaciers and wet aquifers, which dramatically diminished in the last years, (Lasage et al., 2015)

The importance of research based on abiotic stress, for Peru, is important because agriculture represents about 96% of family farming, weighing 82% being produced primarily internal market, and 14% for exportation, the rest 4% is for companies inside the country, (Marlene, 2021), (Libelula, 2011).

To deal with problems of water scarcity, plants have mechanisms that protect them from abiotic stress and start to activate a multiple drought tolerance system, that starts to work in different ways as combined or isolated actions, and one of the consequences is an increase in the production of reactive oxygen species (ROS), and hydrogen peroxide (H_2O_2), superoxide anion ($-O_2$), (Demidchik et al., 2014), that happen in the chlorophyll system, however, antioxidants keep in advance under close control the ROS levels. (Kaur & Asthir, 2017)

Under stress conditions, electrolyte leakage by detected, and K^+ efflux is one of the signals and the main cause for this reaction, frequently under stress, all these reactions are interconnected. (Demidchik et al., 2014), Additionally, some hormones like ethylene (ET) and abscisic acid (ABA), are the defense signal and are switched on, which implicates the signaling of H_2O_2 , tied with abscisic acid (ABA), (Huang et al., 2018).

To discuss the complex of drought tolerance, we have to understand that several trait pathways are implied, under stressed conditions. The results in our experiment were applied in a model plant of *A. thaliana*, genotypes Col-0 wild type with full genome sequence and *Atnced3* mutant Type deficient in ABA, to compare the effect on gene

expression, endogenous phytohormones, and antioxidants, to improve drought stress tolerance, induced by PGPRs *Brevundimonas vesicularis* strain S1T13 and *Pseudomonas koreensis* strain S4T10, this was applied by combining both strains and inoculated in the model plant according to **Table 1**.

4.1. Recovery for Drought-Stressed Plants, Under Drought Effect

One of the most crucial characteristics of plants is their ability to recover from drought stress, after 24 hours, the recovery for the experimental group shows 100% recovery for Col-0 (drought+S1T13+S4T10), and drought was 60% of the recovery, compared to *Atnced3*, shows a 60% of the recovery for (drought+S1T13+S4T10) and drought was 20% of the recovery.

4.2. Chlorophyll SPAD contents in leaves of *A. thaliana*

One of the most vital biological processes on Earth is photosynthesis. It modifies the planet's atmosphere and supplies the consumable energy that is necessary for practically all living things., (P. Wang et al., 2017).

Under drought stress, the contents of chlorophyll are affected clearly and a clear result is the efficiency of photosynthesis to examine how drought conditions affect the chlorophyll content of the plants.

The results for chlorophyll content, for Col-0 in SPAD, show a significant difference for the experimental group of (c) drought+S1T13+S4T10 with 7.5%, followed by (d) with 5%. The results for Chlorophyll content, of the *Atnced3*, show a small difference for (c) drought+S1T13+S4T10with 5%, followed by (d) drought with 4%, the effect on (a) for both genotypes was improved significantly even without any stress effect, in case of chlorophyll *a* and *b* the correlations of behavior were same as a previous explanation and is showed in **Figure 11**.

4.3. Electrolyte Leakage, Hydrogen Peroxide (H₂O₂) and Superoxide Anion (-O₂)

Reactive oxygen species (ROS) are produced by normal aerobic metabolism in plants, in several locations as a photosynthetic and respiratory system, as hydrogen peroxide (H₂O₂) and superoxide anions (-O₂), (Anjum et al., 2022).

Electrolyte leakage under drought stress is illustrated in **Figure 12 A**, For Col-0, (a) drought is 80 uS/cm², followed by (b) drought+S1T13+S4T10 is 55 uS/cm², the value for (b) was moderate the K⁺ efflux for this genotype. For *Atnced3*, (a) drought is 95 uS/cm², followed by (b) drought+S1T13+S4T10 is 70 uS/cm², also in (b), the K⁺ efflux was moderate, in (a) the leakage is very high that shows more stress. To discuss between Col-0 and *Atnced3*, the higher electrolyte leakage in *Atnced3* is ABA pathway-related gene, *AtNCED3*-deficient mutant, which has a less antioxidant defense, and the chlorophyll does not work properly.

H₂O₂ levels under drought stress are reported in **Figure 12 B**. For Col-0, (a) drought, is 3.8 μ mol/g FW, followed by (b) drought+S1T13+S4T10 by 1.9 μ mol/g FW. For *Atnced3*, (a) drought is 4.3 μ mol/g FW, followed by (b) drought+S1T13+S4T10 by 2.3 μ mol/g FW. At high quantities, H₂O₂ can deactivate enzymes and photosystems by oxidizing proteins, lipids, and nucleic acids. The stress of drought causes oxidative damage to plant cells, which signal it is possible that the decline in photosynthesis activity.

In addition, superoxide anion (-O₂), illustrated in **Figure 12 C**, has a significant difference for Col-0, (a) drought, is 68 μ mol/g FW, followed by (b) drought+S1T13+S4T10 by 49 μ mol/g FW. For *Atnced3*, (a) drought is 78 μ mol/g FW, followed by (b) drought+S1T13+S4T10 by 58 μ mol/g FW. In this case, bacterial inoculation plays an important role by increasing the antioxidant levels that control stress in plants. The levels in the control group under normal conditions (H₂O₂ and -O₂) for both genotype plants, are in normal conditions without any apparent changes in the gene expression.

4.4. Antioxidants System of Superoxide Dismutase, Peroxidase, Glutathione, and Polyphenol oxidase

In a stress condition, the first and most effective antioxidant enzyme is, superoxide dismutase (SOD), which is illustrated in **Figure 13 A**, in Col-0, (a) drought+S1T13+S4T10 is 70 U/mg protein, followed by (b) drought, at 40 U/mg protein. For *Atnced3*, (a) drought+S1T13+S4T10 is 56 U/mg protein, followed by (b) drought, 40 U/mg protein. The levels in the control group under normal conditions (c) are the same for both genotypes without any abnormal enzymatic production.

Glutathione is a small and potent intracellular antioxidant molecule, shown in **Figure 13 B**, this molecule for Col-0, (a) drought+S1T13+S4T10, has 40 U/mg protein, followed by (b) drought, 28 U/mg protein. For *Atnced3*, (a) drought+S1T13+S4T10 is 25 U/mg protein, followed by (b) drought, 18 U/mg protein. The control group under normal conditions does not have a significance between their water and bacterial application.

The enzyme of peroxidases actively oxidize (ROS), is illustrated in **Figure 13 C**, for Col-0, (a) drought+S1T13+S4T10 has 70 U/mg protein, followed by (b) drought, 40 U/mg protein. For *Atnced3*, (a) drought+S1T13+S4T10 is 50 U/mg protein, followed by (b) drought, 38 U/mg protein. The control group under normal conditions does not have significance between them by water and bacterial application.

In a Symbiosis environment, polyphenol oxidase substances play an important role illustrated in **Figure 13 D**, for Col-0, (a) drought+S1T13+S4T10 is 65 U/mg protein, followed by (b) drought, 42 U/mg protein. For *Atnced3*, (a) S1T13+S4T10+drought is 55 U/mg protein, followed by (b) drought, 30 U/mg protein. The control group under normal conditions does not have significance.

4.5. Characterization of *AtAO3* and *AtABA3*, and ABA Content

For molecular characterization under drought stress, the role of PGPRs is important, the gene markers result for *AtAO3* in **Figure 14 A**, for Col-0, (a) drought, has

higher expression, followed by (b) drought+S1T13+S4T10. For *Atnced3*, (a) drought, has a higher expression, followed by (b) drought+S1T13+S4T10. These results clearly show the role of PGPRs to help the plant maintain the overexpressed *AtAO3* at the lower expression in both genotypes compared to the (b) drought where the stress is more severe and expressed by the ABA stress hormone. The control group under normal conditions does not have significance between them under water and bacterial inoculation.

For molecular characterization of *AtABA3* expression shows a high accumulation as shown in **Figure 14 B**, for Col-0, (a) drought, has higher expression, followed by (b) drought+S1T13+S4T10. For *Atnced3*, (a) drought has a higher expression, followed by (b) drought+S1T13+S4T10. This result clearly shows the role of the dual application of PGPRs help to maintain the expression levels at optimal conditions under drought stress reducing the overexpress *AtAO3* in both genotypes compared to drought-stressed plants. The control group under normal conditions does not have significance, which means healthy plants.

For abscisic acid (ABA) hormone, **Figure 14 C**, the concentration in the experimental group, for Col-0 (a) drought is 700 ng/g FW, followed by (b) drought+S1T13+S4T10, 450 ng/g FW. Compared with *Atnced3* (a) drought is 600 ng/g FW, followed by (b) drought+S1T13+S4T10, which is 400 ng/g FW. The control group under normal conditions does not have significance between them under water and bacterial application.

ABA hormone pathway, plays an important role in the stomatal opening and closing, in stressed plants this function is blocked and overproduce ABA, in plants with PGPR application the ABA maintains the ability of stomatal control, by signaling the closure in stress conditions to reduce the evaporation and dehydration of the plants.

4.6. Characterization of *AtDREB2A* and *AtDREB2B*

The molecular characterization of drought-related element bindings *BREB* family genes, in plants exposed to drought, the results for *AtDREB2A* is illustrated in **Figure 15 A**, for Col-0, (a) drought+S1T13+S4T10 has high gene expression, followed by (b) drought. In the case of *Atnced3*, (a) drought+S1T13+S4T10 is slightly different compared to (b) drought. The control group in both genotypes does not show any gene expression with the related marker.

In Col-0, the *AtDREB2B* gene marker, **Figure 15 B**, (a) drought+S1T13+S4T10, has high gene expression, followed by (b) only drought. In the case of *Atnced3*, (a) drought+S1T13+S4T10 is slightly different compared to (b) drought, and according to the evaluation in the control groups, it does not appear significant gene expression with the related marker.

The DREB genes are activated once the drought effect is signaling into the cell plants, by the oxidative accumulation, and ABA hormone production, to activate the antioxidant system and photosynthetic activity in optimal conditions

5. CONCLUSION

According to the results, this study was focused on the effect of PGPRs by dual application of *B. vesicularis* strain S1T13, and *P. koreensis* strain S4T10, to know their influence on *Arabidopsis thaliana* plants of genotypes Col-0 wild type and *Atnced3*, ABA pathway-related gene, *AtNCED3*-deficient mutant, by evaluating the percentage of recovery, chlorophyll content, electrolyte leakage, and oxidative compounds, antioxidants, ABA related genes, and *DREB* drought-related gene expression. By understanding the mechanisms of the abiotic stress effect, we decide and use the capacity of S1T13 and S4T10, to reduce that effect. The first parameter measured was recovery percentage, and after 24 hours in Col-0 was 100% followed by *atnced3* at 60% compared to drought the recovery was very low. The chlorophyll content was higher in the experimental group with a dual application, of drought+S1T13+S4T10 in Col-0 and followed by *atnced3*, determined with SPAD, and it was also contrasted with the photosynthetic pigments of chlorophyll *a* and *b*, showing healthiness and improve the phenotypic characteristics in plants mentioned before, but lower than group control. The damage caused by reactive oxygen species (ROS) such as hydrogen peroxide H₂O₂ and superoxide anion -O₂, in treatments with drought+S1T13+S4T10, the effect in Col-0 and *atnced3* has reduced positively the levels of electrolyte leakage by controlling the K⁺ efflux, and the defense system was helped by the action of the PGPRs, in comparison with plants under drought the ROS activity was very high. Antioxidant levels were increased by the effect of accumulation of (ROS) activity, the immediate response to remove this oxidative accumulation was activated by the defense system in plants in the experimental group, in Col-0 and *atnced3* by the treatment of drought+S1T13+S4T10 by releasing compounds of superoxide dismutase to catalyze the -O₂ in H₂O and O₂, peroxidase catalyzes the H₂O₂ in H₂O, glutathione protects the membrane and prevents oxidative damage, and polyphenol oxidase, which actively increased their antioxidant capacity system, releasing enzymes that control oxidation damage and symbiosis between

the roots and (PGPRs), in both genotypes was high, but in the other hand, the plants under the drought treatment the antioxidant system was low. The protein-coding genes related to droughts such as *AtAO3*, *AtABA3*, and *ABA*, had a lower expression because these genes are related to ABA hormone production, which is a stress hormone signaling on plant cells, which controls the stomatal activity, and the S1T13+S4T10 helps in that function, in the experimental group of Col-0 and *atnced3* has reduced the overexpression of ABA related genes, that maintain the healthiness of the plants. In plans exposed to only drought, the expression was higher, and ABA-related genes were overexpressed, making them unable to control the stomatal closing functions and the drought effect was severe. ABA pathway-related gene, *AtNCED3*-deficient mutant, still produces the *AtNCED3* gene induced by the PGPR effects. A clear effect on the expression levels of drought-related element bindings genes of *AtDREB2A* and *AtDREB2B* was overexpressed in the experimental group, under-treatment of drought+S1T13+S4T10 of Col-0, and *atnced3* activating the antioxidant system, to reduce drought effects, compared with plants under drought. In the case of *atnced3*, the expression was lower compared with only drought because the ABA pathway-related gene, *AtNCED3*-deficient mutant, the stomatal functions are lower compared with Col-0 wild type.

We conclude the PGPRs of S1T13 and S4T10, applied in this study, have a potential effect on the recovery after a drought effect was positive, the chlorophyll content was improved by maintaining the photosynthetic functions in a drought process, the reactive oxygen species (ROS) was rapidly kept under relative control by the effect of PGPRs, by activating the antioxidants system synthesizing the enzymes to protect the plants. The functional genomics of ABA-related genes of *AtAO3* and *AtABA3* was playing a role in the stomatal functions, to control the effect and diminish the damage by drought, drought-related genes such as *AtDREB2A* and *AtDREB2B* was increased, activating the defense system in a stress event, by maintaining the physiologic and biochemical functions properly.

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애기장대(*Arabidopsis thaliana*) 에 *Brevundimonas vesicularis* 와
Pseudomonas koreensis 의 동시 처리와 유전자 발현, 식물 호르몬 합성 및
항산화 시스템의 조절을 통한 가뭄 스트레스 내성 향상

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(초 록)

기후 변화는 전세계적으로 가뭄(건조)현상을 증가시키고 있으며 주로 농업 생산을 유지하는 데 필요한 물의 가용성에 영향을 미칩니다. 본 연구는 식물 성장 촉진자 뿌리박테리아(PGPR), *Brevundimonas vesicularis* 균주 S1T13 과 *Pseudomonas koreensis* 균주 S4T10 를 *A. thaliana*(애기장대), Col-0 및 *Atnced3* (ABA 결핍)돌연변이에 처리하고 이들의 건조 내성 조절에서의 역할을 조사하였다. 건조 관련 유전자(*AtDREB1*, *AtDREB2*, *AtA03*, *AtABA3*, ABA)의 발현 및 엽록소 함량, 전해질 누출, 활성산소종(ROS) 조절, 항산화 시스템 조절 및 표현형적 특성 및 복구에 대한 능력을 건조 스트레스 처리 후 확인하였다. Col-0 및 *atnced3*에 대한 S1T13+S4T10 의 복합 처리 할 경우 건조 처리 후 식물의 회복은 PGPR 처리 효과로 생리적 기능이 회복되는 것을 확인하였다. 총 엽록소 함량은 SPAD 로 측정되었으며 엽록소 a 및 b 의 광합성 색소와 대조되어 S1T13+S4T10 을 접종한 식물에서 건강한 상태를 보였으며 건조 조건에서 엽록소의 최적 수준을 유지하고 광합성 능력을 증가시켜 결과적으로 식물의 표현형 특성을 개선하였다. 과산화수소(H_2O_2)및 슈퍼옥사이드 음이온 $-O_2$ 와 같은 활성산소종(ROS)으로 인한 손상이 감소했으며 K^+ 유출에 따른 전해질

누출 수준도 감소하였다. 항산화 능력은 (ROS) 축적의 효과에 의해 증가되었으며, 이 산화 축적을 제거하기 위한 즉각적인 반응은 H_2O 및 O_2 에서 $-O_2$ 를 촉매하는 SOD 관련 방어 시스템을 활성화함으로써 이루어지는 것을 확인하였다. 추가적으로 H_2O_2 를 촉매하고 GSH 등을 통해 막과 산화 손상을 방지하고, 항산화 능력 체계를 활발하게 증가시키는 폴리페놀 산화효소의 조절을 통해 뿌리와 PGPR 의 공생과 산화 손상을 조절하였다. ABA 경로와 관련된 단백질 코딩 유전자 *AtAO3* 와 *AtABA* 의 발현이 개선되었고 식물을 보호하기 위해 잎에서 세포의 팽창과 생리 기능을 유지하는 ABA 호르몬이 낮아지고 기공의 개폐 조절에 직접적으로 관여하였다. 식물을 보호하기 Col-0 과 *atnced3* 의 *AtDREB2A* 와 *AtDREB2B* 의 가뭄 관련 요소 결합 유전자 발현 수준에 대한 명확한 효과는 S1T13 과 S4T10 의 이중 적용에 의해 항산화제의 방어 시스템을 활성화하여 높은 발현도를 보였고, 이는 건조에 대한 빠른 대응을 유도하는 자연적으로 일어나는 현상이다.