Characterization of Drought Responsive Genes in Tomato Genotype(s) Under Drought Stress Conditions

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This thesis is submitted in part completion of the requirements for the master's program in Plant Biotechnology

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I hereby confirm that the research work titled " Characterization of Drought Responsive Genes in Tomato Genotype(s) Under Drought Stress " is solely my own effort. This work has not been submitted elsewhere for evaluation. Proper acknowledgment and referencing have been provided for any materials sourced from external references.

Kalsoom Ali

Master of Science in Plant Biotechnology

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Acknowledgment

I begin by expressing my gratitude to Almighty Allah (God) and the Prophet Hazrat Muhammad Mustafa (peace be upon him) for their blessings and guidance throughout my research journey. It is through Allah's help that I have been able to complete this work, and I acknowledge that His blessings far exceed what I deserve.

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Kalsoom Ali

Dedication

“I would like to express my heartfelt dedication of this remarkable achievement

to my beloved husband.”

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List of Abbreviations

DER	Dehydration responsive element
ROS	Reactive Oxygen Species
H2O2	Hydrogen peroxide
$^{\cdot}OH$	Hydroxyl radicals
NO	Nitric oxide
O2.-	Superoxide anion
$1O_2$	Singlet oxygen
APase	Acid Phosphatase
SOD	Superoxide dismutase
POD	peroxidase
PCR	Polymerase Chain Reaction
RT	Reverse Transcriptase
TAE Buffer	Tris base-acetic acid EDTA Buffer
qRT PCR	Quantitative Real-Time Polymerase Chain Reaction
SD	Standard Deviation
CAT	Catalase
APX	Ascorbate Peroxidase
MDA	Melo-dialdehyde
NCBI	National Centre for Biotechnology Information
MW	Molecular Weight
Chl	Chlorophyll
TF	Transcription factor
PBS	phosphate-buffered saline

ABSTRACT

Tomatoes are widely consumed worldwide as a horticultural crop. Abiotic stresses limit crop production on a global scale, and drought poses significant challenges to food security. Crop productivity and growth are affected by drought, one of the most important factors that impact plant morphology, at physiological, biochemical, and molecular levels. In drought response, a complex signaling network leads to gene upregulation. *DERB* (Dehydration Responsive Element Binding) transcription factors play a vital role in crop development and stress-responsive pathways. This study aims to assess the impact of drought on various aspects of tomato plants, including morphological traits (plant height, root length, shoot length, number of branches), physiological traits (root/shoot length, leaf area, relative water content, SPAD value), and biochemical traits (superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, malondialdehyde content), along with gene expression. The results confirmed significant variations in these parameters for *Solanum l.* x. T-1359 under both drought and well water conditions. Notably, the expression of *DERB3* and *DERB4* significantly increased under drought stress. The results confirmed that there are significant variations in agronomic, physiological, and biochemical parameters *Solanum l.* x. T-1359 under drought and well-water conditions. A significant increase in DREB3 expression was observed under drought stress conditions, with a relative fold change of 14.62 and 6.67 in the leaf and 9.09 and 6.04 in the root when compared to control conditions on the 7th and 14th days, respectively. The expression of DREB4 also increased significantly under drought stress conditions, with a relative fold change of 14.62 and 6.67 in the leaf, and 9.09 and 6.04 in the root, respectively, when compared with the control condition on the 7th and 14th days.. The performance of Solanum l. x T-1359 used in the study showed significant differences in all studied traits. Moreover, By collectively delving into morphological, physiological, and biochemical facets, employing advanced methodologies, and focusing on *DERB3* and *DERB4*, a promising avenue emerges for the enhancement of current tomato cultivars or for the meticulous screening of drought-resistant tomato genotypes.

Keywords: SPAD, qRT, Morphology, Physiology, Antioxidants, *DERB3*, *DERB4*, Drought

Chapter 1

INTRODUCTION

1.1. *Solanum lycopersicum:* An Economical Crop and a Model Plant

Solanum lycopersicum, generally known as tomato, is an exceedingly grown vegetable species around the world Agricultural importance and edible fruit . Belonging to the Solanaceae family, Tobacco, potatoes, eggplants, peppers, and petunias are all closely related to tomatoes. The tomato is a widely popular vegetable crop that contains a wide range of health-promoting compounds. Model organisms are ideal for studying how genes affect biological processes and for researching nutrient metabolism . In 1753, Carolus Linnaeus introduced the scientific name *Solanum lycopersicum* for tomato. However, *Lycopersicon esculentum* was given its genus namein 1754 by Philip Miller. Studies of chloroplasts at the molecular level and nuclei indicate a closeconnection between the tomato and *Solanum tuberosum* (potato). These crops are members of the Solanaceae family of the Plant Kingdom, which comprise frugal important crops like *Capsicum annuum L*. (chili pepper) and *Solanum melongena L*. (eggplant) (Maqsood et al., 2022).

America, southern Europe, the Middle East, and India cultivate tomatoes, which are one of the most popular fruits in the world. China, Japan, and Southeast Asia have seen an increase in tomato production in recent years (Gerszberg et al., 2015). Mediterranean regions such as Italy, Spain, and southern Europe are much involved in its production due to their temperate and tropical climate . The tomato crop, which started in western South America, was localized in Central America. In recent years, to increase productivity, fruit quality, and resistance to biotic and abiotic stresses, selective strains have been developed. This is because of the crucial role that tomatoes play as a food crop . The Mediterranean weather conditions found in Europe and America experience less droughts and salt stresses, which leads to more tomato production with minimal difficulties for farmers. Tomatoes are considered an important element in a healthy diet because of their abundance of bioactive compounds . Production programs in tomato growing areas have prioritized genetic development of the crop, with a focus on developing and adapting varieties that are acceptable for local conditions. Besides being an important vegetable crop

and India and widely used in local cuisine, tomato production in Pakistan remains low due to a unavailability of locally developed and adapted varieties, as well as poor genetic material. Therefor efforts have been made to raise the genetic potential of tomatoes through breeding programs, aiming to increase production, disease resistance, and overall quality of the crop . Tomatoes are a versatile food that can be used as fresh in salads or cooked in sauces and soups to complement meat, vegetable, and fish dishes. Among other processed foods, they can also be found in ketchup, sauces, purees, pulp, and juices. Tomatoes are essential economically as both dried and canned products. In addition, they provide an excellent source of calcium, iron, and vitamin C, with a 100g serving containing 31mg of vitamin C . Among the many antioxidants found in tomatoes are vitamin C, vitamin E, carotenoids, and polyphenols like kaempferol and quercetin. These antioxidants are extremely effective in encountering the dangerous active oxygen species (ROS) in the body. The antioxidant property of ascorbic acid allows it to neutralize free radicals immediately, Superoxide, hydroxyl radicals, oxygen singlet radicals, and hydrogen peroxide are some examples of free radicals. Lycopene, a carotenoid that provides tomatoes with their traits red color, enhances tomato nutrition and marketability. The level of lycopene in tomatoes has been connected to the quality of the fruit, and many studies have depicted its beneficial effects on health. Furthermore, the combination of different antioxidants present in tomatoes can provide additional health benefits to individuals who consume them. In addition to global warming, water scarcity, and a growing population are contributing factors. It is therefore imperative that there is sufficient food on our planet for everyone (Parveen et al., 2019).

Its limited resistance to salt, temperature, light, and drought makes it highly susceptible to environmental stress, particularly from salinity and drought. These stressors can guide to negative effects on the plant such as increased root-to-shoot ratio, slower growth of both shootsand roots, reduced leaf area and number, and premature leaf aging, ultimately reducing crop yield . Tomatoes are widely regarded as a leading model for genetics, breeding, and genomicresearch due to their enormous agricultural and economic importance (Choudhary et al., 2018). The analysis of descriptive statistics has shown important variation in several important characters of tomatoes, Fruit length, fruit width, pedicel length, number of inflorescences production per plant. The results of these studies indicate that it is possible to improve these traits by tapping into existing tomato germplasm (Ali, et al., 2017).

1.2. Tomato Life Cycle and Cultivation Areas in Pakistan

The tomato is very popular and largely cultivated worldwide, with a preference for moderate climatic conditions and the ability to thrive on fertile, well-drained soil (Koop et al., 2022) . Tomatoes are a widely cultivated and significant vegetable internationally, with China, India, the USA, and Turkey being the foremost producers, accounting for 70% of the world's gross production. Besides its agricultural importance, Pakistan ranks 36th globally in terms of tomato production and 52nd in tomato exports. In fact, Pakistan's contribution to global tomato output, exports, and growing areas is relatively small, constituting 0.3%, 1.3%, and 0.3 (Ahmad & Ahmad, 2022). Tomatoes are a very valuable and popular vegetable crop with important economic and health benefits due to their anti-oxidative and anti-cancer characteristics. Their production and use carry on increasing globally. Over 164 million tons of tomatoes are produced on nearly 4.8 million hectares, ranking tomatoes seventh in the world. Tomatoes now rank second among the world's most important vegetables thanks to extensive breeding effort (Parveen et al., 2022).

Tomatoes started in Central and South America. It is the third-largest vegetable planted around the globe, following by potatoes and onions Considering the area and processing first. Plants generally reach a height of 1-2 meters (3-6 feet) because of their weak stems (vines), which demand support. Local varieties, when fully matured, typically reach a height of 2 feet. Although tomato plants can be perennial, they are normally grown as annuals due to determinate tomato plants having a lower sugar content (M. Z. Khan et al., 2022). Tomatoescan be cultivated in the summer season with an ideal temperature range of 25-29 °C, but they can also be grown during the winter season . The growth of tomatoes is characterized by a rapid rate, typically ranging from 80 to 150 days, as indicated in (Table1).

Tomatoes, often taken as a "fruity vegetable," are the second most widely grown vegetable crop in Pakistan. They are normally grown during the summer season in either open fields or environmentally controlled greenhouses. In tomatoes, we find significant amounts of vitamins A and C, along with lycopene, an antioxidant that cannot be found in other Solanaceae plants. Additionally, they contain 94.28 grams of water and 0.712 mg of calcium, niacin, and niacin. Beside their nutritional value, tomato production in Pakistan are often low .

Stresses are abiotic as well as biotic including abiotic and biotic stresses (Chohan et al., 2015) . Tomatoes production is well when the temperature is between 15.6°C to 20°C, and during the day, 25°C to 30°C. However, if the temperature is too low (below 10°C) or too high (above 35°C), it can defect the growth of the tomato plants (Silva et al., 2022).

Drought and salinity can negatively impact the development of tomatoes, causing delays in the flowering stage, distortion of fruit shape, and decreased nutrient levels due to lower moisture content, as well as hindered seed germination (Fatah, Mubarik, & Aqsa, 2020). However, tomatoes are a versatile crop that can be grown year-round in many regions, and are able to adapt to various soil types, particularly those with high water capacity such as a mixture of peat and compost. Organic nutrients and optimal pH levels also contribute to higher fruit production and yields Nevertheless, tomato production can be affected by regional factors and climate conditions, such as the monsoon season and extreme heat in June to August, which may result in reduced output. In this scenario, regions with higher elevations and colder, drier seasons are preferred over lower lands because frost can harm tomato yields in harsh winters. However, due to Pakistan's humid climate and limited access to high lands, tomato production is favored in lowlands, resulting in a significant increase in tomato prices. Tomatoes are crops that require a lot of labor, but they have high production rates and are commercially attractive, with a relatively short lifespan. As compared to cereal crops, tomato production provides producers with slightly higher profits and creates more job opportunities for rural workers (Caruso et al., 2022).

Tomatoes are a highly popular horticulture crop worldwide, cultivated over 5 million hectares and producing over 182 million tons. Pathogens and new diseases have spread rapidly due to several factors, however, In addition to monoculture factors, intense selection, domestication over recent years, international trade of infected propagating materials, and climate change. These factors have enabled for organisms to survive in unfavorable environments, leading to the emergence of new diseases .In this situation, it is better to grow tomatoes in places with higher altitude and colder, drier seasons to avoid damage from winter frost. But in Pakistan, it's too humid in those areas, so they grow tomatoes in lowlands instead. This has caused the price of tomatoes to go up. Growing tomatoes is a good way for farmers to make money and create jobs for people who live in rural areas because it requires a lot of work but can be profitable. It's better than growing cereal crops in terms of earning money and providing job opportunities (Fatah et al., 2020).

There are several types of tomatoes that are commercially grown, including but not limited to (Roma, Rio Grande, Money Maker, Nadir, Pakit, Persimmon, and Naqeeb) (Maqsood et al., 2022).

1.3. Tomato Statistics

Tomatoes are not only used raw as a vegetable or in salads, but they are utilized in the production of various food products such as ketchup, sauces, pulp, purees, and juices. Both canned and dried tomatoes are important processed food items that play an important part in the economy. Tomatoes are an easy source of calcium, iron, vitamin C (providing 31mg per 100g), and vitamin A (Fatah et al., 2020). Tomato juice contains terpenes, a type of volatile compound that originates from the fruit. Chemical processing involves the Millard reaction, lipoxygenases, and carotenoid oxidation. Thermal treatments can trigger these reactions, leading to the production of more volatile chemicals (Servili, et al., 2000) . Pakistan, despite having the lowest per capital greenhouse gas (GHG) emissions, is one of the top five countries in the world that are facing severe Changes in climate (Bashir et al., 2022). The sustainable development of Pakistan's agriculture industry is critical for the country's rural development and food security. Not only does it provide employment opportunities, but it also generates forex. Agriculture contributes 22.7% to the GDP of the country and provides raw materials for industry. The horticulture sector accounts for 12% of the state's agriculture GDP, with vegetables being the most important horticulture product. Among the most widely grown vegetables in Pakistan are tomatoes, potatoes, chilies, onions, carrots, turnips, peas, cauliflower, and gourds, t covers 78% of the total area and produces 81% of the total. Pakistan exported 8.80 million tons of vegetables during the fiscal year 2019-20, an increase of 16.1% over the previous year (GoP, 2020). To produce high-quality tomatoes, a cool and dry climate is essential, which makes the tomato crop increasingly profitable due to its short growing season and expanding cultivation area. Among the most popular vegetables in the world, vitamins, minerals, fibers, and amino acids are abundant in tomatoes. including iron, phosphorus, vitamin B, and vitamin C. Additionally, the antioxidant lycopene, naturally present in tomatoes, has been shown to inhibit the development of various cancers. Tomatoes are consumed fresh in salads, soups, curries, sauces, .etc., while tomato products such as ketchup, juice, and puree have a high market value (A. Khan, Qadar, & Awais, 2023).

Water use by domestic, industrial, and agricultural sectors will increase by 23% by 2025, which poses a significant threat to food security worldwide. In densely populated regions like Chinaand Pakistan, irrigated agricultural output is expected to face a severe water problem. In areas where rainfall is insufficient, irrigation is often seen to boost agricultural production. However, due to a scarcity of fresh water, some farmers resort to irrigating crops with lower quality water. Salinization, especially when salinized water is used for irrigation, poses a significant challenge for agricultural fields. Currently, salinization affects 33% of the world's irrigated lands, and it can have detrimental Plant growth effects by influencing physiologicalactivities such as photosynthesis and transpiration. Plant mortality can result from ion toxicityand osmotic stress caused by salt stress., reducing transpiration, and ultimately hindering plant development by decreasing photosynthesis. The severity and duration of salt stress have a direct correlation with plant growth. Numerous studies have explored the effects of salt stress and techniques to mitigate its harmful impacts (She et al., 2018).

1.4. Tomato Production in Pakistan

To check the time series data for tomato production, it is important to analyses the stationarity of the states. ADF unit root tests were conducted, and results are presented in Table 2. The results indicate that tomato prices(PT) and rainfall (R) series are stationary at the level with trend since the null hypothesis can be rejected. Besides, the temperature series is nonstationary, both with and without trend at the 5% and 10% levels of significance. To achieve stationarity, the temperature series demands differencing. In the end, the analysis shows that tomato prices and rainfall are stationary at the level with trend, while temperature requirements differencing to attain stationarity significant limitations the process of growing or cultivating tomatoes (FAO, 2023).

1.5. Major Restraints in Tomato Production

In the coming couple of decades, the world population is anticipated to rise significantly, leading to a rise in food intake. Crop production may be affected by climate change. Tomato nutritious fruit generally used in processed foods and consumed raw or cooked, climate change. metabolic interactions, causing significant impacts on the plant's physiology (Kaleem et al.,

2018). threatens this region particularly due to its short life cycle. Tomato growth and development can be adversely affected by several factors, drought, temperature, salinity, light, pests, and microbesare examples of these factors. Plants can be affected by these pressures in a variety of ways, suchas their morphology, transcription, and metabolism. Automatically Overall crop productivity is reduced as a result (Maqsood et al., 2022).

Global warming is the rise in Earth's surface temperature is referred to as global warming and ocean surfaces caused by the accumulation of greenhouse gases, primarily CO_2. Climate change refers to the resulting impact on the planet's climate system, leading to more frequent and severe heat waves, cold snaps, and flooding (Rivero et al., 2022). Tomatoes are highly exposed to ecological stressors, including heat, cold, salinity, and drought. Among these, drought stress has an important impact on tomato production, leading to reduced export rates and increased local prices. Drought negatively affects fruit development and delays blooming, as tomatoes are water-rich fleshy fruits. Additionally, water scarcity decreases photosynthesis, disrupting the vegetative phase and hampering tomato growth. As a result of decreased production rates caused by drought stress, the stomatal openings on leaves close, reducing turgor pressure and causing leaf curling (Sahoo et al., 2013).

Tomatoes are highly sensitive to temperature changes. Exposure to high temperatures can damage their photosynthetic process by disrupting the electron transport chain and the photosystem. This results in destabilizing electrolytes, intermolecular interactions, and metabolic pathways. As a result, plant functions become less stable during heat stress. Both the vegetative and reproductive phases of tomato plants slow down in hot weather, ultimately reducing tomato production and growth. Additionally, the heat stress can cause the fruit to become drier, further impacting tomato yield (Zhao et al., 2020). Temperatures that are extremely low, the production of crystals in the cell wall can cause damage to the cell membrane, leading to the release of cytosol and ultimately plant cell death. Additionally, changes in temperature, carbon dioxide levels, and drought stress can trigger the activation of the ROS pathway in plants, which can have negative effects. In this pathway, cellular ions can be disrupted and ROS, hydroxyl radicals, and hydrogen peroxide produced. Abiotic, biotic stressors activate the ROS pathway in plants (Tanveer et al., 2020). Salinity stress affects ionic equilibrium of plants and has a pronounced effect on salt-sensitive crops like tomatoes. This stress disrupts the photosynthetic pathways an Ecological change is a

global phenomenon, its impacts manifest differently in different areas and can lead to the co-occurrence of multiple distinct environmental stressors for instance, Certain parts of the Midwest have experienced flooding as well as high temperatures in recent years. While significant portions of the US West have experienced severe drought stress in combinationwith heat waves. The drought and high salinity levels in many parts of Australia and Northern Africa, on the other hand, have resulted in elevated temperatures and salinity levels in many regions. Climate conditions often coincide with harsh soil conditions, in which nutrients are lacking, soil salinity is high, pH levels are extreme, and Heavy metals, microplastics, pesticides, herbicides, antibiotics, and persistent organic pollutants are abundant in the environment. Climatechange has been linked to heat waves, droughts, and floods, as well as various diseases and insectoutbreaks. The individual abiotic and biotic stresses stated earlier can each pose an important risk to agricultural production. Climate change is complex and multifaceted, so the simultaneous interaction of multiple stresses may threaten Global food security, crop production, and major crops. Recent studies indicate that climate change will negatively affect Rice, sorghum, rapeseed, cassava, rapeseed oil, cassava, maize, and sugarcane are among the crops. While there are some differences between crops and regions, overall, these crops produce 1% fewer consumable food calories (Rivero et al., 2022).

1.6. *DREB* Genes in Tomato

DREB genes are vital in providing stress tolerance to tomato plants which are extremely sensitive. Stress is caused by environmental factors such as salinity, drought, heat, and cold. Promoter elements of these genes contain cis-regulatory elements called the Drought Responsive Element (DRE), which plays a crucial role in regulating gene expression under stress conditions (Maqsood et al., 2022). Abiotic stresses like salinity, droughts, and extreme temperatures greatly influence crop growth, survival, productivity, and distribution. In response to environmental cues, plants alter their cellular, physiological, biochemical, and molecular processes. It is important for plants to adapt to abiotic stresses through the *DREB* protein. DRE/CRT cis-elements bind APETALA2 (AP2), which targets stress-responsive genes independent of ABA. Recent research has extensively studied the use of *DREB* proteins and related regulatory genes and abiotic stress tolerance elements, suggesting the potential of generating transgenic plants to manage these stresses in crop plants (Singh & Chandra, 2021).

1.7. Morphological Characterization

The tomato is an edible fruit belonging to the Solanaceae family and is native to South America. Despite its high tolerance to high temperatures, it can be harmed by frosts as a vegetable, tomatoes are commonly consumed as a berry due to the thick pericarp that contains many seeds. The fruit contains locules or cavities that vary in number and influence the size and shape of the fruit. Tomatoes can be classified as determinate or indeterminate based on their growth patterns. The quality of tomato fruits is mainly assessed by physical traits, firmness, and taste. Breeding programs have focused on developing yield, fruit size, appearance, disease resistance, firmness, and shelf life. Traits of tomato germplasm through morphological and agronomic traits is helpful in the development of new varieties, hybrid seed production, and identification of desirable genotypes (Ali et al., 2017).

Tomatoes belong to the Solanaceae family of flowering plants and are mainly grown in tropical and subtropical regions during late spring and early summer. Besides, they are preferably cultivated during autumn and winter as they are less vulnerable to diseases during this time. With their appealing color, taste, and nutritional value, tomatoes are widely cultivated across the world today. They are mainly known for their rich content of the antioxidant lycopene, which has been associated with a decreased risk of cancer and benefits cardiac patients. Additionally, tomatoes are considered a valuable addition to a balanced diet because of the presence of essential nutrients Vitamins A and C, potassium, fiber, and iron . Changing environmental factors, Drought, extreme temperatures, . Salinity, flooding, and metal toxicity impact crop productivity. Plant development and growth are hindered by drought, which can result in lower yields and even plant death. In response to drought stress, plants complete their life cycles before soil dehydration, close their stomata, and reduce their leaf area. Water stress, however, can decrease net photosynthetic rate and stomatal conductance. Due to this, to cope with water deficit conditions, plants produce compatible solutes that act as osmotic adjustment and osmo-protectants, as well as antioxidant compounds. These physiological and biochemical mechanisms are crucial for plant survival and productivity under water stress conditions. Drought is a significant problem that affects crop production on about 25% of arable land worldwide. The negative impact of water shortage on

Solanaceae family, the winter season provides a conducive atmosphere for production. However, the occurrence of water deficit or moisture stress during this period, especially in the northwestern regions, poses a significant challenge to tomato cultivation. As a result of drought stress, optimal strategies vary significantly. Different species of plants may respond differently to drought stress, even within the same species how long it lasts, and how the plant is growing and developing. As drought stress progresses, plants respond by closing their stomata and rolling their leaves, which can make it difficult to measure their physiological responses. Often, SPAD chlorophyll meters are used to assess drought tolerance as a rapid and cost-effective method. Water stress not only affects the morphology of the plant, but also its metabolism, Growth and yield can be adversely affected by this (Sarker et al., 2020).

Figure: 1 Drought can lead to different consequences for tomato plants**.**

Table: 1 illustrates the duration of growth periods for tomatoes.

Stage	Development Stage	Duration (Days)
0	Establishment and seed germination phase	25-35
1	Vegetative Phase	25-30
2	Flowering Phase	25-30
3	Fruit Formation Phase	25-30
4	Ripening Phase	05-20
5	Total Days	105-145

Table: 2 The seasonal variations in tomato production across regions in Pakistan.

Province	Major Area of Production	Sowing Season	Availability
Balochistan	Bolan, Kharan, Lasbila, Turbat, Sibi, Quetta, Loralai, Qila Saifullah, Khuzdar, Mastung, Pishin	Rabi Kharif	Nov to March July to Oct
Khyber Pakhtunkhwa	Mardan, Swat, Dir, Malakand, Chitral, Mansahra, Haripur, Charsada Peshawar, Charsada, Noshera, Tank, DI Khan, Mardan, Malakand	Rabi Rabi	Nov to March Nov to March
Punjab	Southern Punjab, Centeral Punjab, Rahim Yar Khan, Khushab	Rabi Rabi	April to May May to June Nov to Dec Jan to March
Sindh	Badin, Karachi, Thatta, Nawab shah, Mir Pur Khas, Hyderabad, Umerkot, Noshero Feroz	Rabi	Nov To March

1.8 Research Objectives

Following objectives were specified for the identification and characterization of Tomato Genotype(s):

1. Morphological and physiological characterization of *Solanum l. x.* T-1359 under drought stress conditions.
2. To evaluate antioxidant activities in *Solanum l. x.* T-1359 under drought stress conditions.
3. Expression profiling of drought responsive *DERB3* and *DERB4* genes in *Solanum l. x.* T-1359 under drought stress conditions.

Chapter 2

LITERATURE REVIEW

2.1. Tomato Response to Environment Alteration and Abiotic Stress

Globally, many efforts have been made to model future crop production under warming conditions because of the significant impact of ecological change on vital crops. The acquiring of global sustainable development goals mainly depends on confirming food security and eradicating hunger. However, the effect of ecological change has made food production increasingly hard. Several extreme weather events have occurred in the last few decades,such as prolonged and heavy precipitation, droughts, and heatwaves, making the situation more complex. Climate change is the result of biotic and abiotic stress negatively impacting agricultural productivity. Climate change and ecological variability can be evaluated through an examination of the frequency and intensity of stress events, their impacts on daily life, and their effects on crops (Raza et al., 2019). Agricultural productivity in developing economies can be adversely affected by unfavorable weather conditions and high unemployment rates. To encounter this issue, scientists have developed innovative methods for managing these challenges and sustaining plant growth and production, despite the effects of rising temperature s and CO_2 levels, as described in the research (Rosenzweig et al., 2014).

Ecological change and the rising human population are exacerbating the limitations on agricultural production and leading to ecological effects, which include Abiotic stresses negatively affect plant growth and development. Developing countries in low latitudes are prone to face the most vital negative results of climate change. To tackle these challenges, researchers are making efforts to establish adaptation techniques and develop climate-smart crops that can bear these stresses. Extreme light is one type of abiotic stress that plants can face, and it can harm photosynthesis, resulting in oxidative stress and affecting crop yield and growth. Therefore, studying abiotic stressors is crucial in developing strategies to address the limited availability of food and maintain a healthy environment (Pereira et al., 2016).

2.1.2. Effects of Drought stress

Plant development can be severely affected by abiotic stressors caused by environmental conditions. Crops undergo a variety of adaptations to cope with these stressors, Morphological, physiological, biochemical, and molecular changes take place during the process . Drought stress has become a major concern worldwide for agriculturalproducers. Plant growth and development are adversely affected by its multifaceted nature at various stages. High temperatures, reduced rainfall, declining groundwater levels, and limited water resources all contribute to the severity of the climate. It is possible for plants to undergo a variety of biochemical, physiological, and genetic reactions if there is not enough rainfall or soil moisture (R. Zhou et al., 2017)**.**

The effects of drought can be seen in the disruption of normal physiological processes and the alteration of metabolism, resulting in limited crop production. This, in result, can lead to stunted growth, plant damage, or even death. Environmental stress is a significant factor that limits agricultural production worldwide, with only around 10% of arable land taken stress-free. Environmental stresses, such as drought, are a critical factor in the gap between crop yield and potential performance. Nearly 25% of agricultural lands worldwide are affected by drought and other environmental stresses, a significant risk to crop production. A drought occurs when physical and environmental factors combine to cause stress in plants, resulting in a reduction in productivity (Fathi et al., 2016). Plants can detect changes in environmental factors, particularly deficiency of water in the soil, and respond accordingly to adapt to drought conditions. Deficiency of water signals are transmitted from roots to leaves by abscisic acid (ABA) accumulation. Drought stress responses in plants are controlled by both ABA-dependent and ABA-independent mechanisms. There is no central nervous system in plants roots are connected to shoots via their vascular system and integrate information about stress from both underground and above ground sources. As a result of drought stress, various mechanisms are activated, including ROS, mRNA, and phytohormones are some of the signals that can be transmitted by hydrostatic pressure, electricity, calcium waves, and ROS. There have been numerous studies that have demonstrated that these mechanisms are significant in regulating plant drought responses. Transmitted from roots to leaves by abscisic acid (ABA) accumulation. Drought stress responses in plants are controlled by both ABA-

dependent and ABA-independentmechanisms. There is no central nervous system in plants roots are connected to shoots via their vascular system and integrate information about stress from both underground and above ground sources. As a result of drought stress, various mechanisms are activated, including ROS, mRNA, and phytohormones are some of the signals that can be transmitted by hydrostatic pressure, electricity, calcium waves, and ROS. There have been numerous studies that have demonstrated that these mechanisms are significant in regulating plant drought responses (Takahashi et al., 2020).

There are many factors that can negatively impact the growth and development of crops, including a drought. When plants experience drought, their stomata close, hindering the movement of carbon dioxide, and resulting in increased levels of oxygen and the production of ROS. These ROS can damage the plant's membranes, leading to disruptions in plant development, photosynthesis, and respiration. Moreover, drought stress can impair the building blocks of cells, such as lipids, proteins, carbohydrates, and nucleic acids, due to the damaging effects of ROS. The limited availability of water also reduces turgor pressure, leading to delayed cell growth. Additionally, drought stress adversely affects the actions of photosynthetic enzymes, reducing metabolic efficiency, and ultimately leading to the destruction of the photosynthetic apparatus .Crop production is facing a significant challenge due to drought stress conditions, which are impacting food security worldwide. As a result of rapid and severe changes in climate, the situation has become worse. Drought is one of the most important stress factors affecting crops' growth and development. Drought stress alters the basic morphology, physiology, and biochemical processes in plants, highlighting the need to identify these effects and implement improved crop management strategies (Iqbal, Singh, & Ansari, 2020).

2.1.3. Consequences of Extreme Heat

Physiological, morphological, and biochemical effects of temperature on plant growth and development. Heat stress can have a profound impact, specifically when joined with other stresses. For instance, high temperature increases water loss from transpiration and evaporation, resulting in drought stress. Additionally, heat stress can impede plant emergence and hinder seed germination, which may eventually lead to death (Ostmeyer et al.

2020). Plant growth, metabolism, and productivity are adversely affected by high temperature stress, which is a significant environmental stressor across the globe. Plant growth and development are highly sensitive to temperature, making plants susceptible to high temperatures. The response of plants to heat stress varies, depending on factors such as the intensity and duration of the stress and the type of plant species involved (Hasanuzzaman et al., 2013).The heightened levels of greenhouse gases, such as CO_2, in the atmosphere are leading to a forthcoming climate characterized by elevated temperatures and notable fluctuations in precipitation patterns. Along with a general rise in average annual temperatures, there has been a surge in the frequency, duration, and intensity of intervals marked by unusually high temperatures. Regions across the globe have already documented a trend of elevated frequency and severity of heatwave events (D. Wang et al., 2016).

Plant reaction to combined stressors can differ from those to individual stressors. Plants may close their stomata in response to drought stress, reducing the diffusion of carbon dioxide (CO_2) into the leaf, resulting in an imbalance between the Calvin-Benson cycle and the light reaction. It, in turn, can inhibit plant photosynthesis. On the other side, under heat stress, plant photosynthesis can be restrained primarily by affecting biochemical reactions . Heat stress results in plants to become thermos-unstable by reducing the number of electrolytes within the cell, because of rise in cellular membrane permeability. Heat stress produces reactive oxygen species, which damage membranes and result in ionic toxicity. The result is programmed cell death. When plants are exposed to heat stress and ion toxicity, they activate antioxidant enzymes and signaling pathways to counteract these negative effects. Some examples of these enzymes include peroxide, catalase, glutathione reductase, ascorbate peroxide, and superoxide dismutase. In conditions of heat stress, these enzymes and pathways act together to neutralize reactive oxygen species and prevent damage to the cell (Maqsood et al., 2022).

2.1.4. Impacts of Salinity Stress

Salinity is an abiotic stress that affects plants in the environment and production, with poor cultivation methods and ecological changes leading to a steady rise in salinity levels on arable lands. This has resulted in devastating worldwide results, with an estimated 50% of arable land

at risk of being lost by the mid-21st century. Salinity has already affected 1,125 million hectares of agricultural land on a global scale. Posing a big threat to agriculture. In China, 12.3 million hectares of agricultural land have been affected by salinity (Kumar et al., 2021). Due to its effects on crop yields, salinity reduces crop production by one third on 0.3 to 5 million acres of agricultural land every year Soil salinity is an important global challenge that badly affects agricultural production, and it ranks as the second most serious problem. Salinity of the soil can occur from either primary natural process, such as atmospheric precipitation and deposition from seawater, or secondary anthropogenic processes like week drainage, miss-managed water management, and carried irrigation with brackish ground water as soil salinity increases, an annual expansion of 0.3-1.5 million acres is expected. Due to this expansion causes crop productivity declines by up to 20% (Evelin et al., 2019).

Salinity stress adversely affects the development and production of plants, including water stress, cytotoxicity, and nutrient deficiencies. Plants subjected to salt stress have lower water holding capacity and are less able to absorb essential minerals because of an increase in osmotic pressure in the soil solution relative to their cells. Additionally, the direct penetration of sodium and chloride ions into plant cells may result in toxicity to cell membranes and metabolism. These primary effects can lead to decreased cytosolic metabolism, membrane function, and cell expansion. Besides, salt stress can induce oxidative stress by generating ROS, Consequently, calcium and potassium permeability cation channels at plasma membranes may exhibit anomalies Activation of proteases and endonucleases can accelerate due to potassium ion loss, as a result, programmed cell death occurs (Mushtaq et al., 2021).

2.1.5. Role of ROS under Abiotic Stress

Climate change, drought, high salinity, and heavy metal exposure are some of the factors that limit crop productivity and sustainability globally. These stressors impact normal plant growth and hinder the formation of yields (Waqas et al., 2019). Environmental stressors adversely affect crop yields and plant growth around the world. Globally, 45% of agricultural lands are affected by drought, while 19.5% of irrigated agricultural land is saline. The world's rice crop is also affected by flash floods to the tune of 16%. In field situations, combined abiotic stresses can cause even greater crop yield reductions than single abiotic stress,such as drought and heat. The effects of climate change are exacerbating these challenges, and increasing warming, droughts,

floods, and storm events are predicted. Consequently, crop yields will be further reduced, particularly in the tropics and subtropics (Onaga & Wydra, 2016). Plants in natural environments are vulnerable to various abiotic stressors that can significantly affect their growth and development. When multiple stressors occur at once, it can result in further negative impacts on crop production. Abiotic stresses such as drought and heat can be mitigated by plants by activating specific genes, accumulating solutes, and ROS. Plant cells produce ROS when stressed, but they are notuseful under normal conditions. ROS has the potential to damage the plants because of their reactivity, but they can also activate and control defense pathways that protect against both biotic and abiotic stresses. It's important to maintain a delicate balance between these two results, whichdemands constant monitoring. If ROS accumulates in the cell, it could harm the plant's health . Under normal developmental conditions, plant cells have a strong arsenal of antioxidants and scavenging enzymes that work together to keep ROS levels at bay, beside their continuous production through cellular metabolism . ROS levels can increase significantly when plant cells experience stress due to the downregulation of antioxidant capacity in specific cellular compartments. While it is known that ROS can cause harm to cellular components, it is also recognized that they play a crucial role in cellular signaling (Taria et al., 2022).

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Plants undergo adaptation to their environment over period, which leads to the development of organized pathways for tackling the challenges. Moreover, ROS could be used as a signaling molecule for activating defense cascades. Oxidative damage to lipid peroxidation occurs when ROS levels are high, causing damage to the cellular membrane and important components such as proteins, DNA, and RNA. This damage can cause oxidative stress and ultimately result in cell death. To encounter this toxicity, plants produce a variety of proteins that reduce various cellular activities and aid in ROS detoxification. These proteins include peroxiredoxin, catalase, ascorbate peroxidase, and glutathione peroxidase . ROS detoxification is regulated by multiple metabolic pathways. One such pathway involves the utilization of free, temporary forms of metals, like Fe^{2+}, which can mitigate by inhibiting the Fenton reaction, the levels of ROS are reduced . Peroxisomes, apoplasts, mitochondria, and chloroplasts produce peroxidases, oxidases, NADPH, RBOH, and ROS. Within the cell, the ROS gene network works to balance the production and elimination of ROS through various cellular processes (Onaga & Wydra, 2016).

There are several factors that can affect the level of ROS in cells, including their type, their developmental stage, the amount of stress they are experiencing, and the efficiency of their ROS homeostasis mechanism. Different transcription factors and MAP kinase pathways are triggered by these variations in ROS levels . Flood tolerance, on the other hand, is regulated by GA and ethylene-mediated signaling, while salt tolerance is regulated through ROS signaling. Plants also use sugar accumulation, hormonal regulation, and gene expression to adapt to cold temperatures. To cope with different environmental stresses, plants have developed sophisticated physiological, biochemical, and molecular adaptations. When plants experience drought stress regularly, they have a kind of memory that helps them respond better to the stress in the future. Researchers have found that plants respond differently to a single episode of drought stress than to repeated drought

stress. Arabidopsis produced certain types of molecules in response to drought stress . Molecular and genetic studies have shown that plants possess intricate regulatory systems that allow them to adapt to stress and tolerate it. These regulatory systems operate at various levels and are interlinked. This involves sophisticated networks of regulatory methods ranging from alternative splicing and transcriptional regulation to simulations and ubiquitination regulators. The mechanisms control processes such as chromatin remodeling and membrane transport, which are critical for maintaining cellular ion homeostasis Flood tolerance, on the other hand, is regulated by GA and ethylene-mediated signaling, while salt tolerance is regulated through ROS signaling. Plants also use sugar accumulation, hormonal regulation, and gene expression to adapt to cold temperatures. To cope with different environmental stresses, plants have developed sophisticated physiological, biochemical, and molecular adaptations. When plants experience drought stress regularly, they have a kind of memory that helps them respond better to the stress in the future. Researchers have found that plants respond differently to a single episode of drought stress than to repeated drought stress Arabidopsis produced certain types of molecules in response to drought stress . Molecular and genetic studies have shown that plants possess intricate regulatory systems that allow them to adapt to stress and tolerate it. These regulatory systems operate at various levels and are interlinked. This involves sophisticated networks of regulatory methods ranging from alternative splicing and transcriptional regulation to simulations and ubiquitination regulators. The mechanisms control processes such as chromatin remodeling and membrane transport, which are critical for maintaining cellular ion homeostasis (Chen, Hu et al. 2016).

2.1.6. Role of Transcriptional Factors against Abiotic Stress

Plants always face extreme temperatures, excessive salt levels, drought, pathogen infections, and other environmental variables that have a drastic impact on the yield and biomass production. With the rising effects of worldwide ecological change, abiotic stresses are anticipated to worsen. Drought and salinity are the two main stressors that affect plant growth. Despite this, plants have developed complex defense mechanisms that allow them to accurately deal with abiotic stressors and resist pathogen invasions. Plant resistance to abiotic stressors is influenced by many transcription factors (TFs). The expression of defense-related genes can be controlled by these TFs. TFs have also been investigated for their regulatory functions in stress

Transcriptional factors, protein phosphatases, and kinases are among the molecules that make up the regulatory group. These molecules work together to manage or adjust the biological process or pathway being discussed. the transcription factors in the regulatory group include DREB, bZIP, AREB/ABF, bHLH, MYB, and C2H2. Protein phosphatases include protein phospholipases and phosphoesterases, and kinases include MAP kinase, receptor protein kinase, transcription regulation protein kinase, and CDP kinase. In plants, transcription factors (PTFs) bind to distinct DNA sequences called cis acting elements (CREs) that regulate gene expression. These CREs are generally placed in the promoter areas of genes that have been specifically targeted for regulation. By binding to these CREs, PTFs can exert control over gene expression, a process that can be influenced by both extracellular and intercellular signaling mechanism (Z. Wu et al., 2016).

Gene expression plays a crucial role in a variety of biological processes in plants, including growth, reproduction, development, metabolism, differentiation, and adaptation to different environmental conditions. ABA dependent and ABA-independent signaling pathways.Involved in regulating genes involved in responding to stress in Arabidopsis. Arabidopsis microarray studies revealed that the *DREB/CBF* regulon reacts to abiotic stress in several ways. In response to various stressors, transcription factors regulate plant developmental processes, making them promise genetic targets for crop improvement. Plants rely on them to respond to different environmental cues as key molecular switches (Agarwal et al ., 2006).

2.1.7. Role of Osmolyte Accretion under Abiotic Stress

To encounter the negative effects of water scarcity in crop production, the buildup of osmolyte substances, generally referred to as "osmotic adjustment" or "osmoregulation," is frequently suggested as a strategy. Drought, salinity, heat, heavy metals, light, pesticides, and cold are among the environmental stresses that plants face (Serraj, Sinclair, & environment, 2002). Various environmental stresses can affect plants, including drought, salinity, heat, heavy metals, light, pesticides, and cold. In addition to stressing plants, human activities that damage agricultural ecosystems can exacerbate the problem by increasing salt, drought, ozone, and metal concentrations. Reactive oxygen species can cause significant crop losses and pose an important threat to agricultural systems worldwide. It is possible for some crops to experience yield

reductions of up to 50% in some cases. Plant development can be slowed by abiotic stressors affecting biochemical and physiological processes such as photosynthesis, antioxidant systems, and hormone signaling. To tackle these problems, researchers are exploring innovative solutions such as genetic engineering, breeding, and improved crop management strategies . During stressful conditions, plants accumulate organic solutes such as mannitol and galactinol that act as osmolytes and increase their resistance to stress .Stressed plants tend to accumulate proline as well (Sharma et al., 2019). Proline is an essential amino acid that accumulates during times of stress. Examples of these stress factors include dryness, salinity, heat, and low temperatures. Under conditions of dehydration, it scavenges (ROS) and serves as a structural component of membranes and proteins. Moreover, proline can tolerate high osmotic pressure and provides a valuable source of nutrition (Chun et al., 2018).

2.1.8. Transcription Factors Combating Abiotic Stress

Plants generally face high temperatures, excessive salt levels, drought, pathogen infections, and other environmental variables that have a drastic impact on their yield and biomass production. Abiotic stresses are expected to worsen because of climate change worldwide. Plant growth and development are adversely affected by salinity and drought, among other stressors. Plants have evolved complex defense mechanisms to survive abiotic stresses and pathogens. There are several transcription factors (TFs) that contribute to abiotic stress resistance in plants. TFs can regulate the expression of genes related to defense. The regulatory functions of various TFs in stress have also been investigated (G. Wang et al., 2016). Transcriptional factors, protein phosphatases, and kinases are among the molecules that make up the regulatory group. These molecules work together to manage or adjust the biological process or pathway being discussed. DREB, bZIP, AREB/ABF, bHLH, MYB, and C2H2 are examples of transcription factors in this group. here are also kinases and protein phosphatases in the regulatory group, such as CDP kinase, Receptor protein kinase, MAP kinase transcription regulation protein kinase, and Protein phosphatase that regulates transcription (Joshi et al., 2016). In plants, transcription factors (PTFs) bind to distinct DNA sequences called cisacting elements (CREs) that regulate gene expression. CREs are typically located in the promoter regions of genes specifically targeted for regulation. By binding to these CREs, PTFs are able to exert control over gene expression, a process that

can be influenced by both extracellular and intercellular signaling mechanism . Plant gene expression is responsible for regulating a wide range of biological processes, including growth, reproduction, development, metabolism, differentiation, and adaptation to environmental conditions (Rattan et al ., 2014).

ABA-dependent or ABA-independent signaling pathways are used to regulate ABA-responsive genes in Arabidopsis via more than 1500 transcription factors. Multiple pathways are involved in responding to abiotic stress in Arabidopsis, as revealed by microarray studies. In particular, the DREB/CBF pathway is one of them. Transcript factors play an important role in controlling. Arabidopsis plants that were genetically modified to constitutively overexpress DREB genes showed a remarkable improvement in their ability to tolerate salinity and drought stress. There seems to be a correlationbetween a combination of genes that respond to stress and a buildup of solutes (Wang et al., 2017).Plant development in response to various stresses, making them potential targets for crop improvement. They act as key molecular switches that coordinate the plant's reaction to differentenvironmental cues (Durán et al., 2017).

2.1.9. DREB Transcription Factor Family

Abiotic stresses like drought, high salinity, and cold cause environmental degradation. On a global scale, this can have a significant impact on crop yields and plant growth. Transcription factors (TFs) play a crucial role in response to abiotic stress. DREB (Dehydration Responsive Element) Binding) regulates the expression of several stress-responsive genes in plants. A cis acting elementknown as DRE/CRT is present in the promoter region of genes involved in abiotic stress responseand interacts with DREB. Through this interaction, DREB enhances plant abiotic stress tolerance (Shao et al., 2004). DREB is a plant protein that reacts to stress by controlling other genes. It has different types that respond to specific stresses like cold or drought.Some types also have unique roles such as managing genes involved in responding to sugar or suppressing genes that respond to pathogens. DREB is also involved in regulating plant growth and water balance. Overall, DREB helps plants cope with stress and adapt to changing conditions (Zhao, Xia, Liu, & Ma, 2014). A transcription factor family called the AP2 includes the DREB gene family. A specific amino acid is found at positions 14 and 19 of the AP2 domain of the DREBgene family valine. The amino acids in these amino acids play a vital role in identifying DREB gene

members . In plants, the AP2 domain contains 60-70 conserved amino acids that are essentialfor stress and defense responses . There are four R residues in the DREB genes, two W residues,and one V residue. These residues are involved in drought response (DRE/CRT). AP2 is phosphorylated by DREB genes due to a threonine- or serine-rich area near the domain. Under abiotic stress, DREB interacts directly with DRE/CRT elements via their basic motif, ACCGAC or GCCGAC. Plant species have been studied to detect the DREB gene family, and the number of DREB genes varies with the genome and gene expansion pattern of the species (Du et al., 2018). DREB genes play an important role in monitoring drought-induced plant reactions. Arabidopsis and tobacco are drought-tolerant when the DREB1A gene is overexpressed .In the same way, the use of the RD29 promoter has been found to raise drought tolerance in wheat. Additionally, studies have shown that the induction of cotton DREB genes in wheat can result in increased tolerance against cold, heat, and drought stress . Transgenic Arabidopsis plants that were genetically modified to constitutively overexpress DREB genes showed a remarkable improvement in their ability to tolerate salinity and drought stress. There seems to be a correlation between a combination of genes that respond to stress and a buildup of solutes (M. et al., 2017).

In Arabidopsis and other plants, DREB genes are found both physically and functionally . Rice, tobacco, pearl millet, strawberries, and pearl millet have all been studied by researchers (Cheng, Cai, An, & Huang, 2013), as well as ananas, wheat, bell pepper, and maize (Liu et al., 2021). To improve Arabidopsis' ability to withstand dry and salty conditions, the DREB3A gene was introduced from Leymus Chinensis. In the same way, research on the DREB2 gene from Broussonetia papyrifera has demonstrated that it assists in protecting against drought and high salt levels. DREB genes play an important role in helping Medicago truncatula cope with cold and freezing temperatures . Salvia miltiorrhiza was genetically modified to overexpress the DREB1B gene under the RD29A promoter, which resulted in an enhanced defense mechanism against drought stress, improved photosynthesis, and a more effective antioxidant system . In Salvia miltiorrhiza verexpression of the AtDREB1C gene enhances drought tolerance . The DREB genes seem to play a significant and have demonstrated the ability to enhance stress resilience in all plant species, and this resilience can be boosted even more by utilizing genetic modification methods to enhance the cultivation of different crops (Shu et al., 2016).

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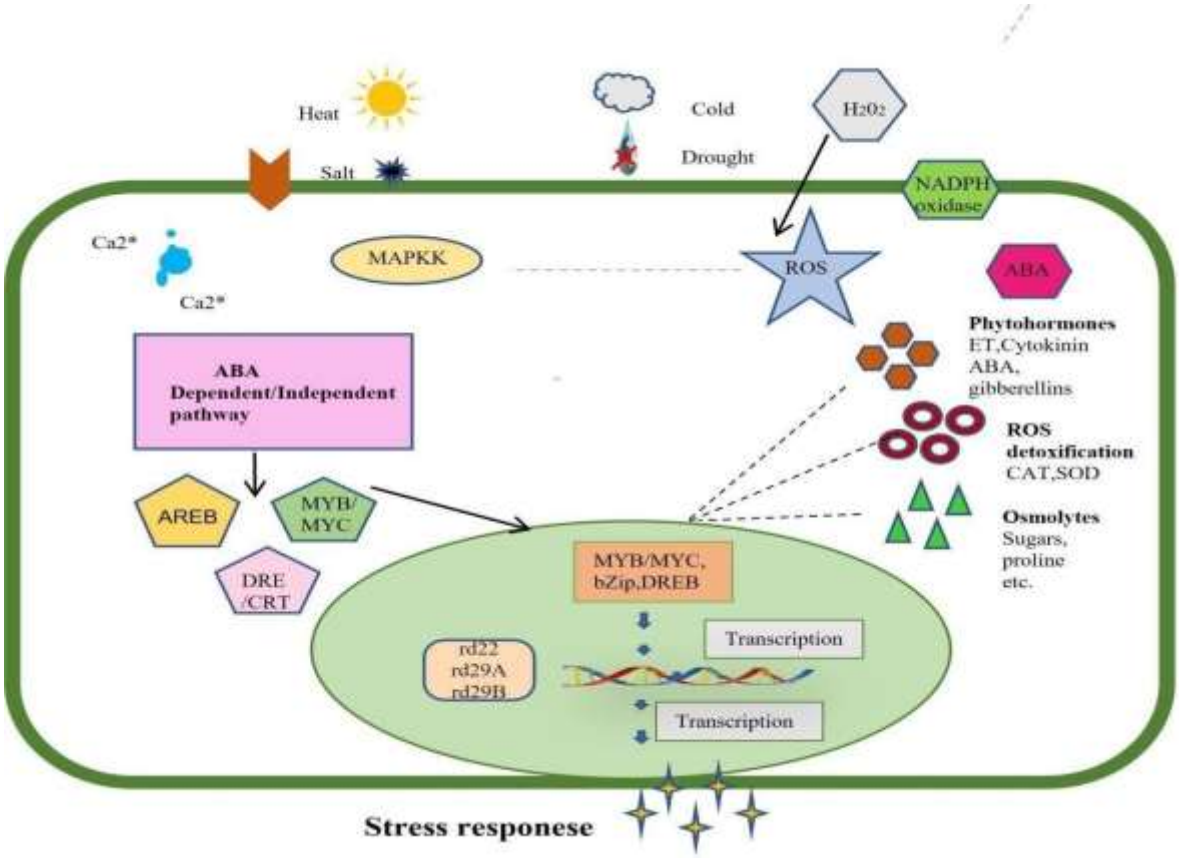

Figure: 2 The activation of transcription factors aids signaling pathways. The primary response involves ROS, phytohormones, and osmolytes, which activate defense-responsive gene families like DREB. These pathways respond to drought, light, salt, and ABA dependent/ independent signaling, normalizing primary responses, and lowering toxin concentration.

Chapter 3

MATERIALS AND METHODS

3.1. Seeds Collection

Solanum l. x. T-1359 seeds were collected at NARC in Islamabad, Pakistan, bythe Oil and Seed Research Department. For our experimental purposes. Similarly, we collected sandy loamy soil from the fields at NARC to be used in this experiment.

3.2. Seed Germination

The seeds were cleaned with a solution containing 70% ethanol for 1 minute and then rinsed with water. They were placed on filter paper to remove any remaining ethanol. After drying the seeds, they were placed in a box, covered in aluminum foil. To break their dormancy, they must be kept in a dark area at a temperature between 20°C and 25°C for 48 hours. After this period, the tomato seeds sprouted and were ready to be planted in the soil. The seeds that had similar germination rates were then transferred into pots.

Table: 3 Experimental designs for morphological, physiological, biochemical andexpression analysis

Day	Control	Drought-Stress treated
0	C1, C2, C3	T1, T2, T3
7	C1, C2, C3	T1, T2, T3
14	C1,C2, C3	T1, T2, T3
21 (Re-watering)	C1, C2, C3	T1, T2, T3

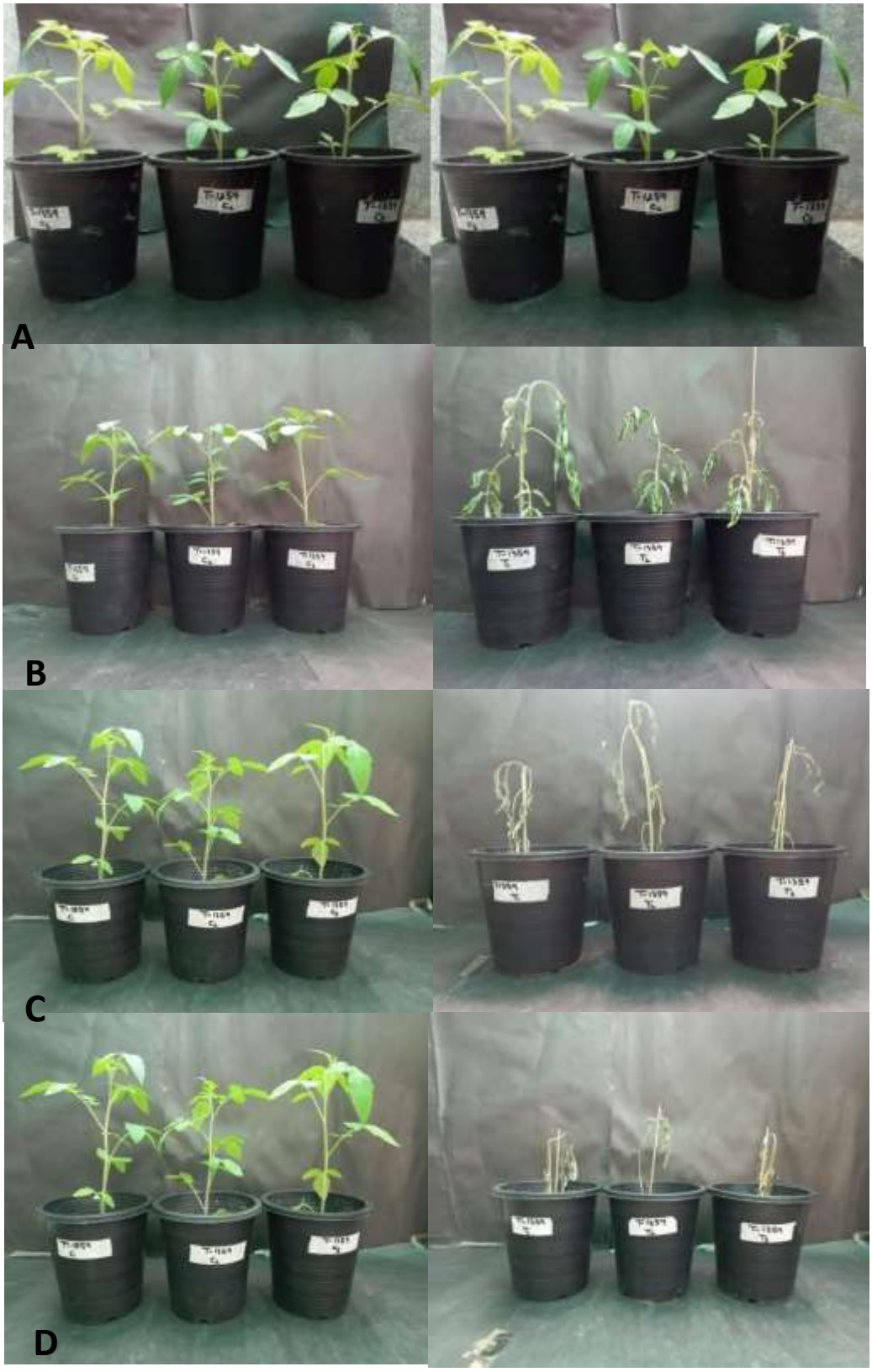

Figure: 3 Experimental plants cultivated in a greenhouse environment at different stages: **A.** zero-day stage; **B.** 7- day stage; **C.** 14-day stage; **D**. Rewatering stage.

3.3. Morphological Analysis of Plant Traits

Different morphological traits were studied on the 45th and 65th days after sowing. On the respective days, leaf numbers were counted, length of the shoots and roots were calculated by a measuring scale. A deionized water wash and measurement of the fresh weight of the root and shoot were performed to determine their weight. In a drying oven, they were dried for two hours at 70°C to remove moisture. After collecting the samples, the dry weights were calculated .

3.3.1. Scanning Electron Microscope

The TESCAN MIRA – SEM microscope was used to examine dry leaf samples using a technique called scanning electron microscopy.

3.3.2. Leave Area

Leaf area was calculated using Image J software. The leaves were manually drawn on graph sheets, which were then scanned using a scanner. The software was used to determine various parameters, including leaf density, area, mean, and median.

3.3.3. Holding Capacity of water

To determine a soil's capacity to hold water. We prepared a pot with multiple drainage holes at the bottom, which were lined with tissue paper. Approximately 1kg of dry soil was then added to the pot. The container, along with the dry soil, was weighed, and the recorded weight was noted. The soil was thoroughly saturated by adding water until it was completely soaked. Subsequently, the soil was left undisturbed until it reached a point where it stopped dripping excess water, indicating that it had reached its water-holding capacity. At this stage, the measurement was recorded, and the water holding Using the formula below, we calculated the capacity (WHC):

where Vw represents the volume of water and Vt denotes the total volume of saturated soil.

$$\text{Water Holding Capacity (WHC\%)} = \frac{Vw}{Vt} \times 100$$

where Vw represents the volume of water and Vt denotes the total volume of saturated

soil. (Wang, Yin, Liu, & Pyrolysis, 20

3.4. Physiological Analysis

3.4.1. SPAD Meter

Plant leaves from the middle section were selected, as they are more representative of the overall chlorophyll content. The plant leaf was placed between the SPAD 502 Chlorophyll Meter sensor area . Multiple measurements across different leaves or different parts of the same leaf were taken and recorded.

3.4.2. Relative Water Content (RWC)

According to the following factors, leaf water content can be determined. Three crucial parameters need to be evaluated. The fresh weight, the turgid weight, and the dry weight of an individual leaf. Leaf edges were carefully trimmed from a plant after it had been carefully selected. Fresh weight was measured immediately. Afterward, the leaves were immersed in distilled water for 36 hours to achieve their full turgidity. To determine the turgid weight of the leaf, it was weighed again. During the drying process, the leaf was heated to 65°C for three hours to determine its dry weight. After drying, the final weight was measured. Calculation of relative water content (RWC).

$$\text{RWC}=\frac{\text{Leaf fresh weight}-\text{leaf dry weight}}{\text{Leaf turgid weight}-\text{leaf dry weight}}$$

3.5. Biochemical assays for enzyme activity

3.5.1 Superoxide Dismutase (SOD)

The antioxidant assay was conducted based on the method described by A fresh plant Kono (1978) sample weighing 0.5g was homogenized using 3ml of phosphate buffered saline (PBS). The homogenized sample was then transferred to a 10ml tube and supplemented with 5ml of PBS

buffer. The tube was then centrifuged at 4 degrees Celsius for 20 minutes at 13000 rpm. The resulting supernatant, containing the plant extract, was collected, and stored at 4 degrees Celsius for 24 hours to be used in the analysis In Superoxide Dismutase. SOD was analyzed with 25ml of enzyme extract mixed with 1ml of PBS buffer, 33ml of EDTA, and 66ml of NBT, riboflavin, and methionine. To measure the absorbance of the resulting reaction solution at 560 nm, a spectrophotometer was used.

The absorbance value determined by the spectrophotometer is used to calculate.

Ae: the antioxidant activity, the following parameters were considered:

Ack : OD of the control tube in light conditions (4000 lux for 20 minutes).The total volume of the buffer solution used to extract the enzyme is V.

A sample's fresh weight is given by W.

The volume of enzyme extract in the reaction solution is Vt.

$$SOD\ activity = \frac{O.D_{control} - O.D \times V}{0.5 \times O.D_{control} \times W \times Vt}$$

3.5.2. Peroxidase activity measurement

Peroxidase (POD) antioxidant assay was conducted following the method outlined by Bergmeyer in 1974. To perform the assay, a fresh plant sample weighing 0.5g was

homogenized using 3ml of phosphate-buffered saline (PBS).Afterwards, we transferred the homogenized sample into a 10ml tube with PBS buffer. The POD determination was performed after centrifugation at 13000 rpm for 20 minutes, followed by storage at 4°C for 24 hours. For the POD analysis, 100µl of the enzyme extract was taken and combined with 2.7ml of PBS buffer, 100µl of guaiacol, and 100µl of 30% hydrogen peroxide (H2O2). To measure the absorbance of the supernatant at 270 nm, a spectrophotometer was used.

Following parameters are used in the calculation:

W stands for Fresh Weight of Sample

The enzyme extraction buffer solution volume V provides a means of assuring that, when the enzyme extract is added to the reaction solution for testing E, the activity remains constant, i.e. E = 26.66 mM/cm2.

$$\text{POD Activity} = \text{O.D}_{\text{value}} \times \frac{V / Vt}{E \times W}$$

3.5.3. Measurement of Catalase (CAT) activity

Catalase (CAT) antioxidant activity was measured using the method described by Aebi in 1984. Fresh plant the homogenized mixture was transferred into a 10ml tube with 5ml PBS buffer added on top. CAT analysis was performed on the extract supernatant after centrifugation at 13000 rpm for 20 minutes at 4 degrees Celsius. An enzyme extract of 100 ml was mixed with 2.8 ml of PBS buffer and 100ul of 30% hydrogen peroxide for CAT. The absorbance of the supernatant was measured at 240 nm. The E value, which represents the activity constant, was determined to be 39.4 mM/cm.

The optical density (OD) represents the activity level.

In this case, W stands for Fresh Weight

The volume of buffer solution used to extract the enzyme is called V.

The amount of enzyme extract used in the reaction solution is denoted by a. The activity constant is denoted by E. This value is 39.4 mM/cm.

$$\text{CAT Activity} = \text{O.D}_{\text{value}} \times \frac{V / Vt}{E \times W}$$

3.5.4. Ascorbate Activity Measurement

The ascorbate peroxidase (APX) level was measured using the method described in (Habib, Chaudhary, and Zia in 2014). To begin, a 0.5g fresh plant sample was homogenized with 3ml of phosphate-buffered saline (PBS). The homogenized mixture was then transferred to a 10ml tube with 5ml of PBS buffer added. At a temperature of 4 degrees Celsius, for 20 minutes, the tube was centrifuged at 13000 rpm. The resulting supernatant, containing the plant extract, was collected in another tube, and stored at 4 degrees Celsius for 24 hours for further analysis of (APX). The enzyme extract was mixed with 2.7ml of PBS buffer, 100ml of 30% hydrogen peroxide (H2O2), and 100ml of ascorbic acid (ASA) for the APX analysis. The supernatant absorbance was then measured at a wavelength of 290 nm. A constant activity (E) of 2.8 mM/cm was determined.

In the given description:

A The optical density (OD) represents the activity level.

W Fresh weight of a sample

V is the total volume of buffer solution used to extract enzyme a, which represents the amount of enzyme extract used in the reaction solution. E, the constant activity, is 2.8 mM/cm.

$$\text{APX Activity} = \text{O.D}_{\text{value}} \times \frac{V / Vt}{E \times W}$$

3.5.5. Measurement of Melo dialdehyde (MDA) Content

The Melo-dialdehyde content (MDA) was determined using the method outlined by T. Chen and Zhang in 2016. Initially, a 0.5g fresh plant sample was homogenized with 3ml of phosphate-buffered saline (PBS). Homogenized mixture was transferred to a 10ml tube and 5ml of PBS buffer was added. A centrifuge at 13000 rpm at 4 degrees Celsius was conducted after centrifuging the tube for 20 minutes. The resulting supernatant, containing the plant extract, was collected in another tube, and stored at 4 degrees Celsius for 24 hours for further analysis of MDA.

The crude protein/enzyme extracts from each sample were added to a 1.5 ml centrifuge tube containing 0.25% TBA (Thiobarbituric Acid). As a reference solution, one ml of 0.25% TBA solution was combined with 100 ml of 100 mM PBS (pH 7.8). After boiling in a water bath for 15 minutes, the mixture turned red. The reaction mixture was cooled on ice for five minutes after boiling. To measure the MDA content, 200 µl of the reaction mixture was pipetted out, and the absorbance was recorded at both 532 nm and 600 nm wavelengths. The extinction coefficient (E) of MDA-TBA at 532 nm was determined to be 532 (mM-1 cm-1) MDA content was measured by pipetting out 200 mL of the reaction mixture and measuring absorbance at 532 nm and 600 nm. At 532 nm, MDA-TBA has an extinction coefficient of 532 (mM-1 cm-1).

In the given description:

The absorbance at 532 nanometers is indicated by A532.

It is measured at 600 nm and represents the absorbance.

The abbreviation "Vr" refers to the volume of the reaction mixture. V represents the total volume of the crude enzyme solution.

In the testing tube, Vt represents the volume of crude enzyme used.

he crude protein concentration (mg/ml) is represented by Cp.

E represents the extinction coefficient (mM-1 cm-1) of MDA-TBA

at 532 nm.Formula for calculating MDA activity:

$$\text{MOD Activity} = (A_{532} - A_{600}) \times Vr \times \frac{V/VT}{E} \times 1000/C_p$$

3.6. Primers Designing

Primers are made by primer plus 3 software (https://www.primer3plus.com/) and verified their qualities by justtBio tool (https://justbio.com/) and primer (https://www.bioinformatics.org/sms2/pcr_primer_stats.html). The primers for the selected DREB and ACTIN genes are given in the table 3 and table 4, respectively.

3.7. RNA Extraction Process

The RNA extraction method used in the study by Vidović and Ćuković (2020) involved some modifications to the Trizol method. The leaf sample was crushed in liquid nitrogen into 0.2g of fine powder. Trizol reagent was added to 1ml of sample Mixing the sample formed a slurry. Following sample collection, the sample was incubated on ice for ten minutes. After incubation, the sample was centrifuged at 14000 rpm for 10 minutes at 4℃. After centrifugation all the debris was settled down and then the supernatant was transferred into a clear Eppendorf and incubated on ice for 5 minutes. Next, Chloroform was then added into the sample and gently mixed. After another 5-minute incubation on ice, once again, at 4°C, the sample was centrifuged for 20 minutes at 14000 rpm for 20 minutes. This resulted in the separation of an aqueous phase that was carefully transferred to a new Eppendorf tube. To precipitate the RNA, 500µl of ice-cold isopropanol was added to the aqueous phase, followed by gentle mixing and a 2-hour incubation at -20℃or 10 minutes at 4℃, a white pellet forms at the bottom of the Eppendorf tube as a result. The pellet was resuspended in ice-cold 75% ethanol and centrifuged for 5 minutes at 9500 rpm after discarding the supernatant. The supernatant was removed, leaving the pellets at the bottom. In the final step, the pellet was dried and resuspended in 2030ul of water free of nucleases. Table 4 shows the specific chemicals and their quantities used in this extraction process.

3.8. The synthesis of cDNA

To synthesize the cDNA, the following procedure was followed the PCR. A thermocycler was used for incubation at 65°C 5 minutes after a short spin to ensure it is well-mixed. Following incubation, ice was placed over each tube immediately. 5x buffer, Ribolock , RNase inhibitor

(10mM), and DNTPS are the remaining reagents. In the same PCR tube, Revert Aid Reverse Transcriptase enzyme was added. The tubes were kept on ice while these chemicals were added. A short spin was performed again on the mixture. In a thermocycler, the mixture was heated to 42°C for 60 minutes and then to 70°C for 5 minutes. DNA synthesized was preserved at -20°C. The chemicals used for cDNA synthesis, as well as their quantities, are listed in Table 5.

3.9. Gel electrophoresis

A 1% agarose gel was used to analyze the PCR products. The agarose gel was mixed with 50 mL of 1X TAE buffer before being added to 0.5 grams. The mixture was heated in an oven until it became clear. Then, 5µl of ethidium bromide was poured over the solution. A casting tray with a comb was filled with the agarose solution. After the gel solidified, the comb was removed, and the tray was placed in a gel tank filled with 1X TAE buffer. The PCR samples were loaded into wells on the gel. For 40 minutes, 80 volts of electric potential was applied to the gel electrophoresis. After electrophoresis, to document the gel, UV light was used. To keep a record of the results, a picture of the gel was taken.

3.10. qRT-PCR

It was performed by qRT-PCR conducted to investigate gene expression. qRT-PCR process involved adding specific reagents (Nuclease-free water, SYBR Green, primers, cDNA) to 0.1ml qRT-PCR tubes. The tubes were then spun to ensure thorough mixing of the reagents. In a qRT-PCR machine, the reaction was carried out under the following conditions: 95°C for 9 minutes, followed by 40 cycles of 95°C for 15 seconds, 62°C for 45 seconds, and 72°C for 20 seconds. The primers used for the reaction can be found in Table 6. The quantities of PCR reagents utilized are listed in Table 9. To ensure reliable results, three biological replicates and three technical replicates were performed for each reaction.

Table: 4 Primers for DREB genes

Primers	Sequence
LeDREB3 -F-RT	GCAGCAGCAGCAGATGATGT
LeDREB3 -R-RT	CCTTGTCGTAAGCCAGAGCA

Table: 5 Primers for universal genes (Actin)

Primers	Sequence
Forward	TATAACGAGCTTCGTGTTGCAC
Reverse	ACTGGCATACAGCGAAAGAACA

***Table:* 6** Shows the reagents used for RNA extraction and their corresponding

No.	**Reagent**	**Quantity**
1.	Trizol Reagent	1 milliliter (or 0.2 grams of sample)
2.	Chloroform	200 microliters (or 1 milliliter of Trizol reagent)
3.	Isopropanol	500 microliters
4.	75% Ethanol	1 milliliter
5.	Nuclease-free water	20-30 microliters

Table: 7 Reagents utilized for cDNA synthesis and their respective quantities.

No.	Reagent	Quantity
1.	Nuclease-free water	4.25 microliters
2.	Oligo dT (18) primer	1 microliter
3.	RNA sample	1 microliter
4.	5X RT buffer	2 microliters
5.	Ribolock RNase Inhibitor	0.25 microliter
6.	10 millimolar (mM) dNTPs	1 microliter
7.	Revert Aid / Reverse Transcriptase enzyme	0.5 microliter
	Total reaction volume	**10 microliters**

Table: 8 The PCR reagents and their respective quantities used in the experiment are listed.

S. No	Reagent	Quantity
1	Deionized water	15 microliters
2	10x buffer reaction	2 microliters
3	MgCl2	2 microliters
4	2.5mM dNTPs	2.5 microliter
5	Actin forward primer	1 microliter
6	Actin reverse primer	1 microliter
7	cDNA	1 microliter
8	Taq DNA Polymerase	1 microliter
	Reaction volume	**25** microliters

Chapter 4

RESULTS

4.1. Morphological Data Analysis

4.1.1. Root and Shoot Length

The average length of tomato plant roots and shoots was measured using a measuring scale under both control and drought stress conditions on the 45th and 65th days. Calculation of the mean, standard deviation, and p-value using Graph Pad Prism software to analyze the data.

The results indicate a decrease in root length in the control treatment compared to the drought treatments. This implies that when plants experience drought stress, their root length tends to grow more compared to the control group. Figure 4 visually presents the differences in root length between the treatments on the 45th and 65th days. The shortest root length was observed in the control group, while the longest root length was observed under drought stress.

In Figure 4, the control conditions at the first day, the seventh day, the fourteenth day, and the 21st day did not show any significant differences. However, the drought conditions at those same time points displayed significant differences. The shortest root length was observed on the -52nd day in the control group. Notably, under drought conditions, T7, T14, and T21 showed significant differences. Additionally, on the 59th day, T14 exhibited even more significant differences under drought conditions. Overall, these results suggest that root length tends to increase under drought stress compared to normal conditions. The results indicate an increase in shoot length in the control treatment compared to the drought treatments. This suggests that when plants experience drought stress, their shoot length tends to grow less compared to the control group. Figure 5 visually illustrates the differences in shoot length between the treatments on the 45th and 65th days. Specifically, the shortest shoot length was observed in the drought stress conditions, while the longest shoot length was recorded in the control group.

In Figure 5, the control conditions at 0 days, 7 days, 14 days, and 21 days show significant differences. However, the drought conditions at those same time points displayed non-significant differences.

The shortest shoot length was observed on the -52nd day in the control group and at 0-days conditions. Notably, under drought conditions, C7, C14, and C21 showed significant differences. Additionally, on the 65th day, C21 exhibited even more significant differences under normal conditions.

Overall, these results suggest that shoot length tends to increase under normal conditions compared to drought conditions. The results indicate that shoot length decreased under drought stress compared to the control condition.

4.2. Weight

4.2.1. Weight of Fresh, Dry Roots and Shoots

As shown in Figure 8, when plants were exposed to drought conditions (T7, T14, and T21) there was a significant reduction in the fresh root weight compared to plants in the control condition (C7, C14, and C21) over a 65-day period. Interestingly, the plants in the control conditions (C14 and C21) had the highest fresh root weight under normal conditions. It should be noted that the effects on root weight were more noticeable under control conditions than under drought conditions. While comparing root results after drying the plant, plants belonging to drought conditions, namely T7, T14, and T21 reduced their root weight rapidly as compared to plants belonging to control conditions namely(C7, C14, and C21).

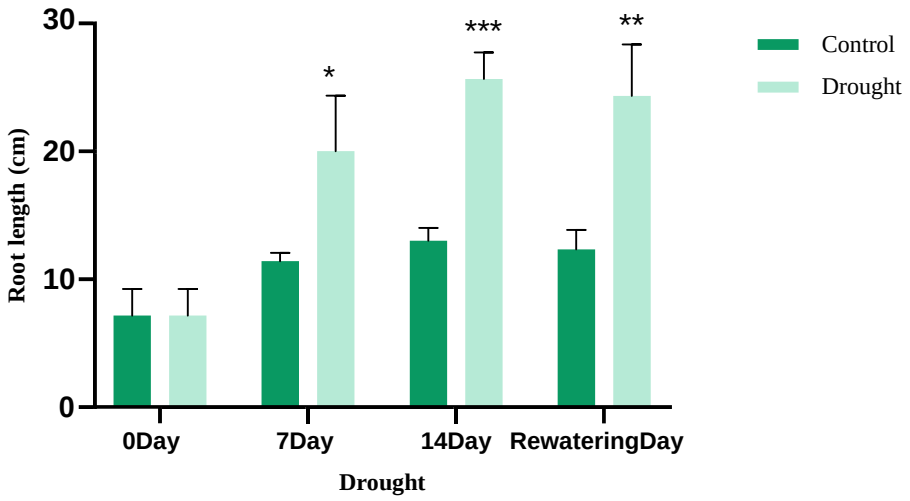

Figure: 4 Shows tomato roots under drought stress. There is a significant drought effect on the root length of plants treated with T7, T14, and T21 compared to controls. Data is presented as the interaction between drought and control conditions. The asterisk sign depicts that the expression of the drought stress group is statistically different from that of control group [**(*p ≤ 0.05)**; then, **p < 0.01, ***p < 0.001, ****p < 0.0001, two-way ANOVA, Bonferroni test].

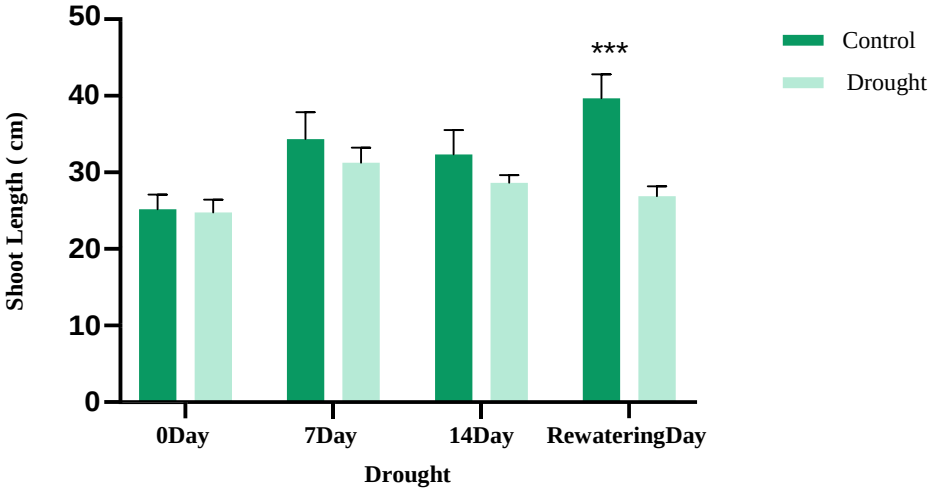

Figure: 5 Shows the length of a tomato plant's shoots under drought conditions. Compared to the control plants, T7, T14, and T21 plants showed significant drought effects on shoot length. Data is presented as the interaction between drought conditions and control conditions. The asterisk sign depicts the expression of drought stress group is statistically different from that of control group [**(*p ≤ 0.05)**; then, ******p < 0.01, ***p < 0.001, ****p < 0.0001, two-way ANOVA, Bonferroni test].

The results indicate that the control group, which received normal watering conditions, had a higher dry root weight compared to the groups experiencing drought. Specifically, the control group C21 showed the most significant differences in dry root weight under normal conditions. Figure clearly illustrates the impact of drought conditions (T7, T14, and T21) on the fresh weight of plant shoots over a 65-day period. The graph reveals a significant decrease in shoot weight for the drought exposed plants compared to those in the control condition (C7, C14, and C21). Notably, the control plants (C7,C14 and C21) displayed the highest shoot weight under normal conditions. As a result of drought exposure, plants' shoot weight was significantly reduced compared to controls. Furthermore, when examining the dried plant results, it becomes apparent that the plants subjected to drought conditions (T7, T14, and T21) exhibited a rapid decline in shoot weight compared to the plants in the control condition (C7, C14, and C21).Drought stress conditions result in a decrease in shoot weight in plants.

4.2.2. Fresh and Dry Weight of Leaves

The results of Figure 12 demonstrate a noteworthy decrease in leaf fresh weight for(T7, T14, and T21) when exposed to drought conditions over a 65-day period. Comparing them with the control group (C7, C14, and C21) reveals that (T7, T14, and T21) experienced more substantial reductions in leaf weight. The 66-day timeframe indicates a decrease in fresh weight for (T7, T14, and T21) while it may indicate an increase in fresh weight for (C7, C14, and C21). The results of Figure 13 indicate a decrease in leaf dry weight for (T7, T14, and T21) over a 65day period. Compared to the control group (T7, T14, and T21) also experienced dry weight reductions.

There was a decrease in the dry weight of leaves under drought stress during 45 to 65 days, with (T7, T14) and (T21) showing reductions compared to corresponding control plants (C7, C14, and C21). These results help us analyze drought stress' effect on leaf biomass. In (T7, T14, and T21), fresh leaf weight

decreased, as did dry leaf biomass, which illustrates how drought adversely affects plant growth. Also, the significant results observed in (C7, C14, and C21) confirm that plant leaf biomass was not affected by these conditions. These findings highlight that drought conditions have a negative effect on plant.

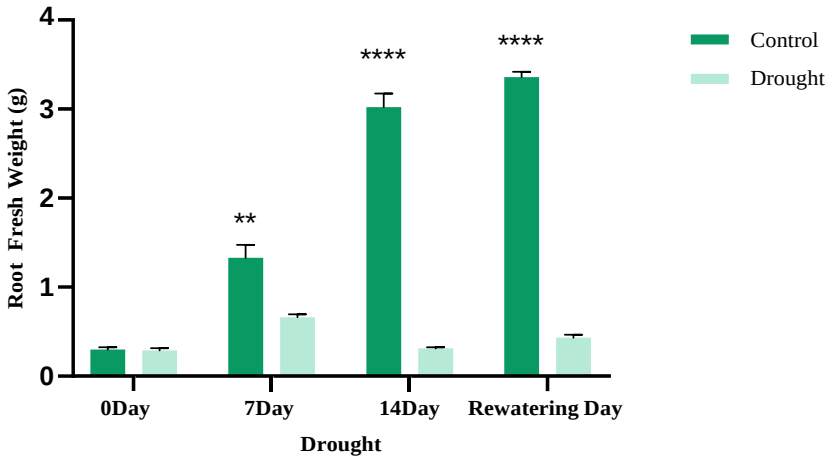

Figure: 6 Shows that plant fresh root weight is reduced when drought stress is present. A plant's responses to control and drought stress conditions are shown. The asterisk sign depicts the expression of drought stress group is statistically different from that of control group [**(*p ≤ 0.05)**; then, ****p < 0.01, ***p < 0.001, ****p < 0.0001, two-way ANOVA, Bonferroni test].

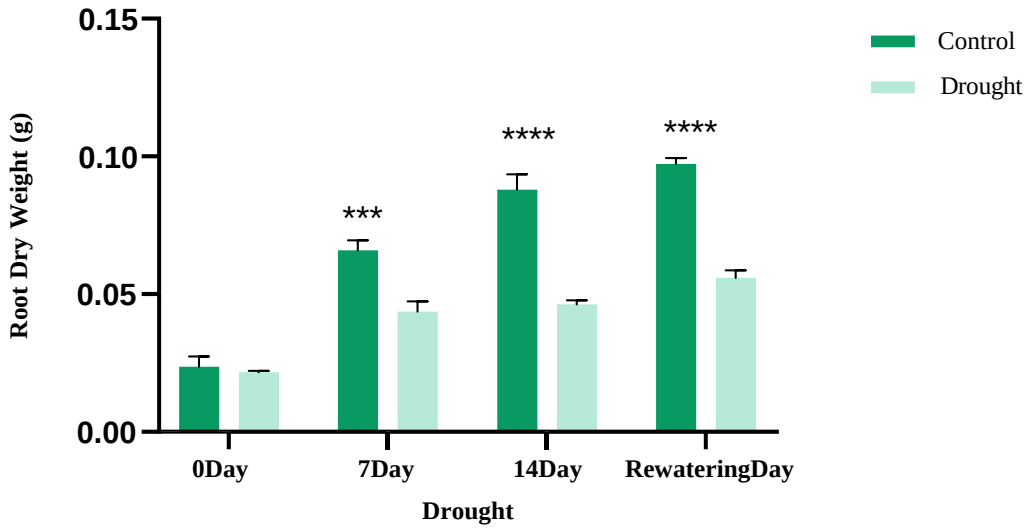

Figure: 7 The dry root weight results show reduced dry matter under drought stress. Data represents control and drought conditions combinations applied to plants. The asterisk sign depicts the expression of drought stress group is statistically different from that of control group [**(*p ≤ 0.05)**; then, **p < 0.01, ***p < 0.001, ****p < 0.0001, two-way ANOVA, Bonferroni test].

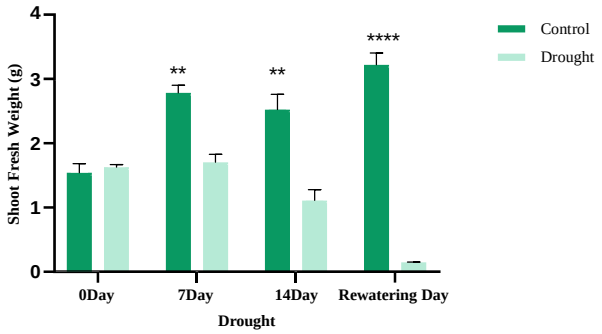

Figure: 8 Fresh Shoot weight in response to drought stress, tomato plants lose shoot weight. There was a significant drought effect on shoot fresh weight of plants treated with T7, T14, and T21 compared to control plants C7, C14, and C21. The data is presented as the interaction of drought and control conditions treatments applied to the plants. The asterisk sign depicts the expression of drought stress group is statistically different from that of control group [**(*p ≤ 0.05)**; then, **p < 0.01, ***p < 0.001, ****p < 0.0001, two-way ANOVA, Bonferroni test].

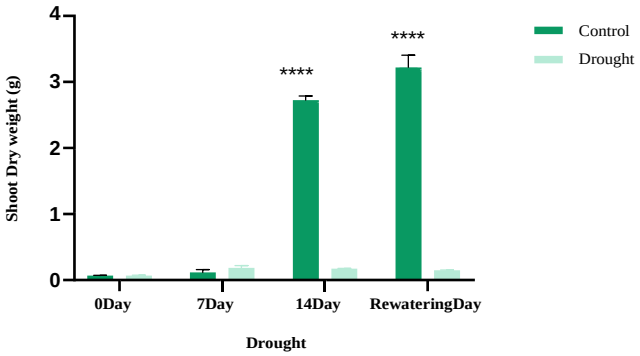

Figure: 9 Dry shoot weight also shows A reduction in drought stress has been observed. In comparison with the control condition, T14 and T17 have less dry mass. The asterisk sign depicts the expression of drought stress group is statistically different from that of control group [**(*p ≤ 0.05)**; then, **p < 0.01,** **p < 0.0001, two-way ANOVA, Bonferroni test].

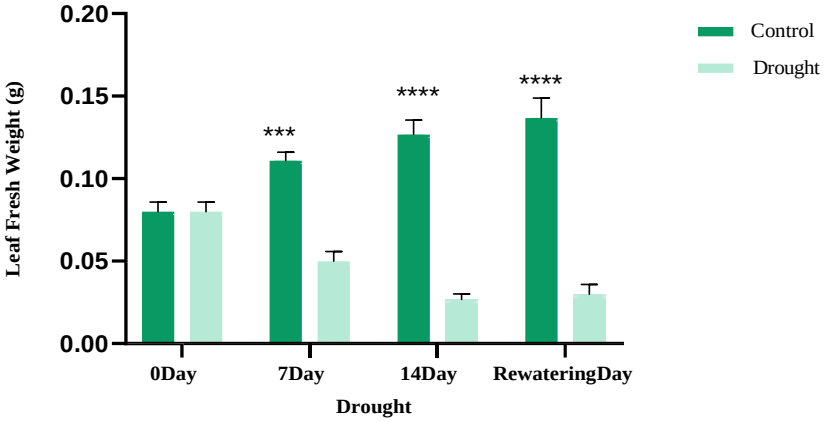

Figure: 10 Leave weight also shows the decline in mass under the presence of drought stress. Data are shown as the interaction of different drought stress conditions and control conditions as applied to plants. The asterisk sign depicts the expression of drought stress group is statistically different from that of control group **[(*p ≤ 0.05)**; then, **p < 0.01, ***p < 0.001, ****p < 0.0001, two-way ANOVA, Bonferroni test].

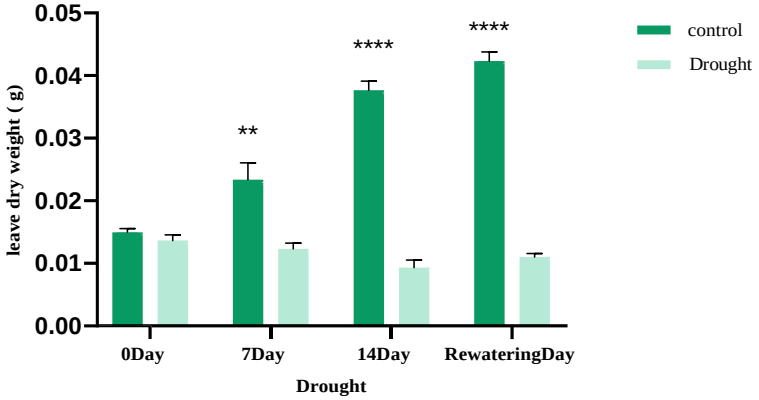

Figure: 11 Dry weight of leaves declines drought stress and indicates that the presence of drought reduces the dry mass of plants. control and drought as treatments applied to plants. The asterisk sign depicts the expression of drought stress group is statistically different from that of control group [(*$p \leq 0.05$); then, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, two-way ANOVA, Bonferroni test].

4.3. Leaf Number

As shown in Figure 21, drought stress reduced the number of leaves. A reduction in leaves was observed in treatments T7, T14, and T21. However, upon analysis, it was concluded that only T21 showed significant results compared to the other treatments on the 65th day.

4.4. Leave Area

A decrease in leaf area reduces photosynthesis and respiration. Drought stress induces a decline in leaf areas in plants. The results presented in Figure 18 indicate that under drought stress conditions (T7, T14, and T21), there was a reduction in leaf area from 52 days to 65 days, compared to the control conditions (C7, C14, and C21). Furthermore, when comparing the drought stress conditions, it is evident that T7, T14, and T21 exhibited significant reductions in leaf area, respectively. Notably, the reduction in leaf area was more pronounced for T14 and T21 under drought conditions compared to the corresponding control conditions.

4.5. Number of Branches

The number of branches decreased under drought stress conditions, as depicted in Figure 17. The reduction in branches was observed in treatments T7, T14, and T21. However, upon analysis, it was determined that only treatment T21 exhibited statistically significant results on the 65th day.

4.6. Relative water content

Relative Water content (%) was highest in C14 and C21 in the control condition. while the lowest relative water content (%) was recorded in T14 and T21 under drought stress.

4.7. Chlorophyll

Chlorophyll is the vital pigment for photosynthesis. Its content is a primary indicator of leaf photosynthesis and plant health. Chlorophyll levels were highest during drought conditions, particularly in samples T14 and T21. In contrast to the control condition, drought stress led to higher chlorophyll levels, indicating a consistent trend toward higher chlorophyll levels under drought conditions.

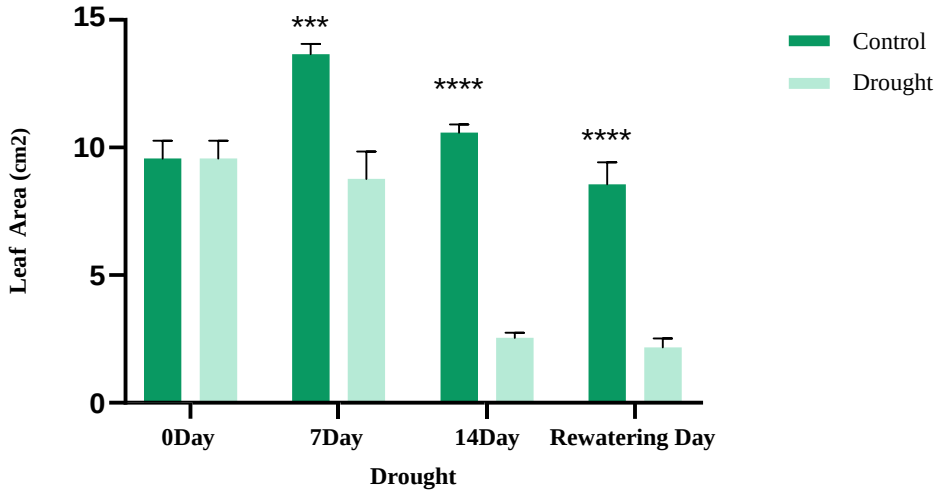

Figure: 12 Shows a reduction in leaf area, indicating that plants at 65 days exhibit a decrease compared to the 45-day period. The data presented in the figure represents the combined effects of drought and control conditions applied to the plants. The asterisk sign depicts the expression of drought stress group is statistically different from that of control group [**(*p ≤ 0.05)**; then, ****p** < 0.01, ***p < 0.001, ****p < 0.0001, two-way ANOVA, Bonferroni test].

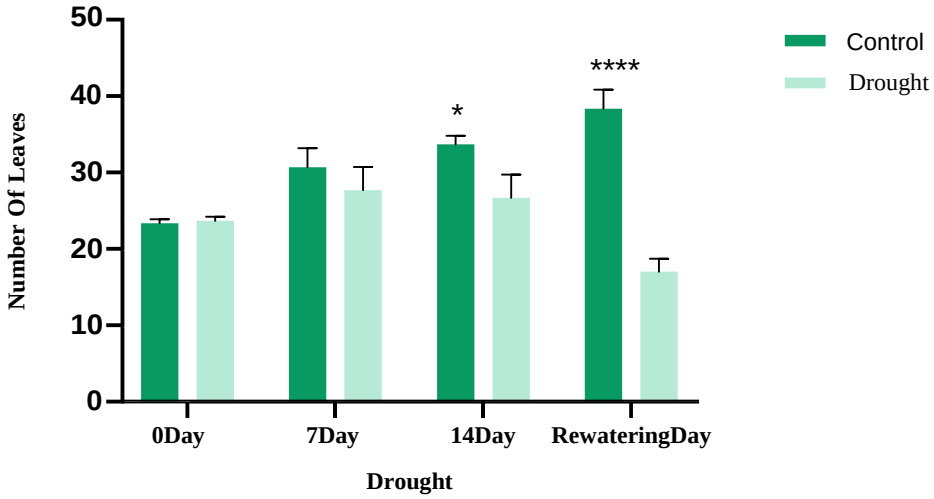

Figure: 13 In drought conditions, the number of leaves is reduced while the control condition promotes plant growth as treatments are applied. The asterisk sign depicts the expression of drought stress group is statistically different from that of control [**(*p ≤ 0.05)**; then, **p < 0.01, ***p < 0.001, ****p < 0.0001, two-way ANOVA, Bonferroni test]

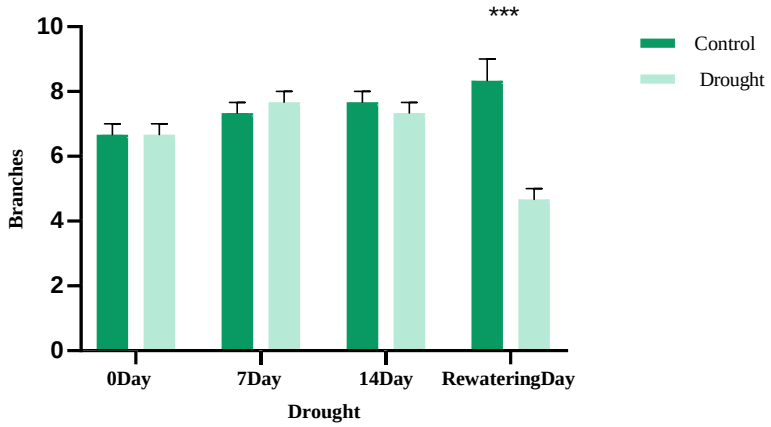

Figure: 14 Shows a reduction in the number of tomato plants branches under drought stress conditions, while the control condition promotes plant growth as treatments are applied. The asterisk sign depicts the expression of drought stress group is statistically different from that of control [**(*p ≤ 0.05)**; then, **p < 0.01, ***p < 0.001, ****p < 0.0001, , two-way ANOVA, Bonferroni test]

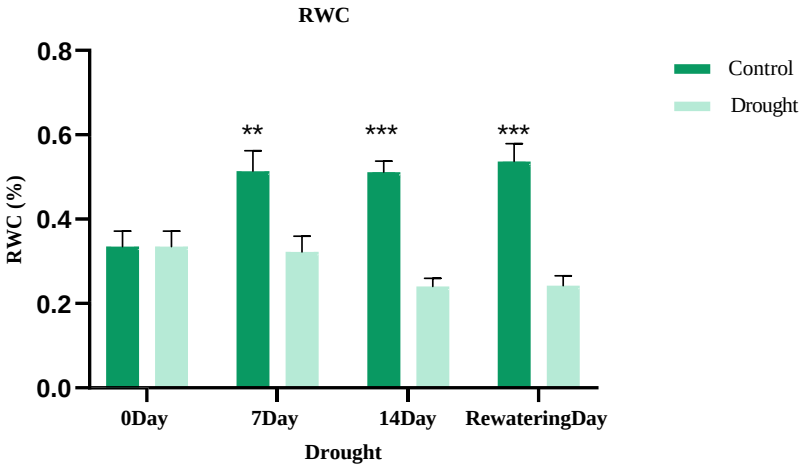

Figure: 15 Drought stress decreases tomato plants' relative water content (%). Relative water content (%) indicates a substantial impact of drought on plants under stressful conditions, especially in T7, T14, and T21 compared to control groups C7, C14, and C21. The data is presented as the combined effect of applying drought and control treatments to the plants. The asterisk sign depicts the expression of drought stress group is statistically different from that of control [**(*p ≤ 0.05)**; then, **p < 0.01, ***p < 0.001, ****p < 0.0001, , two-way ANOVA, Bonferroni test]

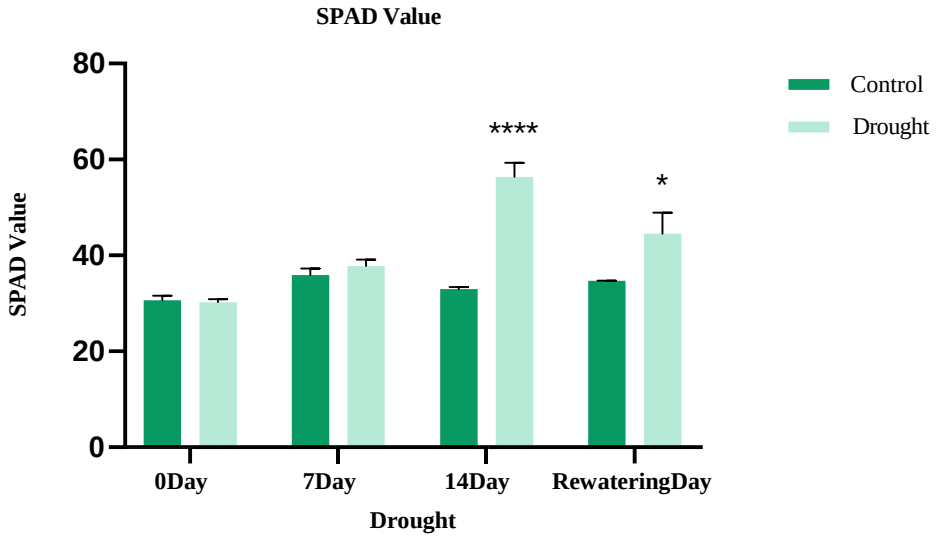

Figure: 16 illustrates the correlation between SPAD-502 values and leaf chlorophyll content under both drought and control stress conditions. As the SPAD values increased, there was a corresponding rise in chlorophyll content. The results show that as SPAD values increased, chlorophyll content also increased, indicating a positive relationship between the two [**(*p ≤ 0.05)**; then, **p < 0.01, ***p < 0.001, ****p < 0.0001, two-way ANOVA, Bonferroni test].

4.8. Biochemical analysis

4.8.1. Superoxide dismutase (SOD)

The antioxidant assay of SOD was measured using the method used by (Kono,1978). UV spectrophotometer analysis was conducted at 560nm. The SOD value was found to be increasing under drought stress. The control plants (C7, C14, and C21) exhibited lower levels of SOD compared to (T7,T14,T21).

4.8.2. Peroxidase (POD)

Peroxidase (POD) Antioxidant assay was conducted using the method given by (Bergmeyer,1974). UV spectrophotometer analysis was performed at a wavelength of 270nm. Results indicated an increase in POD value because of drought stress. POD levels were higher in plants exposed to drought stress (T17, T14, and C21) compared to control plants (C7, C14, and C21).

4.8.3. Catalase (CAT)

According to (Aebi,1984), catalase (CAT) antioxidant activity can be measured by this method. A UV spectrophotometer was used to measure absorbance at 240nm after a 10-second interval. The levels of CAT were significantly elevated drought stress was experienced by samples (T7, T14, and T21) compared to controls (C7, C14, and C21).

4.8.4. Ascorbate Peroxidase (APX)

APX was measured using a method described by Habib et al. (2014). A UV spectrophotometer measured absorbance at 290nm for 10 seconds. APX activity was found to be higher in samples T14 and T21 exposed to drought stress compared to the control samples C14 and C21

4.8.5. Melo-Dialdehyde Content

MDA was measured by this method (T. Chen & Zhang, 2016). A UV spectrophotometer was used to measure the absorbance at 532 nm and 600 nm after a 10-second interval. MDA levels

were significantly higher in samples T14 and T21 subjected to drought stress compared to control samples C14 and T21.

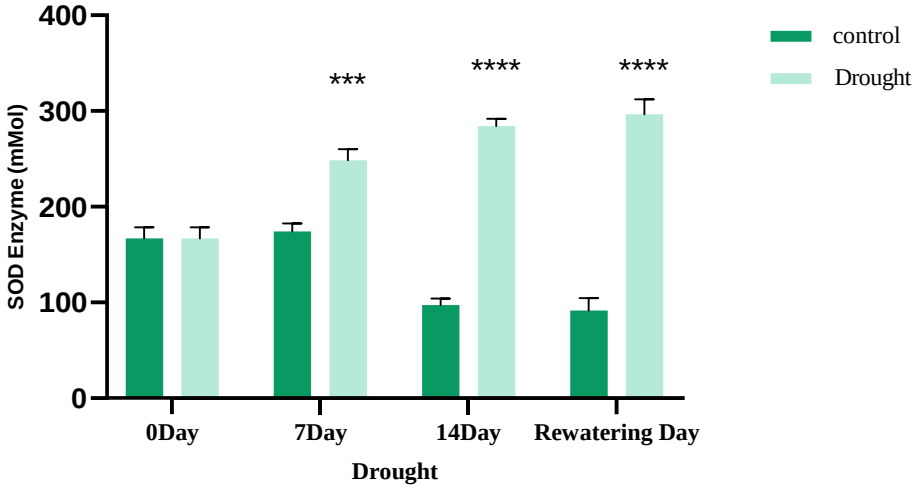

Figure: 17 Shows the interaction of various treatment combinations applied to plants**.** When plant cells experience stress, Reactive oxygen species (ROS) increase. SOD enzyme becomes activated. This enzyme plays a crucial role in converting reactive O_2- ions into hydrogen peroxide. According to the obtained results, SOD is significantly more abundant under drought stress conditio ns than under control conditions. The asterisk sign depicts the expression of drought stress group is statistically different from that of control group [**(*p ≤ 0.05)**; then, ****p < 0.01, ***p < 0.001, ****p < 0.0001, two-way ANOVA, Bonferroni test].

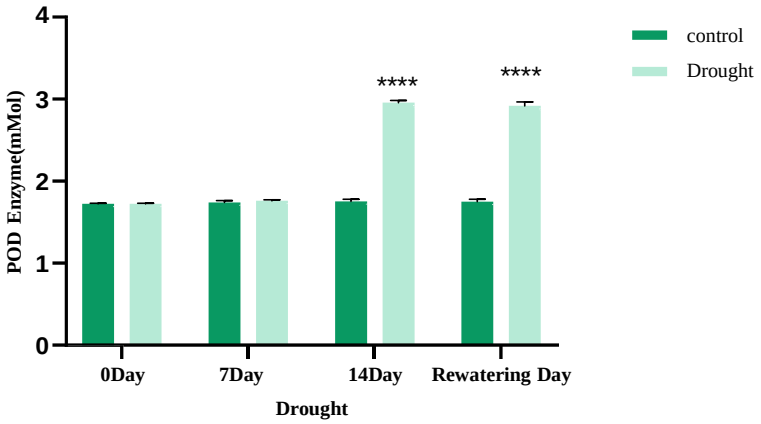

Figure: 18 Shows that the POD enzyme is activated when ROS production increases in plants due to stress. The POD enzyme functions by converting H_2O_2 to H_2O. The results indicate that the concentration of POD was high in plants treated with drought stress, specifically T7, T14, and T21. The asterisk sign depicts the expression of drought stress group is statistically different from that of control group [**(*p ≤ 0.05)**; then, ****p < 0.01, ***p < 0.001, ****p < 0.0001, two-way ANOVA, Bonferroni test].

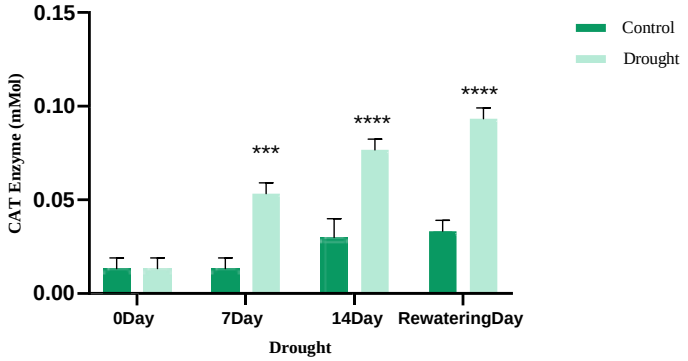

Figure: 19 Shows that the CAT enzyme is activated when ROS production increases in plants due to stress. The cat enzyme functions by converting H_2O_2 to H_2O. The results indicate that the concentration of CAT was high in plants treated with drought stress, specifically T7, T14, and T21. The data represents the interaction between the control and drought stress treatments applied to the plants The asterisk sign depicts the expression of drought stress group is statistically different from that of control group [**(*p ≤ 0.05)**; then, ****p < 0.01, ***p < 0.001, ****p < 0.0001, two-way ANOVA, Bonferroni test].

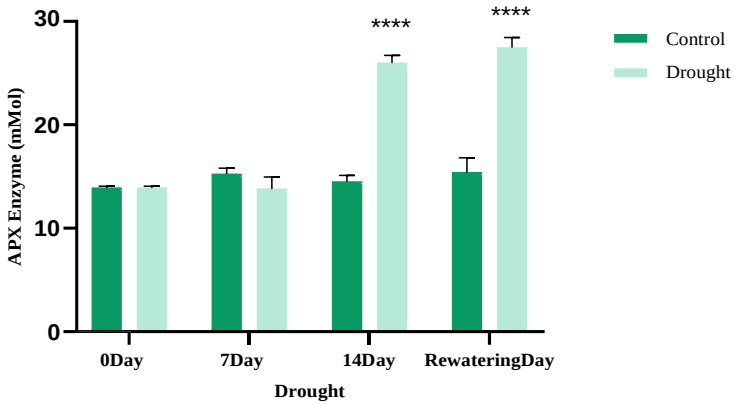

Figure: 20 APX enzyme activity increases with ROS production due to stress. APX converts H_2O_2 to H_2O. Results show higher APX levels under drought stress. Data represents control and drought stress treatments in plants, The asterisk sign depicts the expression of drought stress group is statistically different from that of control group [**(*p ≤ 0.05)**; then, ****p < 0.01, *****p < 0.001, ******p < 0.0001, two-way ANOVA, Bonferroni test].

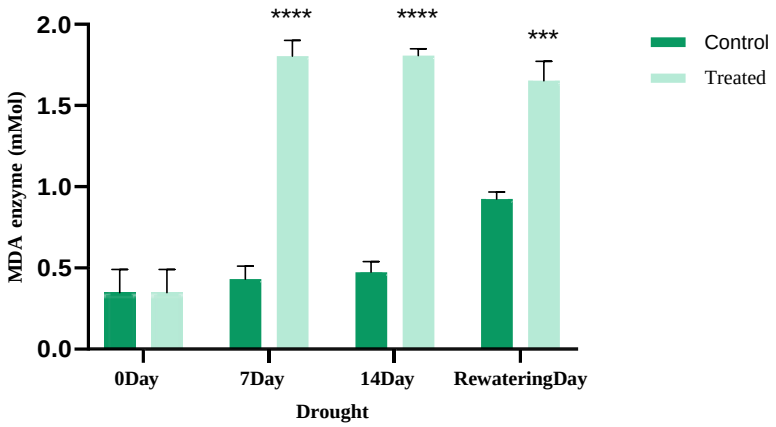

Figure: 21 MDA serves as a commonly used parameter for assessing lipid peroxidation in plant tissue, which typically rises under oxidative stress. The figure illustrates that the MDA content is significantly higher in samples T14 and T21 under drought stress conditions. The data presented represents the interaction between drought and control treatments applied to plants. The asterisk sign depicts the expression of drought stress group is statistically different from that of control group [**(*p ≤ 0.05)**; then, ****p < 0.01, *****p < 0.001, ******p < 0.0001, two-way ANOVA, Bonferroni test].

4.9. Scanning Electron Microscope

Stomatal analyses were examined in Solanum l. x. T-1359, as shown in Figure 26. Under control conditions in the tomato plants,C1,C7,C14 and C21 stomata were of appropriate shape, with well-arranged guard cells and open stomatal pores. On the other hand, plants exposed to drought stress T1,T7,T14 and T21 exhibited nearly closed or completely closed stomatal pores, along with distorted guard cells, in comparison to the control plants.

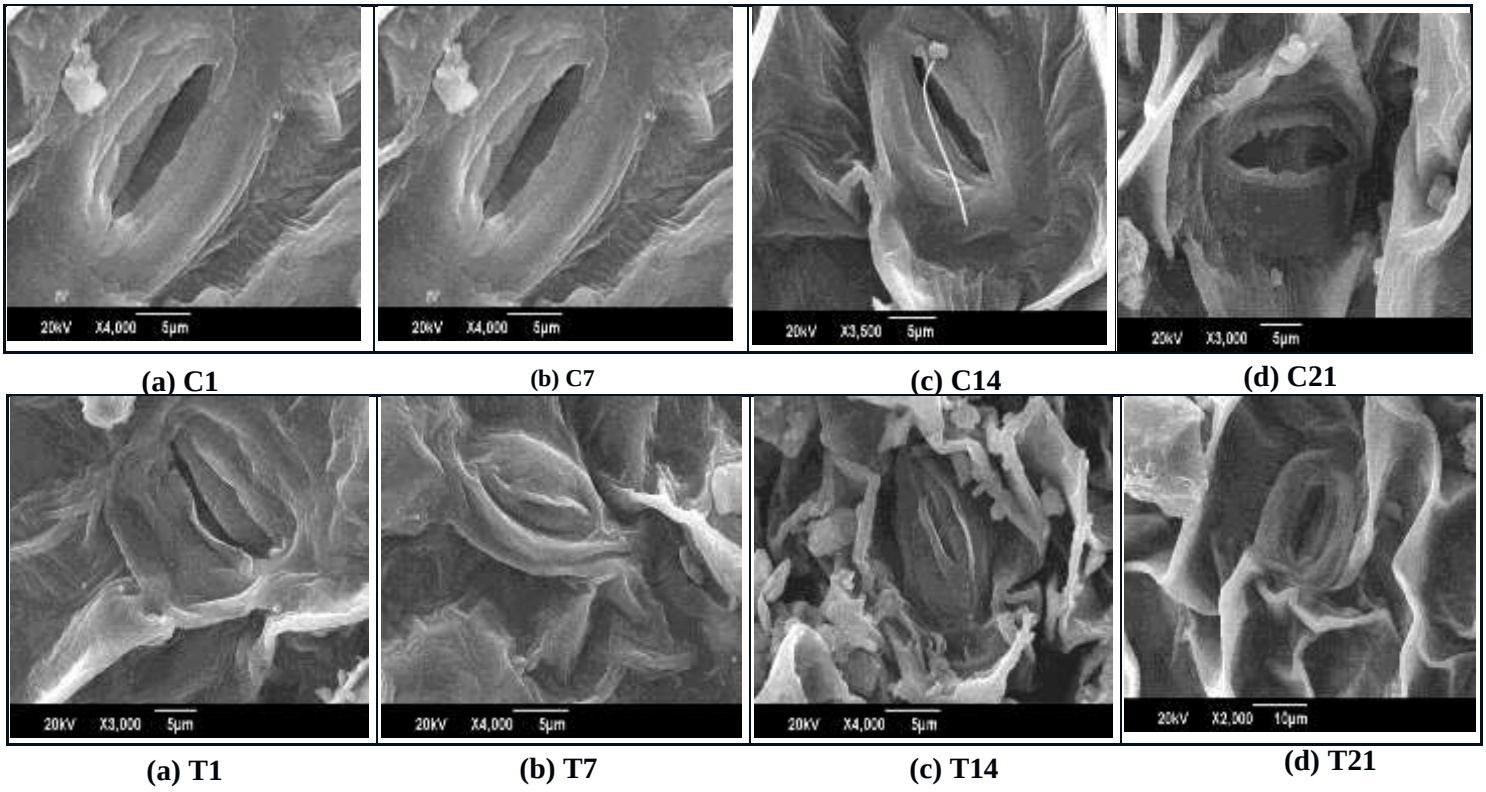

Figure: 22 SEM results confirm the effect of different levels of drought stress on the stomatal response of the *Solanum l. x.* T-1359 under control (a), C0 (b), C7 (c), and C14 (d)21 and under drought stress conditions (a), T0, (b), T7, (c), T14 and T21.

4.10. DERB gene expression analysis

In tomato plants under water deficit conditions, qRT-PCR results demonstrated different expression levels of *DERB3* and *DERB4* genes at different stages (0 days, 7 days, 14 days, and rewatering).

In the early vegetative stage, *DERB3* showed increased relative expression under drought conditions compared to the control (normal watering). At the 7-day and 14-day stage, *DERB3* expression was significantly higher than at other stages. Plants that were re-water exhibited no significant increase in *DERB3* expression.

At the 7-day stage and 14-day stage, there was an enhanced expression of the *DERB4* in tomato under drought conditions. Additionally, Significance expression analysis of *DERB4* was observed in tomatoes root during the rewatering phase from drought stress. In summary, examination through qRT-PCR revealed that these candidate genes, *DERB3* and *DERB4*, play distinct roles under drought stress conditions. Under drought stress conditions, there was a significant increase in the expression of *DREB3*, with a relative fold change of 14.62, 6.67 in leaf, and 9.09,6.04 in root on the 7 day, and 14 day respectively as compared to the control conditions. As same as drought stress conditions, there was a significant increase in the expression of *DREB4*, with a relative fold change of 14.62, 6.67 in leaf, and 9.09,6.04 in root on the 7^{th} day, and 14^{th} day respectively as compared to the control conditions.

The study investigated the response of tomato genes to simulated drought stress using qRT-PCR. The relative expression levels of *DERB3* and *DERB4* genes were observed in drought stressed leaves at 0, 7, 14, and 21 days. The findings revealed distinct upregulation of *DERB3* and *DERB4* genes with increasing duration of drought treatment, indicating their potential specific roles under drought stress. Notably, the highest relative expression was observed for *DERB3* and *DERB4* at the 7-day and 14-day treatment points under drought stress conditions, exhibiting significant differences compared to the control group In summary, the *DERB3* and *DERB4* genes displayed a responsive behavior to external drought stress, showcasing a positive expression pattern. This suggests that these genes may serve specific functions in mitigating the effects of drought stress in tomatoes.

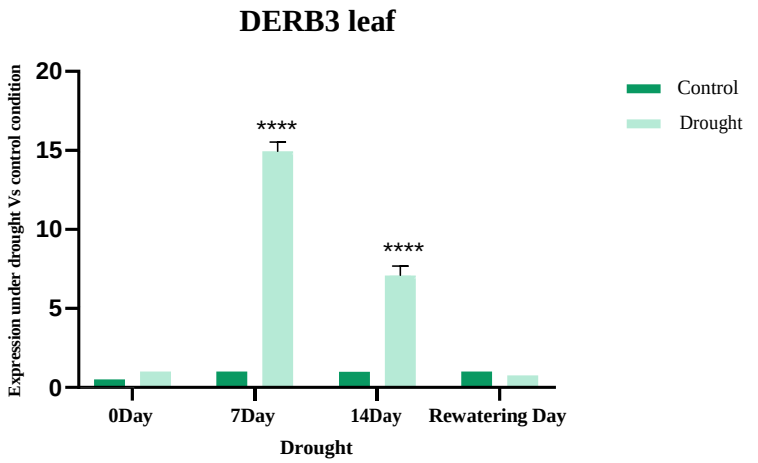

Figure: 22 Shows the relative expression of DERB3 in tomato leaves between 45-65 days under both Re-watered conditions (CO, C7, C14, and C21) and drought conditions (T0, T7, T14, and T21). The asterisk sign depicts the expression of drought stress group is statistically different from that of control group [(*p ≤ 0.05); then, **p < 0.01, ***p < 0.001, ****p < 0.0001, two-way ANOVA, Bonferroni test].Based on the results, it can be concluded that there were significant differences between drought stress stages in terms of the expression of DERB3

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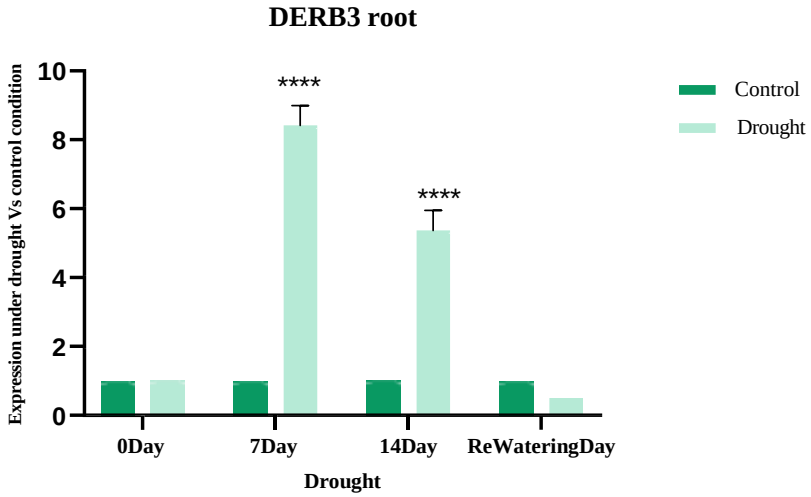

Figure: 23 Shows the relative expression of DERB3 in tomato roots between 45-65 days under both well-watered conditions (CO, C7, C14, and C21) and drought conditions (T0, T7, T14, and

T21) The asterisk sign depicts the expression of drought stress group is statistically different from that of control group [(*p ≤ 0.05); then, **p < 0.01, ***p < 0.001, ****p < 0.0001, two-way ANOVA, Bonferroni test].

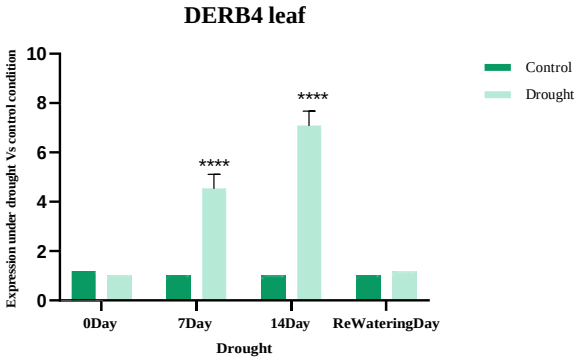

Figure: 24 Shows the relative expression of DERB4 in tomato leaves between 45-65 days under both well-watered conditions (CO, C7, C14, and C21) and drought conditions (T0, T7, T14, and T21). The asterisk sign depicts the expression of drought stress group is statistically different from that of control group group [**(*p ≤ 0.05)**; then, **p < 0.01, ***p < 0.001, ****p < 0.0001, two-way ANOVA, Bonferroni test].

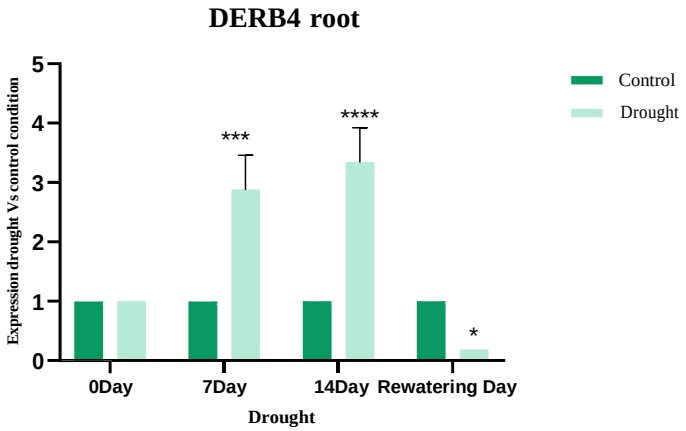

Figure: 25 shows the relative expression of DERB4 in root leaves between 45-65 days under both well-watered conditions (CO, C7, C14, and C21) and drought conditions (T0, T7, T14, and T21). The asterisk sign depicts the expression of drought stress group is statistically different

from that of control group group [**(*p ≤ 0.05)**; then, ****p < 0.01, ***p < 0.001, ****p < 0.0001, two-way ANOVA, Bonferr

Chapter 5

DISCUSSION

Tomato is highly popular and extensively cultivated worldwide, with a preference for moderate climatic conditions and the ability to thrive on fertile, well-drained soil(Nutrition, 2023). Based on previous research, it has been concluded that Drought stress (DR) significantly limits the growth, yield, and quality of tomatoes, As a result of morphological responses, and physiological, and biochemical levels. Among different abiotic stresses, drought stress stands out as a prominent factor impacting tomato plants (Altaf et al., 2022) . In our research, we also observed similar conformational changes in this aspect. Additionally, we investigated the impact of different time points, control conditions, and drought stress on plant growth in our study cases.

In a previous study, drought-stressed tomato genotypes were compared to irrigated plants grown under non-stress conditions for 45 days. Drought stress significantly affected tomato genotype growth, including plant height, branching, root length, shoot length, yield, and related characteristics, as well as physiological traits. A drought-stressed plant exhibits unfavorable effects on meristematic activity, cell elongation, premature abscission of leaves and roots, reduced accumulation of dry matter, and impaired photosynthetic activity. In our own research, we also observed similar trends in terms of conformational changes. Moreover, we investigated the effects of different time points (C1, C7, C14, and C21), control conditions, and drought stress (T1) on plant growth in our study cases (Parveen et al., 2019).

The morphological characteristics of plants, include early germination, plant height, relative root length, root diameter, leaf and root biomass, number of leaves per plant, and number of branches per plant, are significantly impacted by drought stress in an adverse manner(Sarker et al., 2020).

The current investigation suggests the following parameters to consider plant height, the quantity of branches and leaves per plant, chlorophyll content, the percentage of dry matter in leaves, As well as the total number of plants included in the study (Sarker et al., 2020).

Antioxidant enzymes are essential for plants to protect themselves against both living and nonliving stresses. When plants encounter different types of stress, their antioxidant enzymes become more active. This increased activity helps the enzymes efficiently remove harmful reactive oxygen species (ROS) in a precise and effective manner (Altaf et al., 2022) .Drought significantly reduces crop productivity by causing biochemical and physiological changes. As a result of an imbalance between reactive oxygen species production and antioxidant defenses, plants' growth and photosynthesis are adversely affected.

Plant antioxidant enzymes are significantly altered by drought stress. Changes such as these serve as a mechanism for enhanced defense against oxidative damage induced by drought treatment. Tomato leaves exhibited significantly increased levels of superoxide dismutase (SOD) and induced activities of peroxidase (POD), ascorbate peroxidase (APx), catalase (CAT), and malondialdehyde (MDA) (Rohman et al., 2016).

Our study examined the expression patterns of key genes involved in stress-defense pathways in tomatoes to understand how drought stress tolerance is developed. Under varying degrees of drought, we examined two genes, derb3, and derb4, due to their potential involvement in transcription regulation, protein folding, and activation of signal transduction pathways) (Sofo, Scopa, Nuzzaci, & Vitti, 2015)

Through qRT analysis, we monitored the expression levels of these genes over a 60-day period of limited water availability in tomatoes. Our research findings indicate a significant upregulation of *DERB3* and *DERB4* drought stress increases transcript abundance denoted as T7 and T14, in comparison to less severe stress conditions represented by T7 and T21, as well as control conditions, denoted as C7 and C14, at the 65-day mark of under control conditions. Conversely, under control conditions and in their respective parent plants at the same 65-day irrigation deficit period, the fold change of DERB3 and DERB4 was reduced and downregulated. Under drought stress conditions, these results indicate. specifically, T7 and T14, DERB3 and DERB4 genes are prominently expressed, potentially contributing to enhanced physiological growth, increased productivity, and improved antioxidant capacity observed in

drought-tolerant plants containing these genes when compared to their respective counterparts. Consequently, these hybrid plants can be categorized as drought-tolerant owing to the advantageous effects of DERB3 and DERB4 genes under water-deficit conditions (Rai, Rai, Singh, Singh, & Kaushik, 2022).

CONCLUSION

Tomato Plants undergo molecular, morphological, biochemical, and physiological changes during drought stress conditions. Our study investigated how drought impacts tomato genotypes in a comprehensive manner. Several morphological characteristics were considered, the plant's height, the roots' height, the shoot's length, the number of branches, and yield factors all contribute to this. Physiological aspects such as leaf area, relative water content, and SPAD value were also examined. Moreover, the study assessed several biochemical traits, including SOD, total POD, total MDA, and APX. Drought stress significantly alters the activities of antioxidant enzymes within plants. Drought treatment induces these changes as a mechanism of enhanced defense against oxidative damage. The leaves of tomatoes have been found to be significantly higher in superoxide dismutase (SOD) activity, peroxidase (POD), ascorbate peroxidase (APx), catalase (CAT), and malondialdehyde (MDA) activity. DERB3 and DERB4 gene expressions were also analyzed in tomato genotypes. Therefore, qRT-PCR analysis of the DERB genes also showed differential expressions under drought stress at different stages (early stage, 7-day stage, 14-day stage, and rewatering stage). In tomato leaves and roots under drought conditions, DERB3 expression was higher at the 7-day stage than at any other stage. In tomato leaves and roots, DERB4 expression increased after 14 days. Our research concluded that tomato genotype enhanced drought resistance differently, chlorophyll, POD, CAT APX, MDA, DERB3, and DERB4 may be effective mechanisms for drought tolerance in tomato genotypes. According to these results, molecular, biochemical, and physiological parameters are more relevant when screening tomato genotypes for drought tolerance.

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