USAGE OF PLANT GROWTH REGULATORS TO IMPROVE CITRUS DROUGHT TOLERANCE

By

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ABSTRACT

Bright, colorful, fragrant, refreshing, and juicy, citrus fruits are not only delicious for their balanced tart and sweet taste, but they are also an essential part of everyday nutrition, packing powerful health benefits beneath that tough, leathery rind. Citrus fruits are rich in nutrients such as vitamin C, flavonoids, and fiber, which confer vascular protection, reduce inflammation, improve gastrointestinal function and health, and play an important role in preventing diabetes, cancer, and neurological disease. With 270,000 acres in California, citrus is a vital part of human health and significantly contributes to California's economy. However, due to the increasing drought, California is implementing rules to limit water use in the orchards. Reduced water availability in citrus orchards will affect orchard productivity, and growers will need to increase tree water use efficiency. Phytohormones are organic compounds produced by plant cells and control all aspects of the plant life cycle, including the tolerance to abiotic stresses such as drought. Plant growth regulators are synthetic plant hormones, and they are widely used in agriculture to improve tree productivity and fruit quality. However, its application to enhance drought tolerance in citrus under California conditions has not been studied yet. In this research project, we will test the effect of some plant growth regulators such as Salicylic acid, Cytokinin, and Abscisic acid on improving the drought tolerance of citrus seedlings (Nekrich).

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INTRODUCTION

More than 80% of the surface of the world is covered by water. This is discovered in the form of rivers, lakes, ice caps, oceans, seas, and various other water bodies that cover the surface of the earth. Aquifers underneath contain water as well. This water has been present under the surface of the earth for billions of years. Thus, as a result, these water bodies that are found underground are known as finite regenerative resources. Furthermore, water is a renewable resource and thus it does not stay in one place as it is constantly moving throughout the seas, rivers, earth, and the atmosphere in various phases of water. It descends from the clouds in the form of snow or precipitation, then it accumulates on land, in the bodies of water such as the seas and oceans. Thus, it goes through a water cycle. The manner in which the underground water is generated is through the process of the rainfall on the Earth which then makes its way through the soil and surface of the earth by penetration and gets collected in the underground reserves. The cycle is a never-ending process as water keeps going through the cycle when water from streams and rivers reaches the seas, where it evaporates and produces clouds (What Is Drought?).

Based on research and past experience, it is true that water is one of the scarce resources that are present on Earth. However, this is not because of the fact that there is not enough water present on Earth, instead, it is because of the fact that human beings can consume only 3% of the entire water resource that is present on Earth. The human being would not be able to consume the entire water that is present as about ninety-seven percent of the water that exists on Earth is in the form of salt water. Further, salt water is harmful to many plants, animals, and people. Freshwater which is the one that is consumable makes up just the remaining three percent, but it is inaccessible to humans since almost seventy-five percent, of the three percent of freshwater, is frozen in glaciers which is a solid form of water. Thus, there is very limited water that is

available for humans, plants, and wildlife. However, even the limited water is getting scarce now due to the extreme amount of pollution, rising human demand for it, dumping of waste in the water bodies, and changes in precipitation patterns. These factors all contribute to the finite nature of water. Thus, making the scarce water even more scarce (What Is Drought?).

The way the process of the water cycle works is when the water from the water bodies evaporates into the Earth's atmosphere. Then, the next step would be when the atmosphere would carry the water vapor even further up in the atmosphere, and in the right ideal conditions they would form clouds. Then, these clouds are carried from one place to another with the help of the wind. The cool wind that transports the clouds that bring rain in the stream of air plays a huge role in the transportation of water. The structure of the jet stream varies with the season. In simple terms, the jet stream will be carrying climate variables like precipitation and temperature across a different course or in an alternative direction based on what season it is. The importance of Jet Streams is implied by the fact that the regular climate over an area could shift significantly over a while if the jet stream alters its regular behavior or path or is impeded by "ridges" or "troughs" of air in the upper atmosphere. It appears as though the current of air has made an unexpected turn or struck a barrier! (What Is Drought?)

A drought is a possible consequence if the current of air runs into a barrier or makes a specific diversion thus failing to carry clouds that provide precipitation. Numerous factors might alter such jet stream variations. Numerous researchers believe that factors including variations in the percentage of glacier and snow covering, the quantity of plant life (for instance, trees or grasslands) overlaying the earth, the moisture content in the ground, and the temperature of the ocean surface and winds might cause these trends to alter. This change is something that the researchers are currently trying to figure out the answers to these questions (What Is Drought?).

There are several reasons why there are droughts. It may be brought on by prolonged dryness or a lack of precipitation. In the studies of the water cycle and climate change, it was discovered that shifts in the direction of the winds that carry clouds and precipitation across the sky might, gradually, prevent a location from receiving its typical quantity of snow or precipitation. A state of drought in a region may be brought on by areas upstream of you having insufficient precipitation if someone lives somewhere where the majority of the freshwater that is consumed is obtained from a body of water. The residents would have reduced access to the water from the vicinity of the river. Drought is a problem that is largely caused by people. Drought could be caused by the fact that human beings are overconsuming water during the times when the place receives regular rainfall (What Is Drought?).

Drought and Floods

As mentioned earlier, flash floods, severe thunderstorms, and droughts are all common occurrences in the environment. Although the aftereffects, of the damage that is caused by these natural hazards, are capable of doing to homes, vegetation, and the landscape, it might seem bizarre. However, the one distinction between drought and the other natural hazard is that one cannot predict the onset of drought. While on the other hand, the observation of a river's level rise, the wind picking up as a storm approaches, or impending thunderclouds is predictable and could warn the people about the incoming natural hazards. Storms can be viewed on the radar. Drought is a natural hazard that does not have warnings like other unforeseen events like tornadoes and floods (What Is Drought?).

The onset and conclusion of a drought are not immediately apparent, unlike those of a storm, an approaching hurricane, or a major flood. Drought frequently has a sluggish beginning and finish,

and its impacts may not be seen for several weeks, months, or even years. As a drought situation could start and months or even years may pass by before people start realizing it is the drought that is causing the lawn vegetation or flora to begin to appear brown. Several weeks may pass before someone would become aware of a neighboring lake's diminished volume of water. This is not the case for other natural hazards as it does not take several weeks to understand the consequences of floods, tornadoes, or hurricanes (What Is Drought?).

A state of drought is a stretch duration when precipitation levels fall below the average in a certain location. Lack of sufficient precipitation such as rain or snow, can result in decreased soil or groundwater moisture, reduced flow through streams, injury to crops, and water scarcity. The costliest weather occurrences are hurricanes, and following that is drought. It can be challenging to determine when a prolonged drought began or terminated, unlike abrupt weather phenomena like hurricanes, tornadoes, and thunderstorms. It may require weeks to months in order to recognize the first signs of a drought since its impacts might be hard to spot straight away. For the same reason, a drought's termination might be hard to foresee. In certain areas around the world, the drought effects have lasted for a decade and this has a huge negative impact on the population, livestock, and the vegetation of that area (Drought).

There are multiple manners that droughts impact individuals. Water supplies may diminish throughout a period of drought, and access to clean drinking water is necessary for every living thing. If there is a location where water is not available then People would need to find other water resources. In other areas and find ways to access it maybe through a tunnel system or underground water but they do need water to survive. Thus, alternate options have to be acquired. Further, plants require water for proper development as well. Crops have to be irrigation-watered when there is not enough rainfall to naturally irrigate them. Sufficient water

from groundwater, and nearby water bodies is a prerequisite for irrigation. However, during a drought-like situation, the water sources are reduced and may entirely disappear, making it impossible to provide irrigation for crops and leading to their demise (Drought).

Alexandra Cousteau, a National Geographic Emerging Explorer and the brainchild of the Blue Legacy project is one individual studying these issues. spread the message that it is the responsibility of human beings to show concern for the planet Earth and take care of its limited water resources. The famous ocean researcher Jacques Cousteau's granddaughter, Cousteau, predicts that the future of water is going to be a major concern. According to her, people would migrate in the quest for water as a result of water-related issues including drought, storms, floods, and poor water quality. She emphasizes the need for us to take all reasonable steps to safeguard the precious water reserves of the planet (Drought).

Impact of Drought

There are numerous and significant negative consequences of drought. Short-term consequences of drought on health might be detected and evaluated in some cases. However, the delayed onset or chronic nature of drought may lead to longer-term, indirect adverse health consequences that are sometimes difficult to foresee or manage. The long-term health problems for society that may arise from drought include a Lack of water supplies and water of poor quality impacts on nutrition and food security, proper hygiene and sanitation, and the condition of the air. Additional illnesses, such as the West Nile Virus are transmitted by mosquitoes growing in still water (Agriculture).

Water

The water pollution concentrations can rise and could be brought on by diminished stream and river flows. Oxygen levels in water reservoirs are decreased by warmer water temperatures. These concentrations may have an impact on fish, other marine organisms, and the cleanliness and quality of the water. Wildfires brought on by lack of precipitation can cause more silt, ash, charcoal, and woody remains to be washed into surface waterways, where they can kill fish and other aquatic organisms by lowering the oxygen content of the water. Groundwater serves as the main supply of water for several regions of the United States of America. A decrease in rainfall and increasing surface water vaporization over time cause reserves of groundwater to deplete more slowly (Agriculture).

Food and Nutrition

Drought can limit the growing season and create conditions that encourage insect and disease infestation in certain crops. Low crop yields can result in rising food prices and shortages, potentially leading to malnutrition. Drought can also affect the health of livestock raised for food. During drought, livestock can become malnourished, diseased, and die (Agriculture).

Air Quality

Drought may decrease the duration of growth and make it easier for insects and diseases to attack certain vegetables. Low agricultural yields might result in increased food costs and scarcity, which could cause hunger. The physical condition of cattle bred to consume meat can also be impacted by drought. Cattle may suffer from malnutrition, illness, and death during a drought. Drought typically comes with fires in forests, dry weather, and other hazardous situations. Pollen, fumes, and fluorocarbons are a few of the particles in the air that have become suspended

due to fire, soil that is dry, and plant life. These compounds have the potential to aggravate respiratory conditions like asthma by irritating the bronchial passageways and lungs. Additionally, this can raise the chance of developing serious respiratory illnesses including bronchitis and bacterial pneumonia (Agriculture).

Sanitation and Hygiene

Numerous sicknesses are lowered or controlled by having access to water for personal hygiene and sanitation. Although water preservation is necessary due to the current drought, it shouldn't stand in the path of good sanitary practices. There are water-saving techniques for doing one's own personal cleaning, scrubbing your hands and rinsing agricultural produce. One way to cut water usage and keep up routine hand washing along with other beneficial hygiene practices is by installing low-flow faucet aerators in homes and offices (Agriculture).

Infectious Disease

Drought may directly result in a rise in hazardous illnesses. As precipitation declines, viruses, protozoa, and bacteria can contaminate groundwater as well as the water found on the surface of the earth. Water well users may be more susceptible to pathogenic diseases associated with drought. People with preexisting persistent illnesses are among other populations who are in more danger. Should sanitation of the hands is hampered by a perceived or actual scarcity of water, this could lead to acute respiratory and gastrointestinal infections are more likely to be transmitted from one person to another. Thus, the chance of contracting a parasitic illness rises with the shortage of water (Agriculture).

Drought could easily become a breeding ground for bacteria such as salmonella and E. coli. to effortlessly compromise food and pass on illnesses that are infectious. Throughout a period of

drought, nourishment can act as a vector for illness as agriculturalists may utilize water that has been recycled in order to water their agricultural land. However, the issue is that the badly processed water may result in a variety of transmissible diseases (further may also carry bacteria that produce toxins), which would lead to a lot of health problems for populations that are immuno-compromised or labeled as high-risk populations. Additionally, the potential for water to run off the surface usually occurs during the rain situation, and since the land has become bare and really dry, it is hard for the water to penetrate as a result it flows from one area to another via the surface to the land and this could lead to the cross-contamination of crops. It could also lead to the spread of diseases and also the spread of invasive plants. Drought-related water pollution happens due to the run of surface waterways and other forms of aquatic entertainment activities offer additional viral sickness hazards. When the increase in temperature happens due to the intense summers, people become more inclined to engage in activities involving water. People who are in contact with polluted water that is used for recreation have a higher chance of contracting infectious agents that prosper in the humid, shallow reservoirs that are present when there is a shortage of water (Agriculture).

Chronic Disease

These are diseases like asthma and some immune disorders that could easily spread during drought-like situations. As drought situation eventually leads to increases in the concentration of the toxin in the air and also increases the toxin concentration in the stagnant water bodies due to the accumulation of alga and other microorganisms which would eventually lead to other respiratory diseases which could become fatal. Furthermore, stagnant water and low precipitation give birth to various vector-borne diseases such as West Nile virus, dengue, Malaria, etc which are all caused by mosquitos (stagnant water is their major breeding ground) (Agriculture).

Soil Moisture

Based on the article, Soil Moisture by National Integrated Drought Information System, research states that it can be predicted that soil moisture is crucial in the predictor of drought-like situations or fires in the forest, it could also help us indicate if there is a flood-like situation in the immediate future. Thus, these predictions could help us prepare for the future with the proper water management methods. Further, even though there are other types of equipment that could predict drought or flood-like situations, soil moisture has been the best one till now. Based on the definition of soil moisture from the AMS Glossary of Meteorology solid moisture is described as "the total amount of water, including water vapor, in an unsaturated soil." The expression "soil moisture," which is also frequently referred to as "soil water," means that it is the water that is available in the pores of the soil that is present on the land surface; it does not include the groundwater or any other water reservoirs. The soil moisture is based on the elements such as the weather, kind of soil, and nearby plants, that affect the amount of soil moisture. Plants are impacted by the level of soil moisture. Usually, the soil that is present near the roots of the plants is called the root zone. The soil moisture in the root zone goes up to 200 cm of depth in the soil.

Agricultural Drought

Furthermore, based on the article Agricultural Drought by National Integrated Drought Information System, drought occurs when the precipitation rate is lower than the average or normal rate and when the temperature is extremely high, higher than the average temperature. The place of occurrence, scope, and intensity of drought's effects on agribusiness is influenced by underpinning societal and ecological shortcomings, water distribution availability, and crop varieties, among other elements. Based on the report for the Agricultural severe drought-like

weather, from the National Drought Mitigation Center, "links various characteristics of meteorological (or hydrological) drought to agricultural impacts." Agricultural drought is identified and tracked by looking for deficiencies in the process of precipitation variations in current and prospective evaporation rates from the flora and soil and various other interfaces), soil water shortcomings, and reduced access to water.

Importance of Water in Plants

Water plays a huge role in the development of a plant because it is essential to the photosynthesis process and movement of both inorganic as well as organic molecules in the xylem and Phloem of a plant. Despite this reliance, plants only save less than 5% of the fluid that their roots use to develop and expand. The remaining portion is lost by transpiration, which is the process through which water leaves plants and enters the earth's atmosphere. Based on the research, it was found that A single irrigated corn plant that is developing in Kansas may utilize 200 L of water throughout an average summer, whereas some giant tropical rainforest trees can use approximately 1200 L of water in just one day (McElrone).

Furthermore, ninety-five percent of a crop's cells consist of water, making it a critical resource for vegetation. A seed needs water for germination, and once a crop develops, the next role of water is to transport minerals and other nutrients around the entire organism. Inside the tissues of plants, water plays a number of significant roles. The photosynthesis procedure, during which plants utilize solar energy to make their own sustenance, requires water. Trees utilize hydrogen elements from water (H2O) acquired via their root systems and carbon dioxide from the air throughout this entire procedure, releasing oxygen as a side effect. Through their stomates that are present in the underside of the leaves, this exchange takes place (Richmond).

For development and fertilization, minerals and glucose undergo dissolution in water and then they are transported down their concentration gradient, thus they are transported from the source (the place where they are created and are in high-concentration like the roots) to the sink (location when these nutrients are consumed- low-concentration sites like the stems and meristemic areas). Plants of all kinds depend on water for the structural integrity of their cells. Water exerts a continual force on cell walls known as turgor pressure, making the plant sturdy but flexible and enabling it to turn in the direction of the wind or shift its leaves in the direction of the sun to increase photosynthesis rate. Insufficient hydration will result in leaf curling up, discoloration of plant cells, and finally premature death of the plant (Richmond).

Even in times of dryness, the plants have to still continue to photosynthesize in order for these plants to survive. The way process of photosynthesis works is due to the presence of chlorophyll (these are the green pigment that is found in most plants). The energy manufacturers of plant life, known as chloroplasts, are unique plasmid organelles that are filled with chlorophyll pigments. Chlorophyll utilizes sunlight to generate carbohydrates in conjunction with water as well as carbon dioxide (CO2). Thus, these plants convert light energy to chemical energy to make sugar and oxygen as by-products. One of the by-products is really important for the fauna to expand and thrive while the oxygen is released into the atmosphere which is then consumed by other organisms. Thus, the process of photosynthesis greatly requires water. Though there are some plants that try to adapt to the environment when there is a drought-like situation and there is not enough water for them to synthesize however this process may negatively affect the plant. As a result, this would lead to the accumulation of harmful substances known as free radicals in the plants during the process of photosynthesis with limited water. This means that the molecules in the plants will react with everything that the plants come into contact with in order to make up

for the limited water and cause harm for the plants. It also means that vegetative organisms must precisely regulate the ways in which they utilize and consume solar energy. The plants open their pores to let in Carbon Dioxide for photosynthesis but in this process, they lose water due to transpiration. Thus, this leads to plants ensuring that they take in the CO2 but also do not lose too much water which is a challenging task. Thus, for such circumstances, plants have Abscisic acid (ABA plant hormone) which helps the plant to balance the water (Van Der Vyver and Peters).

Plant Adaptations to Drought

Abscisic acid is a plant hormone that is generated and delivered to the stomata, which are small openings called pores found in the lower side of the leaves, during the time when the plant is suffering from insufficient water. Abscisic acid helps to control the opening and closing of stomata by adjusting the turgor pressure—this is the force that the liquids inside a plant cell wall impose on the cell wall. Thus, when the pressure is high it would force the cell walls to open up as there is the force exerted against its wall. The strain increases if there is more water present inside the cell. In order for photosynthesis to occur in spite of a drought-like situation, turgor pressure has to be maintained in a way that ensures that there is a healthy equilibrium between the CO2 absorption and transpiration of water. However, if water availability is still restricted during an extended drought, the plant could ultimately get overwhelmed by the pressures of the situation and this adapted method of photosynthesis as a whole may become ineffective. The issue of water loss while photosynthesis has been successfully avoided by drought-tolerant vegetation by using another adaptation. Some plants open their stomata to just absorb Carbon Dioxide when it is chilly. Following storage, they utilize this CO2 during the day for photosynthesis, this would help to reduce the water transpiration. Because this way the plants are able to afford to keep their stomata closed during the day but they still have enough CO2 that

they accumulated during the night. Thus, this might help them continue their development even though the rate might be slow (Van Der Vyver and Peters).

Furthermore, there are various growth hormones that could help plants to develop their drought resistance. Together biotic as well as abiotic variables have an impact on vegetation, and as a result, many internal alterations take place in crops. These variables have an impact on the efficiency and advancement of plants. Biotic elements are associations between organisms and their environment that can be beneficial or harmful. The positive effects might be advantageous to plants. For instance, organism like Phytohormones helps the development of plant resistance to drought. While, on the other hand, effects such as allelopathy, herbivore impact, or infection by pathogenic organisms in plants are examples of adverse consequences. Multiple chemical elements used in plant protection systems aid in preventing negative consequences. Plant cell walls include proteins (antimicrobial) and secondary metabolic products, and they also have a chemical layer of polysaccharides that is extremely weighted and prevents pathogens from physically penetrating and growing through them (Iqbal et al.).

Abscisic acid, also known as ABA, plays an essential function in the adaptation to drought. External treatment with ABA has been proven to be a successful method of enhancing crop resilience to drought in a number of experiments. External ABA treatment can control the mechanisms of power, amino acid, and lipid metabolic function; encourage the building up of flavonoids, betaine, and various other compounds; enhance enzymatic and non-enzymatic anti-oxidant control structures; and strengthen the photosynthetic efficiency and the relative water content of vegetation to enhance plant development as well as enhance the resistance to the drought for a variety of agricultural crops. Additionally, by stimulating a number of gene transcription factors, sprinkling the seedlings of tobacco using 2 mg/l 10,40 -trans-diol-ABA, an

essential starting point of the biosynthesis of ABA in fungal organisms, improved their ability to tolerate drought of tobacco seedlings. According to certain research, concurrent ABA plus jasmonate/benzyl amino purine therapy is more successful at fostering crop tolerance to drought than any of these treatments alone (Zhang, Hui, et al).

The quick transformation of Abscisic acid into physiologically inactive components has restricted the application of this pesticide, despite the fact that it may be employed instantly as an agrochemical like herbicide or pesticide. Consequently, a variety of ABA-imitative participants have been developed and created in order to get around the drawbacks of using ABA for different agricultural fields. The very initial engineered ABA activator to be found was Pyrabactin, which exhibits various ABA-like behaviors, including the closure of stomatal pores, reduction of the emergence of seeds, and improvement of root conductivity to water (Zhang, Hui, et al).

Based on the research that was completed on the various crops, the result that was found is mentioned below. Thus, the research was done with the hormone Abscisic Acid was experimented on the crop maize (*Zea mays L.*) and wheat (*Triticum aestivum L.*), it was reported that in those crops there was elevated glycine betaine (GB) buildup, and altered water interactions and plant development, and elevated glutathione (GSH) and astaxanthin concentrations. Further, when the hormone Abscisic Acid was experimented on pearl millet (*Pennisetum glaucum L.*), it resulted in increased antioxidant enzyme capabilities. Furthermore, Abscisic Acid also experimented on sweet potato (*Ipomoea batatas*) crops, and the resulting improvement was that the mechanism that was affected was the boosting of the levels of endogenous hormones, promoted the processes of photosynthesis, and controlled the source-sink equilibrium of the carbon conversion enzymes (Zhang, Hui, et al).

Based on another research that was completed on the various crops with the plant hormone called Salicylic acid, the result that was found is mentioned below. Thus, when Salicylic acid was experimented on the barley (*Hordeum vulgare L.*) crop and the wheat (*Triticum aestivum L.*), the result showed that the preservation of the process of photosynthesis, improved antioxidant the defensive end, and improved the breakdown of energy. Further, when Salicylic acid was experimented on the maize (*Zea mays L.*), there was an enhancement of the antioxidant enzyme capabilities, build-up of osmolytes, and endogenous Abscisic Acid in the maize crops (Zhang, Hui, et al).

Among the most significant phytohormones that promote the division of cells and cause changes are cytokinins, which were first identified in 1950. Trans-zeatin, which was primarily separated from corn crops, was the initial naturally occurring cytokinin. According to Li et al. (2016), these types of hormones are crucial for the control of plant development and adaptation to stress caused by drought. Cytokinins have both adverse and favorable effects (Iqbal et al.).

The length and intensity of the stress caused by the drought determine whether the amount of cytokinin levels increases or decreases. The advantages include increased resistance to the effects of stress related to drought. Additionally, it has been suggested that cytokines promote isopentenyl transferase enzyme gene expression in plants with transgenic genes. By limiting the stress-induced senescence of foliage, the transgenic crops demonstrated great resistance to drought. Along with the beneficial consequences of cytokines building up, the detrimental effects of cytokines buildup on the endurance of drought are additionally documented. When the CK oxidase/dehydrogenase (CKX) enzyme catalyzes cytokinins, is overexpressed, and broken down throughout Arabidopsis, the amount of internal cytokinin decreases. Due to the self-sustaining

overexpression of CKX in Arabidopsis, transgenic cultivars with increased resistance to drought and decreased cytokinin concentrations of cytokinin were produced (Iqbal et al.).

The use of cytokinins in the cultivation of crop tissues and the careful examination of the biological aspects of plants, including the development of plant components and the blooming phase. These substances are in charge of promoting multiple mechanisms throughout the expansion and maturation of female gametes and vegetative embryos. In particular, cytokinins additionally play a role in the growth of shoot apex meristems, floral advancement, vessel growth, photomorphogenesis, the sprouting of seeds, and the death of leaves. Additionally, it aids in the induction of a lack of rainfall and unfavorable responses to the environment in plants. Additionally, a variety of hormonal substances and macro-nutrients regulate the synthesis of the genes involved in cytokinin biosynthesis. Cytokinins increase the proliferation of cells in Arabidopsis by competing with auxin (Iqbal et al.).

Furthermore, Gibberellins (GA) are primarily used by plants as hormones that promote growth to give resilience to environmental factors like drought. During every phase of a crop's existence, Gibberellins remain to serve plants. Gibberellins' main function is to promote cell lengthening, which in turn promotes proliferation, thereby accelerating both the young and mature phases of the developmental process of plants. Additionally, it benefits plants' proliferative and reproductive phases. By lowering the levels of gene expression that are involved in gibberellin production. If the GA is high, tolerance to drought is considered to be improved (Iqbal et al.).

Furthermore, there is another plant growth hormone that could help with the drought situation by helping plants adapt in a better way to drought with the help of hormones. The name of the next hormone is Ethylene, this hormone which plays a role to extend the duration of storage of

vegetable and fruit products and flowers that have been cut. Further, ethylene activity is frequently blocked since it influences crucial horticultural and farming features such as the ripening of fruits and vegetables, following harvest physiology, the process of senescence, and abscission. Thus, many times Ethylene inhibitors are used in order to slow down the ripening process which would be helpful during circumstances when the plant is facing a drought-like situation as it would help to slow down the process of senescence and abscission (Schaller et al.).

MATERIALS AND METHOD

Therefore, the purpose of this capstone project was to find the ways in which the different growth hormones: Abscisic acid, Cytokine growth hormone, Salicylic acid, Ethylene Inhibitor, and Gibberellins would react with the seedlings. Further, the next step that was studied was the ways in which these hormones would interact to help the seedlings to adapt to the drought-like situation and be able to survive in the drought-like situation. As drought is believed to become the most prominent hazard to farmers cultivating field crops, and the shortage of water has increased greatly worldwide over the last few years (Arias). Thus, it would be really helpful to develop seedlings that could be minimizing water loss and maximize photosynthesis yield for the seedlings. Thus, this would help to save the seedlings in drought-like conditions and thereby help to save the problem of drought for plants by increasing the drought resistance of the plants. Furthermore, another article called Improving Crop Drought Resistance with Plant Growth Regulators and Rhizobacteria: Mechanisms, Applications, and Perspectives were written by authors such as Hui Zhang, Xiaopeng Sunand Mingqiu Dai. This article describes the research that was conducted to find new ways to form low-cost, environmentally friendly, and strong field operability equipment and methods to improve crop drought tolerance. Furthermore, recently, they have found that there are some exogenous plant growth regulator (PGR) treatments and

microbe-based plant biotechnology that are really beneficial for improving the performance of plants in stress situations like droughts (Zhang et al.). Furthermore, Abscisic acid is something that was researched and found that it is really important for developing the resistance of plants in a drought-like situation. The article describes the benefits of the treatment for ABA. Some of the benefits are to control the enhancing enzymatic and non-enzymatic antioxidant regulation systems, boost photosynthesis efficiency and promote plant growth and enhance drought resistance (Zhang et al.).

Method

Therefore, this is the reason why this research is really advantageous as it would help plants in drought-like situations. Thus, the initial step of the capstone research project was to find a spot in the UCR greenhouse. Then, I was trained on how equipment that would calculate the chlorophyll level would work and how the project research would take place. Then, I was provided with 100 seedlings which consisted of Tango and Cara Cara seedlings which are the different types of citrus plants. I was required to collect the Chlorophyll of all the plants on a weekly basis and record the various observations that were made. Further, the next step was dividing the Tango and Cara Cara seedlings into various groups and spraying the different amounts of the five growth regulators: Abscisic acid, Cytokine growth hormone, Salicylic acid, Ethylene Inhibitor 2-aminoethoxyvinyl glycine (AVG), and Gibberellins. Thus, I observed how 50 of the seedlings trying to survive in the drought-like situation while the other 50 seedlings enjoyed and thrived in the control group and then I recorded all the information. This experiment would consist of a control group and an experimental group (the group that is exposed to drought-like situations). The first group would be sprayed with different concentrations of the five growth regulators: Abscisic acid, Cytokine growth hormone, Salicylic acid, Ethylene Inhibitor, and Gibberellins.

Thus, hypothetically the seedlings would respond differently based on the different growth hormones that they received. The observations from the comparing and contrasting of the different groups would be recorded for all the groups. These experiments would be completed in the UCR greenhouse and the Lindcove Research and Extension Center.

Moreover, the observation that would be made would be based on the measurement of the chlorophyll content which could be noticed by comparing the green color of the leaves and by using a chlorophyll meter (at LEAF CHL BLUE, 0131-58 Ver 1.0., FT GREEN LLC, USA) and the other measurement factor would be the fresh and dry weight of the leaves from the experimental group and the leaves from the control group. Furthermore, there would be a comparison and contrast of the weight to find the result of the experiment. Thereby, this research project would help the research community by understanding the right concentration of the five growth regulators: Abscisic acid, Cytokine growth hormone, Salicylic acid, Ethylene Inhibitor, and Gibberellins to increase drought resistance by lowering water loss and increasing the photosynthesis rate.

Plant material, and experimental conditions

The experiment had been carried out at a greenhouse located at the University of California, Riverside, California. The plants used in this experiment were one-year-old seedlings of CARA-CARA. CV. (*Citrus* × *Sinensis*) navel orange grafted on Rich rootstock and Tango mandarin CV. (*Citrus reticulata*) grafted on C-35 rootstock. Further, there were about 100 seedlings among which 50 seedlings were the control group (untreated group) while the other 50 were the experimental group. The experimental group was the seedlings that would experience the drought-like situation. Both the groups had the same number of plant subgroups that were

untreated (no growth regulators added), treated with abscisic acid, treated with gibberellins acid, and treated with ethylene inhibitor, treated with cytokinins, and treated with salicylic acid. Seedlings were grown in 4-liter pots with a dimension of 12.7×12.7×30.48 cm containing 25% coconut-Coire, 50% peat moss, and 25% sand as a growth medium. The transplants were normally irrigated and fertilized before starting the experimental treatments.

Experimental design and treatments

The experiment design was a complete randomized block design with four replicates. Before exposure to the drought, the seedlings were hand sprayed with various plant growth regulators as indicated in Table 1. Each treatment was performed on eight seedlings and then divided into two groups, the first group of four received the normal irrigation schedule and the second group did not receive any water for five weeks. Plants were monitored during the experiment and data were collected.

Chlorophyll content

The Chlorophyll content was recorded on the mature leaves by using a chlorophyll meter (atLEAF CHL BLUE, 0131-58 Ver 1.0., FT GREEN LLC, USA). Two random mature leaves were chosen per replicate to determine the chlorophyll content index, SPAD readings, and total chlorophyll (μg/ cm^2).

Fresh and dry weight

Four weeks from the exposure to drought, plants were cut above the spoil and fresh weight was determined. Shoots and leaves were left to dry completely, and dry weight was recorded. Furthermore, below is a table of the various concentrations, that were used during the experiment

process, of the five growth regulators: Abscisic acid, Cytokine growth hormone, Salicylic acid, Ethylene Inhibitor, and Gibberellins along with the commercial name of the various growth regulators.

Table 1: Treatments used in the study.

Treatment	**Concentration ion**	**Commercial name**
Untreated	—	—
Cytokinin	**6 ppm**	**Kimzal**
Salicylic acid	**100 µM**	**Salicylic acid**
Gibberellic acid	**20 ppm**	**ProGib**
Ethylene Inhibitor	**100 µM**	**ReTain - AVG**
Abscisic Acid	**100 µM**	**ProTone**

RESULTS AND DISCUSSION

The experiment on Tango Mandarin revealed a significant impact of drought on plant growth. The untreated plants exposed to drought for five weeks showed a 59% reduction in fresh weight. This indicates that Tango Mandarin is highly susceptible to drought stress. However, the results also revealed that the application of plant hormones can mitigate the negative effects of drought on plant growth. The application of cytokinin, Salicylic acid, Gibberellic acid, ethylene inhibitor, and Abscisic Acid to the Tango Mandarin plants had a positive impact on their growth under drought conditions. The reduction in fresh weight in treated plants was lower compared to untreated plants. The highest reduction was observed in the cytokinin treatment (58%), followed by Salicylic acid (56%), Gibberellic acid (57%), ethylene inhibitor (39%), and Abscisic Acid

(39%) (Figure 1). The data suggest that plant hormones play a crucial role in the regulation of plant growth under drought stress.

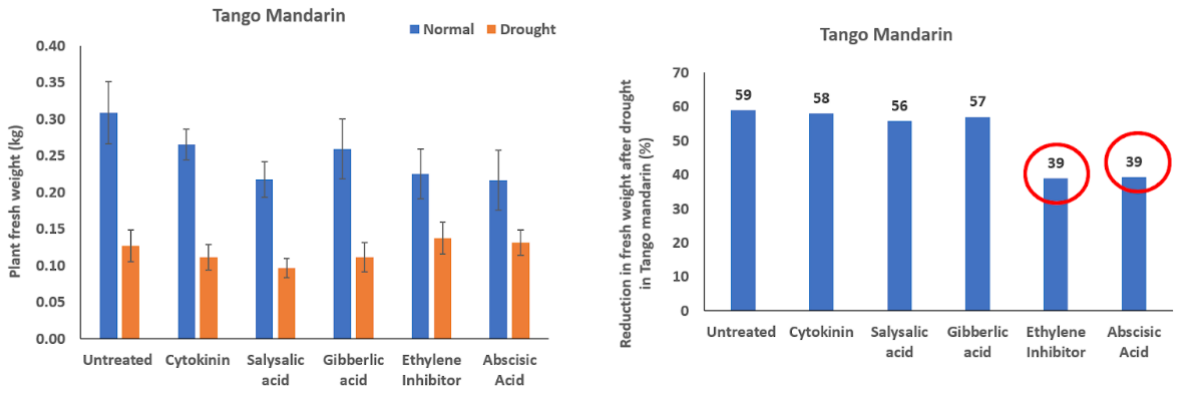

Figure 1: Effect of plant hormones on the response of Tango mandarin fresh weight to drought.

Furthermore, figure 2 shows the difference in the dry weight to drought for the tango mandarin seedlings and it can be noticed that the ethylene inhibitor and the abscisic acid have the lowest plant dry weight difference between the normal (control) group and the drought (experimental) group. Furthermore, based on Figure 2, the untreated plants had a 43% reduction in dry weight, whereas the hormone-treated plants showed a much lower reduction in dry weight. Further, the percentage reduction in dry weight after drought in Tango Mandarin it was observed that the plants treated with the growth regulators Ethylene inhibitors (4%) and Abscisic acid (9%) have the lowest reduction in dry weight which is a good sign and proves that these growth regulators positively affect the seedlings are being affected by the drought situation. Thus, it can be concluded these plants were assisted by the growth regulators and which helped them survive better in a drought-like situation. Furthermore, the reason why abscisic acid and ethylene inhibitors had the biggest impact was because of the fact that abscisic acid helps to control the opening and closing of the stomata. Thus, this helps to control the water loss via transpiration

during the opening of stomata in order to take in carbon dioxide which is needed for the photosynthesis process to occur (Brookbank et al.). Thus, this minimization of wasting water via transpiration helps the seedling to utilize that water to continue another metabolism in the plants and helps it survive better in a drought-like situation. Further, since ethylene is a growth regulator that increases the senescence of the plants, if there growth treatment of an ethylene inhibitor is used it would help to reduce the rate of senescence due to the drought and help the plant to survive longer in a drought-like environment. Thus, the plant dry weight results showed that the hormone treatments had a positive effect on plant growth (Schaller et al.).

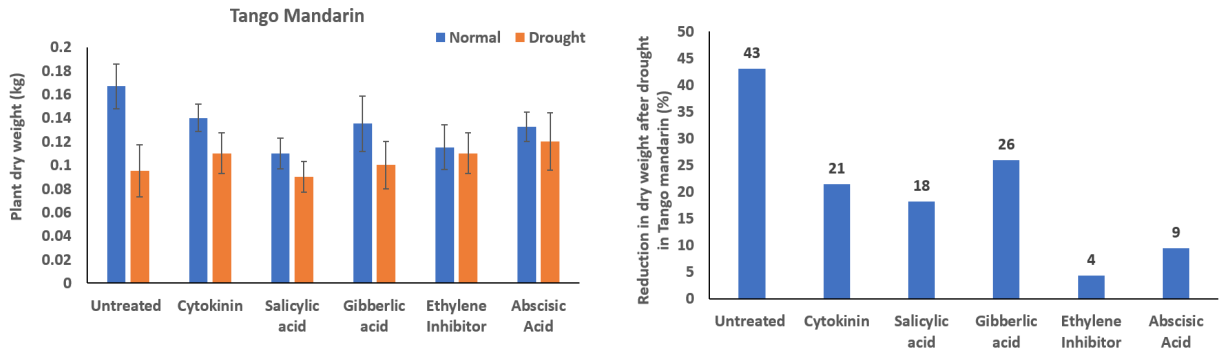

Figure 2: Effect of plant hormones on the response of Tango mandarin dry weight to drought.

Overall, the study revealed that the ethylene inhibitor (AVG) and Abscisic Acid were the most effective treatments in mitigating the negative effects of drought on Tango Mandarin growth. The ethylene inhibitor reduced the senescence process associated with drought conditions, which maintained the plant growth rate. In contrast, Abscisic Acid played a crucial role in reducing water loss, which led to higher drought tolerance. These results suggest that the application of plant hormones could be a potential strategy for improving Tango Mandarin and drought tolerance and enhancing crop productivity in drought-prone regions.

Similarly, the plant dry weight results, for the Cara Cara seedlings, showed that the hormone treatments had a positive effect on plant growth. The untreated plants had a 43% reduction in dry weight, whereas the hormone-treated plants showed a much lower reduction in dry weight. The highest reduction was observed in the cytokinin treatment (20%), followed by Salicylic acid (7%), Gibberellic acid (2%), ethylene inhibitor (10%), and Abscisic Acid (10%) (Figure 3). These results indicate that the hormone treatments were effective in maintaining the plant and growth rate even under drought conditions. Thus, based on the reduction in dry weight after drought in Cara Cara oranges, it was observed that Salicylic acid and Gibberellic acid had the lowest reduction while ethane inhibitor and abscisic had the same amount of reduction in dry weight percentage. The reason for seedlings treated with Salicylic acid and Gibberellic acid to have the lowest is because Salicylic helps to induce the defense mechanism for the plant when the plant faces some type of pressure stress from the environment or any other factor and manner in which the plants defends itself is through using its mechanism such as morphological, physiological and biochemical methods (War). Thus, when the seedling faced the environmental stress of drought, seedlings that were treated with Salicylic acid were able to adapt better in that situation and this is why the reduction percentage is lower for these particular groups or plants. Further, the reason why the Gibberellic acid-treated plants have only a 2% reduction in dry weight is that Gibberellic acid help in the lengthening of plants by cell division thus by controlling the cell proliferation, seed germination and it helps in the development of the flower of the plant which requires a lot of energy. Thus, the control of the gibberellic acid growth regulator helped the seedlings to adapt in a way that it was able to tolerate the drought-like situation. Thus, using the energy for germination and proliferation in the most efficient manner for the plant to survive in drought stress from the environment. Thus, this might be the reason

why the Cara Cara orange oranges dry weight after the drought had the lowest weight reduction percentage (2%) (Gupta and Chakrabarty). In addition, the plant dry weight data showed a more apparent trend than the fresh weight. Thus, the untreated plants showed a 28% reduction in dry weight, while the plants treated with cytokinin, Salicylic acids, Gibberellic acid, ethylene inhibitor, and Abscisic Acid treatments showed reductions of 20%, 7%, 2%, 10%, and 10%, respectively (Figure 3).

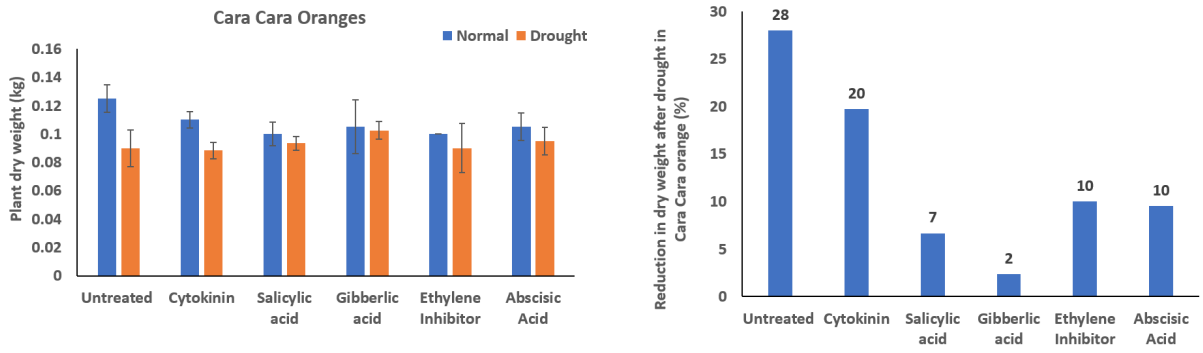

Figure 3: Effect of plant hormones on the response of Cara Cara oranges dry weight to drought.

In Cara Cara Oranges, the effect of drought on plant growth was similarly observed as in Tango Mandarin. The untreated plants (control group) experienced a significant reduction of 51% in fresh weight when exposed to drought conditions for five weeks. However, when treated with various plant hormones, the negative impact of drought was reduced to some extent. The results showed that the cytokinin, Salicylic acid, Gibberellic acid, ethylene inhibitor, and Abscisic Acid treatments resulted in reductions of 57%, 44%, 47%, 54%, and 44% in fresh weight, respectively (Figure 4). Thus, it was observed that the cytokinin growth regulator had a negative effect on the reduction percentage of the fresh weight after drought in Cara Cara orange saplings. As the reduction percentage of fresh weight was highest in cytokinin (57%) even higher than the

reduction percentage of untreated or the control group which was 51%. Furthermore, Salysalic acid, Gibberellic acid, and Abscisic acid had the lowest reduction percentages and this is because the seedlings treated with Salicylic acid and Gibberellic acid were 44% and 47% which are really low compared to the untreated or control group which was 51%. The reason is that Salicylic acid helps to induce the defense mechanism for the plant when the plant faces some type of pressure stress from the environment or any other factor and manner in which the plant defends itself is through using its mechanism such as morphological, physiological, and biochemical methods (War). Thus, when the seedling faced the environmental stress of drought, seedlings that were treated with Salicylic acid were able to adapt better in that situation and this is why the reduction percentage is lower for these particular groups or plants. Further, the reason why the Abscisic acid-treated plants have only a 44% reduction in fresh weight after drought in Cara Cara orange is that Abscisic acid controls the stomata closing and opening and this helps in controlling the water loss due to transpiration (Brookbank et al.). Thus, helps the plant survive better in a drought-like situation. Thus, this is why the reduction percentage of weight is lower in Abscisic acid-treated plants. Further, the reason why the reduction percentage of weight is also lower in ethylene inhibitor treated seedlings which would reduce the senescence process associated with the drought condition, thereby maintaining the plant and the growth rate.

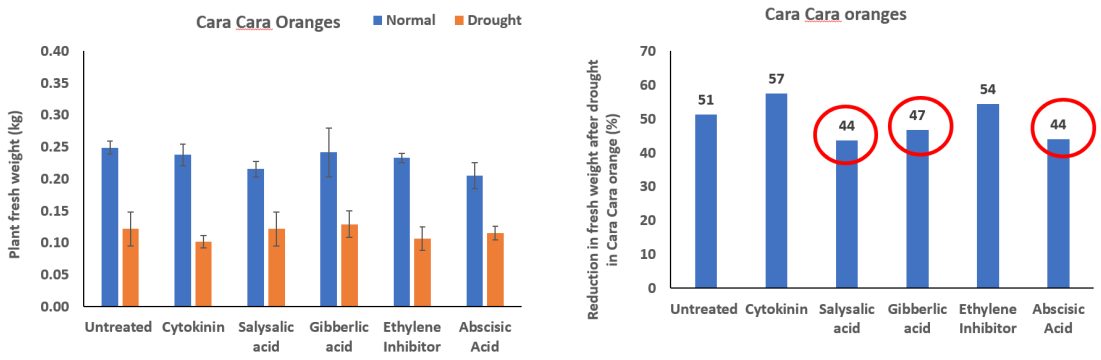

Figure 4: Effect of plant hormones on the response of Cara Cara oranges fresh weight to drought.

Furthermore, when comparing the chlorophyll levels in the control groups and experimental groups, the results were observed in Figures 5 and 6. Thus, when comparing the differences in the chlorophyll content among the different treatments, the trend was not prominent. as they were very minor. Despite these differences, the reduction weight difference between the control and experimental groups, there is enough evidence to prove that all the hormones had a positive effect on the Cara Cara Mandarin tolerance to drought. The most effective treatments were found to be the ethylene inhibitor (AVG) and Abscisic Acid. The ethylene inhibitor reduced the senescence process associated with the drought condition, thereby maintaining the plant and the growth rate, while Abscisic Acid played a role in reducing water loss, resulting in higher drought tolerance. Overall, the results indicate that using these plant hormones can effectively reduce the negative impact of drought on the growth of Cara Cara Mandarin and potentially other citrus crops.

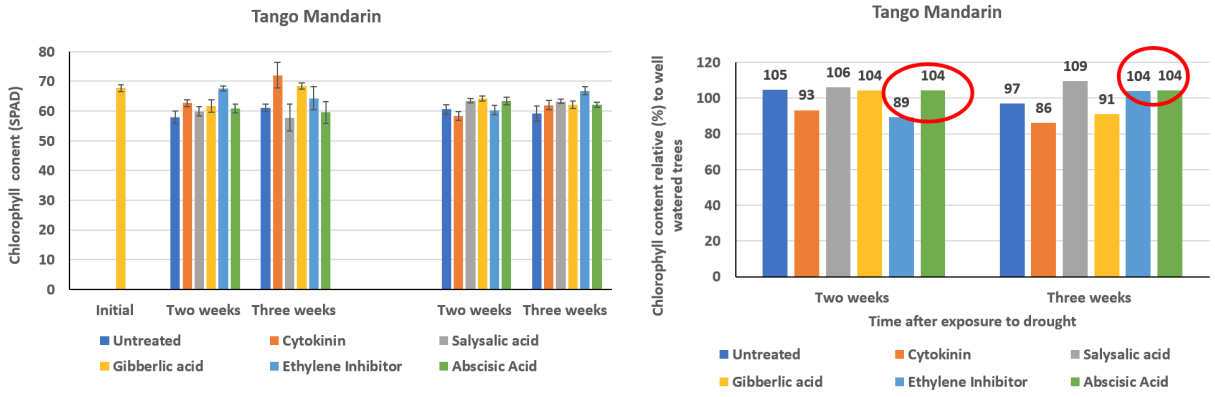

Figure 5: Effect of plant hormones on the response of Tango mandarin leaf chlorophyll content to drought.

Furthermore, the results revealed that the application of hormone treatments had a limited effect on chlorophyll content. Although some differences were observed among the treatments, the trend was not significant. This suggests that the hormone treatments had a minor impact on the photosynthetic process of Tango Mandarin and Cara Cara oranges seedlings under drought conditions. For Figure 5, the chlorophyll content relative to well-water plants is about 89% (Ethylene inhibitor) and 104% for Abscisic Acid growth regulator treated seedlings for the tango mandarin seedlings. For Figure 6, the chlorophyll content relative to well-water plants is about 110% for cytokinin-treated seedlings, 106% for Gibberellic acid-treated seedlings, and 104% for abscisic acid-treated seedlings. However, though the difference in chlorophyll content is much very significant, it helps to understand the observation that the growth regulators do positively impact the plant to survive in a drought-like situation.

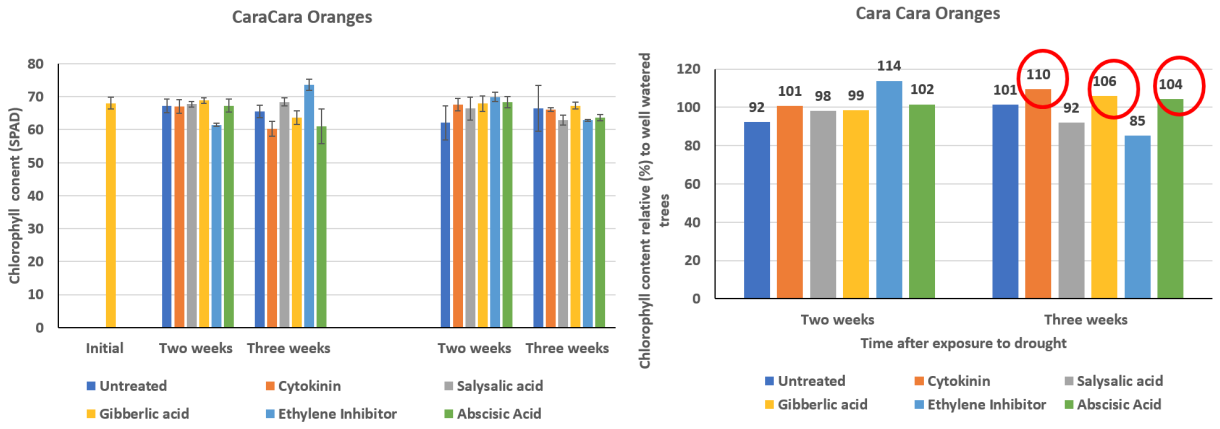

Figure 6: Effect of plant hormones on the response of Cara Cara oranges fresh weight to drought.

Dry weight is a critical trait to measure in plants as it reflects the amount of biomass that has been accumulated through photosynthesis. During photosynthesis, plants produce various polysaccharides, which are essential for plant function and survival. The net result of plant metabolism is translated into the dry matter, which measures the plant's overall health and condition. Plants that are more tolerant and resilient to abiotic stress are able to maintain their performance and keep their various metabolic processes running efficiently.

However, when plants are subjected to abiotic stress such as drought, they must allocate their energy towards protecting their organs and tissues against the stress. For example, under drought stress, plants will close their stomata to reduce water loss, which is used to obtain carbon dioxide from the air and perform photosynthesis. As a result, the reduction in photosynthesis translates into a reduction in plant energy and overall metabolism, which ultimately results in a decrease in plant dry matter. Plant hormones play a crucial role in helping plants respond and adapt to various environmental stresses, including drought. One of the essential functions of plant hormones is to improve the signaling pathways that trigger a cascade of events that activate all the defense mechanisms against abiotic stress. By stimulating the production of specific proteins

and enzymes, plant hormones can help plants better tolerate and overcome the effects of drought stress. For example, cytokinins can stimulate cell division and promote the growth of new leaves and roots, while abscisic acid can reduce water loss and promote stomatal closure. By improving plant hormone signaling pathways or treating the plant with them, we can develop new strategies for improving plant tolerance to abiotic stress and ultimately enhance crop productivity and sustainability.

CONCLUSION

Lastly, based on the experiment, it has been proven that the growth regulators do positively impact the seedlings to a great extent in a drought-like environment. There are some growth regulators that have a much larger positive effect than the other growth regulators. In the tango mandarin seedlings, it was observed that the Ethylene inhibitors and Abscisic acid had a much bigger impact in helping the seedlings to survive in the drought-like environment in comparison to the other growth regulators. Further, for the Cara Cara Orange seedling, Salicylic acid, Abscisic acid, and Gibberellic acid growth regulators had a much bigger impact than the other growth regulators. Thus, it can be stated that the growth regulators have different impacts on different plants and the growth regulators have an overall positive impact on the seedlings to adapt to a drought environment.

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