Identification and Isolation of DREB Gene(s) from *Solanum lycopersicum*

By

Aneela Mustafa

Registration # 00000327805

Supervisor

Dr. Faiza Munir

DEPARTMENT OF PLANT BIOTECHNOLOGY

ATTA-UR-RAHMAN SCHOOL OF APPLIED BIOSCIENCES (ASAB)

NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY (NUST)

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A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science in Plant Biotechnology

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Registration # 00000327805

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Dr. Faiza Munir

Supervisor's Signature

DEPARTMENT OF PLANT BIOTECHNOLOGY

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(ASAB)NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY
(NUST) ISLAMABAD, PAKISTAN.

2022

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Titled: Identification and Isolation of (DREB) gene(s) from Solanum lycopersicum be accepted in partial fulfillment of the requirements for the award of **MS Plant Biotechnology** degree with (B+ grade).

Examination Committee Members

1. Name: **Dr. Alvina Gul** Signature:

2. Name: **Dr. Rabia Amir** Signature:

Assistant Professor
Department of Biosciences
Capital University of
Science and Technology

3. Name: **Dr. Erum Dilshad** Signature: Erum Dilshad

Supervisor's name: **Dr. Faiza Munir** Signature:

Dr. Faiza Munir
Assistant Professor
Atta-ur Rahman School of
Applied Biosciences (ASAB)
NUST - Islamabad

Date: 22/.8/2022

Dr. Muhammad Faraz Bhatti
Head of Department (HoD)
Deptt of Plant Biotechnology
Atta-ur-Rahman School of Applied
Biosciences (ASAB), NUST Islamabad

Head of Department

Date

COUNTERSINGED

Date:

Dr. Hussnain A. Janjua
Principal
Atta-ur-Rahman School of
Applied Biosciences (ASAB)
NUST, Islamabad

Dean/Principal

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Signature: ________________

Dr. Faiza Munir
Assistant Professor
Atta ur Rahman School of
Applied Biosciences (ASAB)
NUST - Islamabad

Name of Supervisor: **Dr. Faiza Munir**

Date: ________________

Dr. Muhammad Faraz Bhatti
Head of Department (HoD)
Deptt of Plant Biotechnology
Atta-ur-Rahman School of Applied
Biosciences (ASAB), NUST Islamabad

Signature: ________________

HOD Plant Biotechnology: **Dr. Muhammad Faraz Bhatti**

Date: ________________

Dr. Hussnain A. Janjua
Principal
Atta-ur-Rahman School of
Applied Biosciences (ASAB)
NUST, Islamabad

Signature: ________________

Principal ASAB: **Dr. Hussnain A. Janjua**

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Dr. Faiza Munir
Assistant Professor
Atta ur Rahman School of
Applied Biosciences (ASAB)
NUST - Islamabad

Signature of Supervisor

Dr. Faiza Munir

Assistant Professor

Atta-ur-Rahman School of Applied Biosciences (ASAB)

National University of Sciences and Technology (NUST)

Islamabad, Pakistan

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Aneela Mustafa

Master of Science in Plant Biotechnology

Registration # 00000327805

Copyright Statement

Acknowledgement

In the name of Allah, the most Gracious and the most Merciful, all praises to Allah Almighty whose bounteous blessings and glory enabled me to complete this project. I am proud of being the follower of the Holy Prophet Hazrat Mohammad (P.B.U.H), the most perfect and exalted among and every human on earth to seek and acquire knowledge.

I would like to express my gratitude and appreciation to my esteemed supervisor Dr. Faiza Munir for her dedicated support, consistent guidance, motivation and patience throughout the research period. Her insightful comments and recommendations helped me to complete this project. In short, this landmark would have been impossible without her constructive suggestions and valuable feedback.

I would also like to pay my gratitude to Principal, ASAB Dr. Hussnain A. Janjua and HOD Plant Biotechnology Dr. Muhammad Faraz Bhatti for their incredible efforts to create an amazing research atmosphere. I am sincerely thankful to my Guidance and Examination Committee (GEC) members, Dr. Alvina Gul and Dr. Rabia Amir for their great feedback. I am proud to be a part of NUST. I started this journey as a learner and keen observer. I have come out of it as much learned and knowledgeable. It has given me confidence to do more professional work and contribute to my respective field.

I am also thankful to my friends **Warda Jabbar**, **Somia khan**, **Laila-Tul-Qadar** and **Noor-Ul-Huda** for their support and wonderful company throughout my journey at ASAB, NUST.

Last, but not least, I wish to express my heartfelt love and thank you to my father **Ghulam Mustafa** and mother **Bashiran Bibi** and my brothers, **Nadeem Mustafa**, **Ghulam Murtaza** and **Ameen Mustafa** who have also been a father figure to me in my life. I acknowledge the love and support of my sisters-in-law **Sadia Nadeem**, **Binish Murtaza** and **Tabbasum Amin**. I would like to send love to my nieces and nephews **Abeera Nadeem, Abdullah Ghumman**, **Rayyan Nadeem**, **Abeeha Murtaza**, **Bareera Nadeem**, and **Haram Nadeem.**

Aneela Mustafa

Dedication

“This research project is dedicated to my beloved parents and brothers*”*

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Abstract

Abiotic stressors, such as high temperature, dehydration, and excessive salt concentration reduce crop viability, development, and productivity. Under extreme drought stress condition, tomato growth is restricted which results in low plant yield. To develop plant resistant to different stresses, beneficial functions of DREB genes are studied. Dehydration Responsive Element Bindings are crucial crop drought responsive genes that play a key role in enhancing plant drought tolerance. DREB genes interact with a DRE/CRT cis-element located in the promoter region. DREB genes involve ABA independent pathway to regulate drought in plants. The DREBs play significant roles in the control of plant responses to abiotic stressors. *An in-silico* analysis is conducted that involved retrieval of tomato DREB sequences and other monocot plants from the kingdom plantae. In order to evaluate the evolutionary relationship and compare the *Solanum lycopersicum* plant to monocot plants, a phylogenetic analysis is performed using the sequences. Additionally, sub-cellular localization shows that DREB genes both in dicots and monocots have similar localization pattern which indicates their similar functioning in them, but these genes vary in concentration, have higher concentration in dicots than monocots, indicating dicots are better candidates in response to drought stress. In addition to what has already been mentioned, multiscale pocket binding structures of SlDREB with comparison to TaDREB have been discovered which indicated that monocot plants contain more amino acids as compared to dicot plants. Protein analysis shows that dicot peptide lengths are shorter as compared to dicot plants. In wet laboratory techniques, tomato DNA was isolated and amplified using two unique gene primers to identify gene in tomato plants. Prior to sequencing the material, it was purified. Sequences are submitted to GenBank database.

Introduction

Human diets have developed significantly since the dawn of civilization. The availability of resources, the environment, and socioeconomic conditions, all influence food choices and consumption. By providing our bodies with calories and nourishment, they require bioactive substances to help us resist the degenerative effects of pollutants and avoid a variety of health disorders. Vegetables, fruits, and legume seeds include carbohydrates, proteins, minerals and vitamins. Additionally, they contain physiologically active substances that are beneficial to one's health Monteiro et al. (2015).

1.1. Solanaceae Family- An Overview

There are over 90 genera in the Solanaceae family, which has between 3000 and 4000 species. The family comprises of valuable cultivated crops, weeds, spices, ornamental plants, and medicinal plants. Despite the fact that many species of this family comprise of powerful alkaloids and some are exceedingly poisonous, nightshades are consumed by numerous civilizations and are even a staple meal for others. Extremely numerous perennial trees and annual herbaceous species inhabit a variety of terrestrial environments, ranging from arid regions to tropical forests. The Solanaceae family has a mainly global distribution, with a concentration in South America. Despite the family's great size, only a few representatives of the Solanaceae have become important food sources in humanoid cultures [potato, naranjilla, pepper, eggplant, tomato, tree tomato], flowers, and pharmaceuticals (tobacco)(Gebhardt et al., 2016)

1.3. Introduction to Tomato Crop

Tomato (Solanum lycopersicum L.) is one of those foods that are consumed by people worldwide due to their numerous health advantages and vital nutrition. Tomatoes are nutrient dense and contains a variety of minerals and micronutrients, thereby being a prime option for nutritional fortification. Tomatoes are high in vitamin C, folate, vitamin A (as provitamin A) and potassium. Tomatoes have a dry matter between 5% and 10% (Vats et al., 2022).

1.3.1. Significance of Tomato

In tomatoes, carotenoids are the main bioactive compound class. Tomatoes contain two types of carotenoids: nonoxygenated carotenes like lycopene and oxygenated xanthophylls like lutein, zeathanxin, neoxanthin, and canthaxanthin. Phytoene accounts for 5.6% to 12.0%, beta-carotene for 1.1% to 11.0%, neurosporene for 0.0% to 0.9%, phytofluorocarbons for 2.5%, lutein for 0.1% to 1.0%, and other carotenoids for 0.1% to 1.0%. The provitamin A forms of carotenoids in tomatoes are -carotene. The types and amounts of carotenoids in tomatoes vary widely according to cultivar, maturity stage, environment, and growth conditions. Tomatoes are an excellent source of all-trans lycopene and other carotenoids. In nature, lycopene can be found in a variety of cis-forms, including 5-cis, 9-cis, 13-cis, and 15-cis. Variety and maturity level dictate the relative amounts. The percentage of cis-lycopene varied from 0% to 8.83 % across two different varieties in one study and from 0% to 14.22% between two other types.(Castellanos-Sinco et al., 2015).

Tomatoes contain flavonoids and phenols. Raw tomatoes contain quercetin, naringenin chalcone, and rutin. Kaempferol, myricetin, and their glycosides are also found in low concentrations in tomatoes. Caffeoylquinic acid are hydroxycinnamic acids found in tomatoes. Tomatoes contained some phenolic acids for example ferulic acids, p-coumaric and their glucosides. Like carotenoids, phenolic compounds in tomatoes differ greatly depending on cultivar, maturity stage, environmental factors, and growing conditions. The enormous use of tomatoes in industrial operations generates an equal number of tomato seeds, peels, and other trash. The potential for edible oil extraction from tomato seeds is enormous (Giannelos et al., 2005).

Along with beneficial fatty acids, Lycopene, beta-carotene, lutein, tocopherols, and polycosanols are only some of the antioxidants found in abundance in tomato seed oil. Apart from these benefits, tomato seed oil was studied for its physiochemical qualities in order to determine its suitability for usage as biodiesel. They reported that tomato seed oil yields roughly thirty five percent of its dry weight. Oil is highly viscous and extremely stable, as well as a low Sulphur and total ash content, while maintaining a similar cetane number and density to other vegetable oils. They determined that the tomato oil can be a possible contender for usage as biodiesel and a renewable source of energy, based on these features (Giannelos et al., 2005).

Tomatoes are the most popular prototype for researching the molecular mechanism of fruit development and composition. In the twentieth century, classical geneticists described random and induced genetic variations for fruit development, fruit morphology, fruit coloring, and fruit composition. Using either positional cloning or the functional genomic strategy, numerous relevant genes were cloned and functionally characterized at the end of the 20th and beginning of the 21st centuries (Gebhardt et al., 2016).

1.4. Tomato Production

Tomato (Solanum lycopersicum L.) fruit is produced all year, and its crop is the world's second most valuable vegetable crop, after potato. The global tomato production is approximately 182 million tonnes, grown on 4.8 million hectares at an average yield of 38 tonnes per hectare. China and India are the world's largest tomato producers. Globally, fresh tomato exports total US$ 8.8 billion, while tomato and tomato-related products exports exceed US$ 13 billion. Mexico is the largest exporter in the world, followed by Spain. The United States of America is the largest importer of fresh tomatoes, followed by Belgium and Russia (Vats et al., 2022; Waiba et al., 2021). Pakistan is ranked 33rd in terms of tomato production. Total tomato cultivated area in Pakistan is approximately 61 thousand hectares, with a production of 569 thousand tonnes and an average yield of 9.5 tonnes per hectare (Poussio et al., 2022). Population growth necessitates more global agriculture. Biotic and abiotic stresses reduce yield. Abiotic and biotic stresses are serious environmental issues that have a big impact on crop yield. Plants have more complex stress responses than animals. Plants absorb solar energy by absorbing CO2 and generating oxygen and organic matter, sustaining the environment. Climate, temperature, soil nutrient conditions, water availability, pests, and illnesses all affect agricultural production. Abiotic and biotic stressors affect agricultural output (Chand et al., 2021).

1.5. Research objectives

1. To identify DREB genes from Solanum lycopersicum through in silico analysis of phylogenetic studies, evolutionary relationship association and its comparison with monocot members
2. To Isolate, amplify and sequence DREB genes from tomato genotypes.
3. To find sub-cellular localization, Heat map expression, protein function and protein-protein interaction analysis
4. To predict the structures of proteins by 3D modelling and validation of structures.

Literature Review

2.1 Abiotic and Biotic Stresses

Population growth requires a global boost in agricultural output. Biotic and abiotic stressors decrease agricultural yield (Wani et al., 2014). To feed an additional 2.3 billion people by 2050, productivity must improve 70% (Tilman & et al, 2011). Plants have more complicated stress response and tolerance mechanisms than animals. Plant biotechnologists must identify a plant's reaction to adversity in its surroundings. Common abiotic stresses include drought, saltiness, and high temperatures. Plants gather solar energy by consuming CO_2 in the atmosphere and producing oxygen and organic matter, thereby supporting the global environment. Climate change, temperature changes, soil nutrient conditions, water availability, pests and diseases are all factors that have an active or passive impact on agricultural production. Biotic and abiotic stresses are serious issues that have a big impact on crop yield (Lata et al., 2011).

Drought, cold, salt, and heat are all examples of abiotic stressors. whereas biotic stresses comprise bacteria, fungi, viruses, nematodes, and insects, among others. Pathogen infection produces a variety of physiological changes in plants, including biomass loss, early blooming, reduced seed set, and the build-up of protective chemical. Agricultural yield is greatly harmed despite plants being constantly subjected to diverse challenges, resulting in complicated response interactions. Abiotic stresses, can cause 50% crop lose worldwide while biotic stresses result in yield losses of up to 35% (Gull et al., 2019). Significant losses in agricultural production are caused by abiotic stress conditions around the world. Individual stressors like as drought, salinity, and heat have all been extensively researched. Abiotic stressors, on the other hand, are routinely applied to crops and other plants in the field. Many crops, for example, are vulnerable to a mix of drought and many other stresses, such as heat or salinity, in drought-stricken areas (Fichman et al., 2020).

The responses of plants to abiotic stress are intricate, dynamical, flexible and elastic. The afflicted organ or tissue dictates the responses of plants to the stress. Roots, for instance, respond to stress in different tissues or cell-specific ways, depends on the stimulus (Zhu et al., 2016). Furthermore, the severity and duration of stress may influence the response's intricacy (acute vs. chronic). To overcome these limitations and ensure food security, it is necessary to develop new climate-aware agricultural varieties (Idupulapati et al., 2021).

2.2. Adaptation to Abiotic Stressors

Understanding how abiotic stressors affect plants the biochemical, molecular, and physiological levels, are vital for increasing crop yield since abiotic stressors cause significant agricultural losses worldwide. Drought, salt, heat, chilling, freezing, ozone, viruses, and UV radiations are the most influential environmental factors on crop yield. The length and course of the stress, plant stage, biotic and abiotic variables can influence the stress response. Some crops may suffer initial damage, but they will recover and survive (Mostofa et al., 2018). The vulnerability and resistance to stress can vary considerably amongst plant species and genotypes. Drought is the most severe of these stresses, resulting in a global decline in agricultural productivity (Golldack et al., 2014).

Plant development, membrane stability, pigment concentration, water balance, and photosynthesis are all influenced. Numerous rivers around the world are drying up on a regular basis, and the majority no longer carry water to the ocean. Following drought, salinity is the next most significant factor affecting the agricultural production. Numerous plant hormones have been identified to protect plant against abiotic stresses. Some hormones have numerous stress-resistance roles; thus, they've been assigned to specific problems and combinations of stresses. In the past, researchers analyzed abiotic stress conditions by examining different stresses such as heat, salt, and drought, as well as the numerous molecular components of plant acclimation. In contrast, natural ecosystems have various conditions. In nature, crops are subject to a number of forces or combinations of situations (Iwama et al., 2006).

The majority of molecular research is conducted in a laboratory or greenhouse in conditions that are not representative of actual field situations. In the laboratory or greenhouse, only one environmental stress is delivered, whereas in the wild, multiple environmental stresses are applied simultaneously (Dolferus et al., 2011). Therefore, it is cited that studying combined stress at once is more beneficial as compared to studying a single stress at a time. Drought and heat stress cost the United States $200 billion in losses (Lamaoui et al., 2018).

1.6. Effect of Drought Stress on Plant

Among the many abiotic pressures impacting agricultural output around the world, drought stress is becoming increasingly important (Hui et al., 2018). Drought is a condition that impacts plants on multiple levels. Drought is a meteorological condition marked by reduced rainfall, declining groundwater levels, and limited water availability, all of which are accompanied by a rise in temperature (DaMatta et al., 2003). As a result, water scarcity causes drought stress, which reduces plant viability. The majority of crops are affected by drought stress, which can result in yield reductions of up to 50%. Second, excessive solute accumulation is toxic and inhibits the function of numerous enzymes, resulting in less efficient photosynthesis and water utilization (Farooq et al., 2012).

As a result of prolonged dehydration, plants show leaf wilting, rolling, and bleaching, eventually dying. Drought stress is especially harmful to reproductive phases like blooming and seed production. As a result of environmental changes, CO2 levels rise, restricting plant respiration and rising the temperature. When the temperature is raised from 15 to 40 degrees Celsius, the plants' respiration rate increases, altering their morphological traits. Drought-induced increases in CO2 levels in the leaf drive synthesis of oxidative stress (ROS), which cause a variety of crop stressors (Salehi-Lisar et al., 2016). Locked stomata limit CO_2 passage inside the leaf, and increasing oxygen levels result in the production of ROS. ROS-produced membrane breakdown disrupts plant growth, photosynthetic reactions, and respiratory mechanism (Qi et al., 2018). ROS during drought damage lipids, proteins, carbohydrates, and nucleic acids. Drought strain reduces turgor pressure, causing slow cell development. Water constraint has an impact on photosynthetic enzymes, reducing metabolic competence and ultimately damaging photosynthetic apparatus (Impa et al., 2012).

1.7. Effect of Salinity Stress

The second most severe global hazard influencing agricultural output is soil salinity (Parihar et al., 2015). Salt accumulation, which can be induced by primary or secondary processes, causes soil to become saline (Läuchli et al., 2007). Primary sources, natural ways of salt disposition. Air precipitation, ocean deposition, and parent rock weathering are the main processes. Some examples of secondary causes are inadequate drainage, poor water management, long-term, continuous irrigation, the use of brackish groundwater, and cultural approaches to irrigation. There is a rapid increase in soil salinity, which can reduce agricultural productivity by as much as 20%, and it is predicted that 0.3–1.5 million acres of cropland will be added each year (Yadav et al., 2020).

Ion poisoning and osmotic stress are two primary repercussions of increased salt content in crops. Under normal conditions, plant cells maintain higher osmotic pressure than soil solution. Due to increased osmotic pressure, plant cells transport soil water and essential nutrients to root cells.. In stress conditions, the soil osmotic pressure rises against plants cell, lowering the plant cells capacity to retain water. In contrast, cell membrane and metabolic activities are affected by sodium and chloride ion movement into plant cells. In addition to impairing cytosolic metabolism, membrane function, and cell development, salt stress causes a variety of secondary consequences (Isayenkov et al., 2019). Salinity stress is caused by the formation of reactive oxygen species after oxidative stress. Calcium and potassium channels in the plasma membrane are often activated when reactive oxygen species are present. Mutations in the DNA caused by reactive oxygen species (ROS) causes cell damage (Ahanger et al., 2017).

1.8. Plant Transcription Factors

Depending on the species, sessile plants have developed a variety of defenses to withstand environmental constraints (Al-Whaibi et al., 2011). Plants are subjected to abiotic and biotic stressors on a regular basis, which are regarded as a significant threat to crop yield around the world. Multiple signaling pathways are activated by plants to modulate stress responses(Wang et al., 2004). Stress-regulatory genes are primarily stimulated to control gene expression in diverse areas of the plant that are under stress (Scharf et al., 2012). There are numerous transcription factors in plants, with about 1500 transcription factors belonging to distinct gene families in Arabidopsis. Plants have unique ways to combat with different abiotic stresses. Abiotic stress-inducible genes in plants have been found and classified. These genes encode osmolyte accumulation, LEA, transporter, detoxifying proteins, and heat shock proteins (Maruyama et al., 2012).

All of these regulate signal transduction pathway. In the promoter region cis acting elements are found to which different plant transcription factors bind to genes and control gene expression. Growth, development, reproduction, differentiation, metabolism, and environmental adaptability need gene expression regulation in plants. An estimated 1500 transcription factors involved in stress-responsive gene expression are located in the Arabidopsis genome. (Yoo et al., 2010). Microarray studies show that Arabidopsis' DREB/CBF regulon responds independently to abiotic stress. Transcription factors are also genetic alternatives for crop improvement because they coordinate plant development in response to stresses (Xu et al., 2011).

Plants respond to environmental changes with a smart, coordinated reaction. RWC, EL, ROS, and free radicals impact cellular homeostasis by reacting with lipids, proteins, pigments, and nucleic acids, producing lipid peroxidation, membrane damage, and lipid peroxidation (LP)(Razi et al., 2021; Xu et al., 2011). ABA hormone reduces photosynthetic activity and limits transpiration (WUE). Stress detection, signal transmission, metabolic alterations, gene expression Reduce stress. Stress-induced genes regulate metabolic proteins and signal transduction genes (Sasi et al., 2021).

Transcriptome study categorizes gene products. Few genes code for water-stress-protecting proteins. These genes are involved in the buildup of solutes, passive transport and water consumption different transport systems safety and stability of cell structures against waterlessness and harm due to reactive oxygen species (Seki et al., 2003). Transgenic plants can be drought- or salt-tolerant by adding or overexpressing late embryogenesis abundant proteins and betaine synthetase (Bhatnagar-Mathur et al., 2008). Regulatory proteins regulate stress signal transmission and gene expression. Stress-related gene promoter trans-elements up regulate downstream genes to increase stress tolerance. ABA independent pathway is involved under drought conditions (Agarwal et al., 2010).

1.6. Regulation of DREB Genes Under Abiotic Stress

Both the inside and the outside of a plant can trigger responses. These responses are mediated by phytohormones, which are a diverse group of signaling molecules produced by plants (Voß et al., 2014). Plant adjust to the changing environments because of the role that mediates growth, development, source/sink transitions, and nutrient allocation plays (Fahad, Hussain, Bano, et al., 2015). When it comes to managing the physiological and molecular responses of plants, phytohormones are absolutely necessary. Phytohormones can exert their effects either where they are produced or elsewhere in plants. The growth and adaptability of plants are both aided by phytohormones (Peleg & Blumwald, 2011). Auxin (IAA), CKs, ABA, ethylene (ET), GAs, salicylic acid (SA), and others are some examples (JAs). Abscisic acid, also known as ABA, is referred to as a "stress hormone" due to its role in the process by which plants respond to abiotic stresses. Plastids have an isoprenoid that is composed of MEP. The dormancy of seeds, the opening of stomata, the development of embryos, and the synthesis of proteins and lipids are all influenced by ABA (Sreenivasulu et al., 2010). It is a common knowledge that Abscisic acid plays an important role in a plant's ability to tolerate stress, as it is a critical signaling molecule in the adaptive plant response to environmental stress. Environmental pressures can cause a rise in the amount of endogenous ABA, which can start signaling cascades and alter gene expression (O'Brien et al., 2013).

According to one study, ABA has an effect on ten percent of the genes that code for proteins. The action of ABA as an internal signal is what allows plants to live long. ABA is able to help plants communicate with their shoots to let them know that their roots are under stress. This causes water-saving anti-transparent behavior in the plant, such as stomatal closure and reduced leaf growth. Under conditions of low nitrogen availability and drought, ABA promotes the root growth and causes structural modifications (Wilkinson et al., 2012). The expression of genes- stress respondents as well as protein synthesis can be regulated by ABA (Verslues et al., 2006). In order to offer drought tolerance, ABA boosts the synthesis of osmo-protectant and antioxidant enzymes while also increasing cell turgor (Sreenivasulu et al., 2012)..

Studies on the production, transport, and signaling functions of auxin (IAA) have been conducted since the early 1900s (Ke et al., 2015). There are four Trp-dependent routes and one Trp-independent pathway in the synthesis of auxin in plants (Mano et al., 2012). IAA, also known as indole-3-acetic acid, is essential for the growth, development, and management of stress in plants. Auxin's importance in plant adaptation is demonstrated by the fact that single-celled green algae generate signal and transport the compound. We have a good understanding of the role that auxin plays in plant growth and development, but not of the impact that stress plays (Kazan et al., 2013).According to research, IAA assists plants in adjusting to the effects of salt stress.(Kazan et al., 2013). It facilitates the production of roots and shoots in plants that are under stress from salt or metals (Egamberdieva et al., 2009).

Salinity was found to reduce the levels of maize IAA, whereas salicylic acid was found to raise those levels (Fahad, Hussain, Bano, et al., 2015). This finding suggests that Signal transduction, perception and stress response require hormonal equilibrium and crosstalk. Rice, Arabidopsis, and soybean, all have auxin response genes that can be induced by auxin. The discovery of novel stress genes may be able to assist crops in surviving abiotic stress (Fahad, Hussain, Matloob, et al., 2015). Endogenous CK levels are affected by both dryness and salinity. Cells and tissues that have been mutated or transgenic and have changed cytokinin enzymes or sensing machinery point to a potential function in agriculture and tolerance to stress (Zalabák et al., 2013).

Absorption and production of CK are both boosted by stress. In contrast to ABA, they activate dormant seeds. Anti-ABA CKs Plants that are under water stress will collect less CK and ABA, which will improve their ratio of ABA to CK. Reduced levels of CK increase apical dominance, which helps plants adapt to dryness by working in conjunction with the ABA's ability to regulate stomatal opening and closing. ET is responsible for the ripening of fruit, the ageing of flowers, the falling off of leaves and petals, and various stress reactions. Both ACC and AdoMet are responsible for the biosynthesis of methionine. ACC synthase is responsible for the conversion of AdoMet into ACC and ethylene (Shi et al., 2012). Both temperature and salinity influence the transpiration rate of plants. The use of ET increases tolerance (Shi et al., 2012).

Heat stress is alleviated by ET. The environmental stress’s effect on ET production is beneficial to plant survival. Ethylene signal effectors modulate gene expression. Both ET and JA contribute to combat abiotic and biotic stresses. They ensure that resistance to diseases and pests is maintained. The production, distribution, and storage of hormones, all contribute to increased plant defense. The effects of ET and ABA on plant growth can either be synergistic or antagonistic (Klay et al., 2014). Methyl jasmonate (MeJA) and jasmonic acid are the two most common cyclopentanone phytohormones (JAs) that are synthesised from membrane fatty acids (JA)(Fahad, Nie, et al., 2015; H. S. Seo et al., 2001). Multifunctional chemicals are active in both the direct and indirect defence mechanisms of plants as well as their reproduction, blooming, fruiting, senescence, and secondary metabolism (J. S. Seo et al., 2011).

1.9. Gene family DREB (Dehydration Responsive Element Binding)

Dehydration responsive element-binding (DREB) TFs promote plant tolerance to abiotic stresses by binding DRE/CRT cis-elements(Rae, Lao, & Kavanagh, 2011). APETALA2/ETHYLENE-RESPONSIVE FACTOR (AP2/ERF) comprises DREB TF family. The AP2 domain, which is 60–70 amino acids long, defines the AP2/ERF superfamily. The 19–22 amino acid YRG region contains the conserved YRG motif that gives AP2 DNA-binding specificity (Mushtaq, Munir, Gul, Amir, & Paracha, 2021). RAYD element in AP2 domain has conserved core area that can form amphipathic -helix. DREB's AP2 domain differs from others in amino acid locations (Ghorbani, Zakipour, Alemzadeh, Razi, & Plants, 2020).

Val14 and Glu19 are conserved DREB residues. 60-70 amino acids are conserved in AP2 domain that are needed for plant stress and defense processes (Ain-Ali et al., 2021). One V, four R, and two W residues are needed for DRE binding in the AP2 domain (Allen et al. 1998A Threonine/Serine -rich preserved area near the AP2 domain is needed for DREB 26 gene phosphorylation (Hrmova & Hussain, 2021). DREB genes have six structural types, A1-A6. DREB transcription factors interact directly with DRE/CRT, which has the core motifs ACCGAC/GCCGAC, and are promising for abiotic stress tolerance in plants(Ren et al., 2019). KIN1 and KIN2 (cold responsive), RD29A (drought responsive)(L. Chen et al., 2013), LEA (Late Embryogenesis Abundant) (Yu Liang et al., 2019), COR15A and COR15B have these sequences in their promoter regions (cold responsive) (M. Li et al., 2013).

The DNA-binding domain of DREBs is crucial for the regulation of the expression of stress-responsive genes via DRE/CRT cis-elements. They play an essential role in providing resistance to a wide range of stressors and display responses that are broadly similar across a range of stress conditions. In response to abiotic and biotic stress, DREBs control stress-responsive gene expression through ABA-independent mechanisms. Identifying the most conserved domains of DREB proteins is important for the creation of genetically modified solutions for crop improvement since these domains are required for the proteins' unique biological activities. Transgenics with increased tolerance to high salt, drought, and/or cold stress can be produced by combining the DREBs with additional promoters. Improve stress tolerance without reducing yield by recruiting stress-induced promoters and transcription factors. Success or failure will ultimately depend on the host plant's genetics.(Agarwal et al., 2006).

1.10. Role of DREB Genes Under Abiotic Stress

DREB genes contribute to drought, salinity, cold, and heat stress responses(Jangale et al., 2019). First-ever Arabidopsis DREB genes have been found. When subjected to cold and dehydration, AtDREB2 and AtDREB1 can act as 2 different proteins(Q. Liu et al., 1998). According to a study, Arabidopsis ABA signaling has little effect on A1 and A2 expression. ABI4, A3 sub-group member, is involved in ABA signaling and sugar signaling.(Shkolnik-Inbar et al., 2011). TINY, related with drought tolerance, and HARDY, associated with salinity and drought tolerance, are the most studied A4 subgroup members(C. Niu et al., 2019). Cold and Drought stress activate A5 subgroup RAP2.4, RAP2.1. and RAP2.4B genes in A6 subgroups address drought, heat, and salinity(Huang et al., 2020).

DREB is connected to drought resistance in several plant species. Overexpressing DREB1A in Arabidopsis and tobacco increased drought tolerance. RD29A promoter created a wheat phenotype. Cotton's DREB gene increased wheat's drought, heat, and cold tolerance. Overexpressing CsDREB in transgenic Arabidopsis boosted salinity and drought tolerance(M. Wang et al., 2017).Arabidopsis (Rae et al., 2011), rice (Zhao et al., 2010), bell pepper (Kong et al., 2019), soybean (Kidokoro et al., 2015), pearl millet (Saha et al., 2014), wheat (X. Niu et al., 2020), maize, chrysanthemum, strawberry, and tobacco have DREB genes (tomato). Diagram showing their abiotic stress responses. Transgenic Leymus chinensis DREB3a improved Arabidopsis salinity and drought tolerance. The DREB2 gene in Broussonetia papyrifera responds to drought and salinity, according to functional analyses(Lata et al., 2011).

BpDREB2 transgenic expression in Arabidopsis improved cold and salinity tolerance. Medicago truncatula studies showed the role of DREB genes in cold stress. ZmDBF3, a maize DREB gene, is upregulated by salinity, drought, heat, and cold. Overexpression of TaDREB3-AI improved heat, dehydration, and salinity stress resistance. The Arabidopsis DREB1B gene under the RD29A promoter improved drought tolerance, photosynthesis, and antioxidant defense mechanisms in Salvia miltiorrhiza. AtDREB1C modification increased drought tolerance in transgenic Salvia miliflora. DREB genes are linked to abiotic or environmental stress responses in plants and designing agricultural stress resistance (Mushtaq et al., 2021).

1.11. ABA-Independent DREB Signaling

Drought is one of the worst environmental pressures and impairs plant processes. Abscisic acid (ABA) is created during water scarcity and aids drought tolerance. ABA induces most drought-stress genes. In ABA or Arabidopsis mutants, dehydration, salt, and cold stimulated additional genes. Some genes may not need ABA in drought, salt, and freezing environments. In a previous work, CRT/DRE elements were linked to ABA signaling. The cor78a/rd29A ABA-responsive element needs CRT/DRE elements. DREBs, save CBF4, are ABA-independent. CBF/DREB1 and DREB2 proteins regulate ABA-independent drought and cold gene expression. The transcription factors for DREB family has conserved domains that allow them to engage in downstream gene interaction in the absence of ABA. DRE's role in ABA-dependent stress regulation suggests crosstalk between the two types of signal transduction pathways, reliant on and independent of ABA. This interaction demonstrates how stress signals and ABA regulate stress-induced genes(Lata et al., 2011). Table 1 shows DREB genes identified in different plants.

Table 1. DREB genes identified in different plant species.

Sr. No	**Plant name**	**DREB genes identified**	**References**
1.	*Hordeum vulgare*	41	(Guo et al., 2016)
2.	Vigna radiata	30	(Labbo et al., 2018)
3.	*Musa acuminate (genome A)*	81	(Lakhwani et al., 2016)
4.	*Musa balbisiana (genome B)*	99	(Lakhwani et al., 2016)
5.	*T. aestivum*	210	(X. Niu et al., 2020)
6.	*Ananas comosus*	20	(Chai et al., 2020)
7.	*Morus notabilis*	30	(X. Liu et al., 2015)

Developing better plant varieties with much higher stress tolerance and adaptation is a simple, cost-effective, and environmentally friendly strategy. Plant genomics and bioinformatics can help plant scientists find and use stress-responsive genes, allowing them to create more stress-tolerant cropsB. There are six A-1 to A-6 DREB genes discovered in Arabidopsis thaliana, or DREB1 to DREB6. The A-1 and A-2 TFs have the most functionally defined TFs(Sakuma et al., 2002). A-1 member AtCBF1 activated due to low temperature. Additionally, DREB1A and DREB1C are reported to stimulate under cold stress. SwDREB1 is involved in the response of sweet potatoes to cold temperatures (Ipomoea batatas)(Kim et al., 2008).

ZjDREB1.4(Feng et al., 2019), from zoysia grass (Zoysia japonica) was overexpressed heterologously in Arabidopsis, increasing resilience to hot and cold temperatures without harming growth. Interaction among DREB1A, DREB1B, and DREB1C with the GCC box of Oryza sativa increases cold stress tolerance(Kim et al., 2008). As a result, DREB1 TFs are primarily associated with the regulation of cold stress. In literature DREB2 has been reported to linked with drought and salinity tolerance. DREB2A and DREB2B of Arabidopsis thaliana belonging to A-2 subfamily of DREB genes are known to stimulate under drought and salt conditions (Sakuma et al., 2002).

GmDREB2 overexpression improved salt tolerance in Arabidopsis without affecting growth retardation (M. Chen et al., 2007). Sugarcane (Saccharum spp. Hybrid) resistance to drought and salinity stress was increased by heterologous overexpression of EaDREB2 (Augustine et al., 2015). A-3 to A-6 proteins, unlike A-1 and A-2 proteins, have yet to be found. The A-4 subgroup gene DREB4.1 in maize (Zea mays) which belongs to A-4 group has been connected to plant development (Li et al., 2018). Desert moss (Syntrichia caninervis) ScDREB8 gene belonging to A-5 subfamily this has been demonstrated to improve Arabidopsis seedling salt tolerance (Yuqing Liang et al., 2017). Overexpression of CmDREB6 gene of A-6 subfamily is linked to improve heat stress in chrysanthemum (Chrysanthemum morifolium) (Du et al., 2018).

Materials and Methods

3.1. *In-Silico* Analysis

3.1.1. Sequence Retrieval

Tomato DREB protein sequences were retrieved through NCBI database using potato protein sequences as query sequence. The protein length, chromosomal position, complete coding sequences, AP domain length and accession number were maintained in an excel sheet. The DREB protein sequences of different plants were retrieved from blast NCBI. The different plants selected for phylogenetic analysis are *Solanum lycopersicum, Sorghum, Arabidopsis thaliana, Zea mays*, *Triticum aestivum,* and *Oryza sativa.*

3.1.2. Phylogenetic Analysis and Motif Analysis

To create phylogenetic trees, the full-length amino acid sequences of tomato DREB genes and other plant gene proteins were aligned. Multiple sequence alignment was executed by ClustalX 2.1. Following alignment, the tree was constructed through NGPhylogeny with 1000 bootstrap. Based on Arabidopsis thaliana protein classification, tomato genes were classified into different classes. The MEME software was used to find conserved motifs in *solanum lycopersicum* DREB protein sequences. The criteria were set as following motif width should be 6-15 amino acid, one occurrence per sequence and motif limit was set 10.

3.1.3. Protein Three-Dimensional Structure Analysis

String database was used to search for protein interactors that interact with the tomato protein. The SOPMA server was utilized so that the secondary structure analysis of SlDREB proteins could be carried out. The I-TASSER was able to build the three-dimensional structures of SlDREB proteins. The Ramachandran plot analysis and PROCHECK server were both employed for the structural validation of SlDREB proteins.

3.1.4. Active Catalytic Sites Analysis

Using the CASTp 3.0 web server the multi-scale pocket structures of SlDREB protein surfaces were identified.

3.1.5. Heat Map Analysis

Using TB tools software heat map of four tomato representative and four wheat representatives was generated and the presence of genes SlDREB1, SlDREB3, SlDREB4A, SlDREB9A and TaDREB3A.1, TaDREB20.1, TaDREB21D.2 and TaDREB26D.2 is found out.

3.2. Molecular Analysis

3.2.1. Sample Collection

Tomato seeds were obtained from National Agriculture Research Centre (NARC), Islamabad. The varieties include (table 2).

Table 2. Different tomato plant varieties germinated in soil.

Sr. No	**Variety Name**
1.	Solanum lycopersicum. var. Roma
2.	Solanum lycopersicum. var. Money Maker
3.	Solanum lycopersicum. var. Rio-Grande
4.	Solanum lycopersicum. var. Pakit

The tomato seeds were grown in a greenhouse. Plants were watered every other day and kept at 18-25°C.

3.2.2. DNA Extraction

DNA was extracted using CTAB and plant leaves. Plant leaves were grounded to fine powder in pestle and mortar. 1ml of CTAB, 2l of b-mercaptoethanol, and a little PVPP (polyvinylpolypyrrolidone) was preheated at 65 degrees Celsius for 30 minutes. After heating CTAB, it was added to grounded leaves and slurry was obtained which then transferred to Eppendorf. The sample has been heated for 30 minutes at 65 degrees Celsius. 750 l microliters of chloroform-IAA (24:1) were applied to the sample after heating. For 10-20 minutes, the tubes were rotated in an orbital shaker. After being centrifuged at 13,000 rpm for 10 minutes, the samples were taken out.

The supernatant (aqueous phase) was shifted to 1.5 ml Eppendorf tube. Cold isopropanol 600 ml was added to the supernatant containing DNA. Ten minutes of 13,000 rpm centrifugation yielded the DNA Pallet. Wash buffer was used to wash the buffer (70 percent ethanol). The pallet was centrifuged for 5 minutes to remove the impurity if there was left any. Inverting the tube, the wash buffer was drained carefully and DNA pellet was desiccated. The DNA was liquified in 50 microliters of T.E. buffer. The DNA quality was examined on an agarose gel and kept at -20 degrees Celsius.

3.2.3. Gel Electrophoresis

DNA was visualized using gel electrophoresis. The formation of an agarose gel with a concentration of 1.5 percent required the heating of 0.75g of agarose and 50 ml of 1X TAE . Following a cooling period of one to two minutes at room temperature, five microliters of ethidium bromide were added to the solution in order to see it under UV light. Following the completion of the preparation of the solution, it was placed onto a tray that contained comb. The gel was allowed to cool down for forty minutes. After the casting tray solidified, it was placed in 1x TAE gel tank. In the first well, a 100 bp ladder was loaded, and in subsequent wells, mixture 3 microliter of DNA sample with 1 µl of loading dye was loaded. Gel electrophoresis apparatus settings were set at 80 volts, 500 mA, and 45 minutes. Gel Doc was used after 45 minutes to visualize DNA bands.

3.2.4 Primer Designing

First, LeDREB3 and StDREB3 sequences were downloaded from NCBI to build primers. Handmade primers followed. After primer design, OligoCalc determined GC concentration, size of primer and Tm.

SlDREB3A primer set

StDREB3(F) GCCCATGGATTCCCAAATCTTTTCAAC

StDREB3(R) GCAGATCTTATAGAGAGGCCCAATCAATTTC

SlDREB3B primer set

LeDREB3(F) ATGGATGATAGGTGGTGGGG

LeDREB3(R) ATCCACTTCTGGATCTTCGG

3.3. PCR Amplification

The PCR reaction mixture was 25 microliters. Following reagents mentioned in table 3 were added.

Table 3. PCR reaction mixture recipe

Sr. No	Chemical Name	Chemical volume
1.	PCR Water	15 µl
2.	10x Taq buffer	2 µl
3.	Mgcl2	2 µl
4.	2.5mM dNTP’s	2.5 µl
5.	Primer (F)	1 µl
6.	Primer (R)	1 µl
7.	DNA sample	1 µl
8.	Taq Polymerase	0.5 µl

Results

4.1. *In silico* Analysis

In this research work, DREB genes in seven diverse plant species were identified though *in silico* studies. The plant species include Potato, Arabidopsis, Millet, Maize, Rice, Wheat and Tomato. To study evolutionary relatedness and sequence similarity A Neighbor- Joining phylogenetic method with thousand bootstrap repetitions was utilized to create a phylogenetic tree for DREB sequences among S. lycopersicum (23 DREB sequences) and other plants using DREB protein sequences from diverse plants (100 DREB proteins). Multiple sequence alignment (MSA) showed that AP2 domain is conserved in all DREB monocot and dicot sequences. DREB. Figure 1 shows the classification of DREB genes into 6 sub-families. Each family is denoted by a different color in the figure. The sub-classes are classified on the *Arabidopsis thaliana* classification basis. The classes are referred as A-1, A-2, A-3, A-4, A-5 and A-6. The biggest subgroup A6 comprised of 54 DREB genes while group A3 contained of 3 DREB genes making it the smallest group in tree.

4.2. Cellular localization

Four representatives from tomato and four from wheat genome sequences was utilized to find cellular localization through DeepLoc server. The occurrence of genes SlDREB1, SlDREB3, SlDREB4A, SlDREB9A is higher in nucleus and mitochondria. The occurrence of genes TaDREB3.1, TaDREB21D.2, TaDREB26A.1 is higher in nucleus but the occurrence of TaDREB20D.2 were lower as compared to rest genes. The amount in mitochondria is remained same figure 3 and 9 explains the cellular localization of representative tomato and wheat DREB genes respectively.

Cellular localization results showed that dicot plants have higher concentration than monocot plants which makes dicot plants a strong candidate as compared to monocot plants.

4.3. Protein modeling

The SOPMA online server was employed to predict secondary structure of tomato DREB genes, the proportion of -helices, extended strands, beta turns, and random coils were estimated in Table 2. Variations in proteins affect their sequences, shape, durability, quantity, and function. Characteristics of different SlDREBs are shown in Table 4. Protein models and their respective

Ramachandran plot for validation are shown in figure 7, 8, 13 and 14 respectively. Figure 8 is showing the Ramachandran plots of SlDREB protein models as structure validation, these structure are less accurate as compared to monocot plants. Figure 14 shows the structure validation of wheat modes, which depicts that monocot plants have more suitable structure, as monocot plants have less amino residue in disallowed regions.

Table 4 SlDREB secondary structure analysis using SOMPA software

Protein	a-helices	extended strands	Beta turns	Random coils
SlDREB1	80 is **36.36%**	24 is **10.91**	11 is **5.00%**	105 is **47.73%**
SlDREB3	53 is **25.85%**	32 **is 15.61%**	6 is **2.93%**	114 is **55.61%**
SlDREB4A	99 is **33.00%**	31 is **10.33%**	14 is **4.67%**	156 is **52.00%**
SlDREB9A	67 is **31.90%**	24 is **11.43%**	10 is **4.76%**	109 is **51.90%**

4.3. Binding pockets predictions

The molecular pockets on the SlDREB protein surfaces were found, defined, and quantified by the CASTp 3.0 server . It is essential for structural versatility and interaction specificity to detect protein binding sites. We hypothesized that the pocket binding positions of SlDREB proteins would provide insight into structural alterations in Solanum lycopersicum that may be related to the functions of DREB proteins. Catalytic ligand binding sites are represented by the red areas. It was possible to measure the surface area and volume of the predicted ligand binding sites. This pocket was shown to have high concentrations of valine, arginine, alanine, glutamate, lysine, tyrosine, isoleucine and leucine. Different binding pocket of SlDREB genes and TaDREB genes are shown in figure 4 and 10 respectively.

Structural comparison between SlDREB and TaDREB further revealed that the conserved binding pocket is same within all proteins but the variation in some structure is due to the presence of additional amino acids in TaDREBs which make TaDREB plots more suitable than SlDREB.

1. The comparison between TaDREB3A.1 and SlREB3 shown that their catalytic binding pocket contain ARG, GLU AND ILE showing amino acids are conserved.

2. TaDREB20, TaDREB21 and DREB26 have ARG similar to SlDREB3 but rest of the SlDREBs i.e. SlDREB1, SlDREB4A and SlDREB9A genes do not have ARG that distinguish monocots and dicots. Table 5 shows the functions of SlDREB genes.

Table 5. Predicted area and volume of the SlDREB proteins' active catalytic site using CASTp.

Protein	Pockets	Area (SA)	Volume (SA)	Amino acids
DREB1	**1**	**186.243**	**151.843**	**ARG, GLY, ILE, GLU,**
DREB3	**1**	**21.63**	**6.473**	**VAL, ARG, PRO, ASN, ALA, LEU**
DREB4A	**1**	**23.071**	**5.683**	**GLU, PRO, LYS,**
DREB9A	**1**	**94.511**	**107.146**	**ASP, LEU, ASN, ALA**

4.4. Protein Association Network

By analyzing interaction networks, protein functions and molecular pathways can be better understood. Analysis of protein-protein interactions reveals its unknown function. We used

STRING to predict SlDREB interactors. The interactions were a physical and functional protein network. Five SlDREB proteins interact with regulatory proteins from genes unrelated to DREB. Protein-protein interaction revealed that they have multifunctional roles and are involved in different regulatory pathway.

The SlDREB1 interactors are NAC1, NAC domain protein, DREB genes tend to bind to the promoter region of NAC protein. SlDREB3 interactions are SIZ1 genes and it interact with SlDREB genes within the same family. The interactor of SlDREB4A is AREB which help the plant in stability during stress conditions. The interactor of SlDREB9A are RAV1, NAC1 and AIB3 which help in plant function during stress conditions. Different protein-protein interaction network is shown in figure 5 for tomato and in figure 11 for wheat.

4.5. Heat Map Analysis

The heat map analysis shows that the presence of genes SlDREB1, SlDREB3, SlDREB4A, SlDREB9A is higher in nucleus and mitochondria and it shows that there is no gene presence in plastid and membrane organelles.

The heat map analysis for four wheat sequences TaDREB3A.1, TaDREB20.1, TaDREB21D.1, TaDREB26D.2 shows that these genes are also have higher levels inside nucleus and mitochondrial regions and the gene TaDREB20.1 has lower level concentration in nucleus as compared to rest genes. DREB genes both in dicots and monocots have similar localization pattern which indicates their similar functioning in them, but these genes vary in concentration, have higher concentration in dicots than monocots, indicating dicots are better candidates in response to drought stress, figure 6 explains the different concentration of DREB genes in different sub cellular organelles.

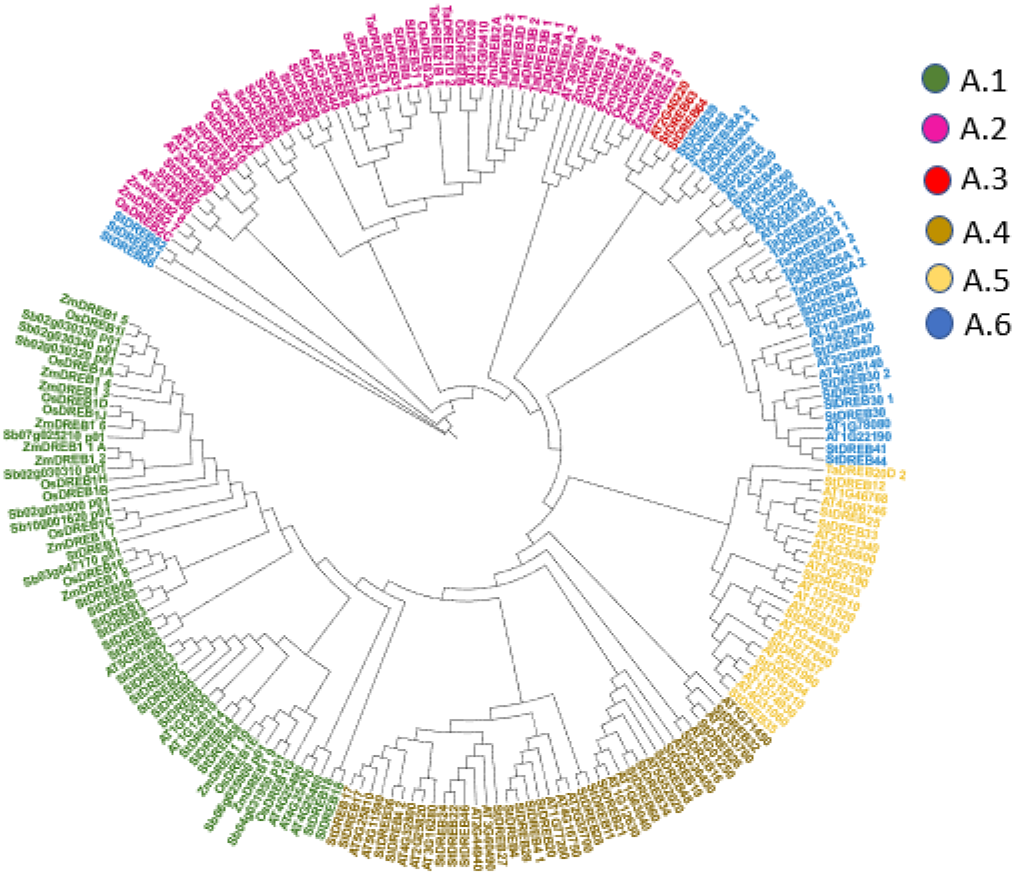


Figure 1. Phylogenetic analysis drawn by Ngphylogeny between tomato and different plants, different plants represents A-1 to A-6 classes, which are present in tomato genome.

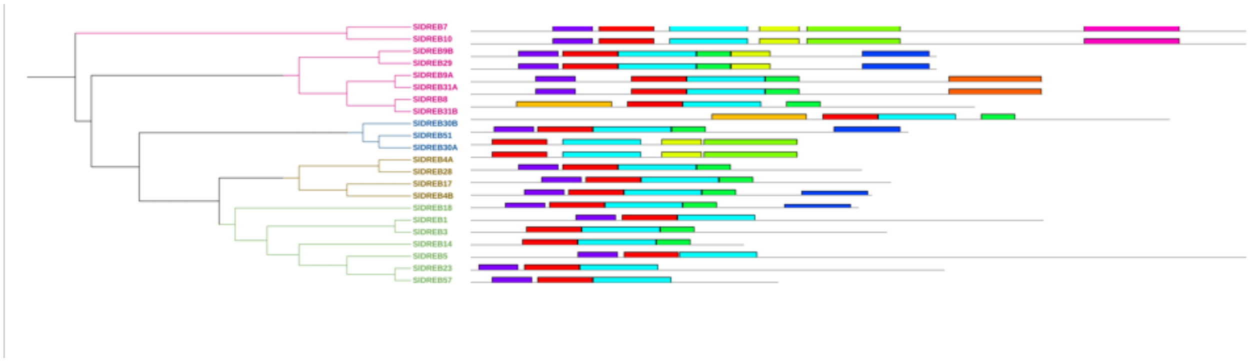
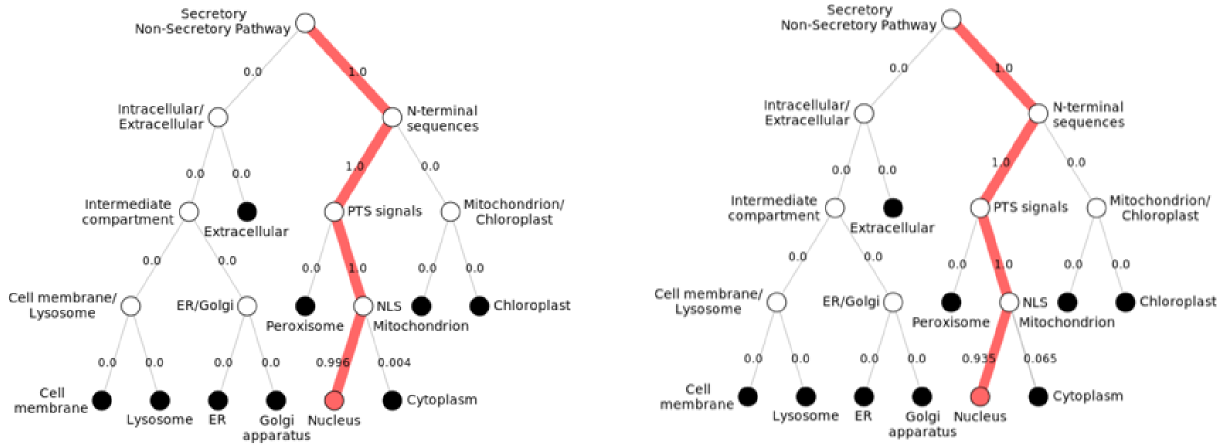
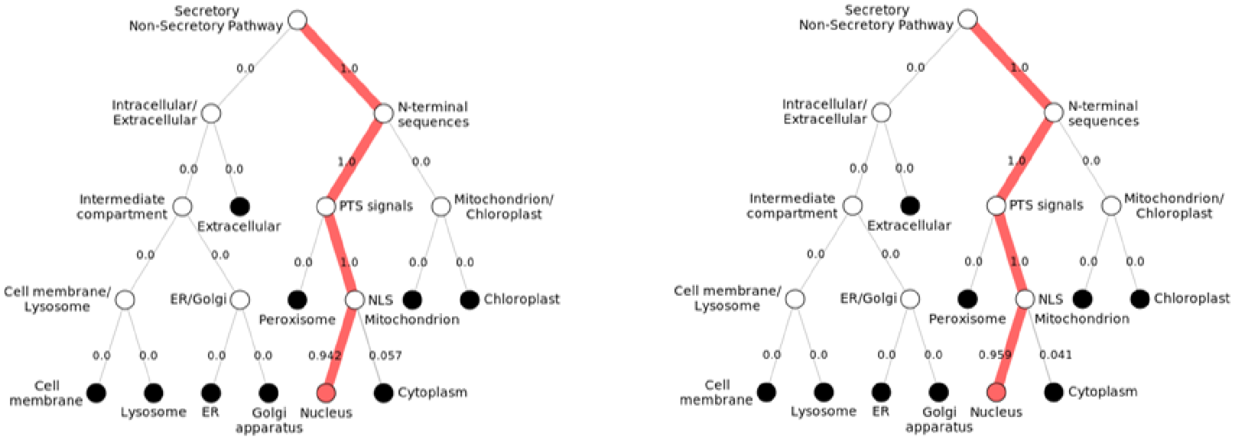


Figure 2 phylogenetic and evolutionary study of SlDREB motif patterns. MEME found conserved motifs in SlDREB proteins. Each of ten expected motifs was represented by a colored box. Grey lines marked non-conserved areas.



Hierarchical Tree of DREB9

Hierarchical Tree of DREB3



Hierarchical Tree of DREB1

Hierarchical Tree of DREB4.

Figure 3. Protein localization of SlDREB1, SlDREB3, SlDREB4A, and SlDREB9A done through DeepLoc server.

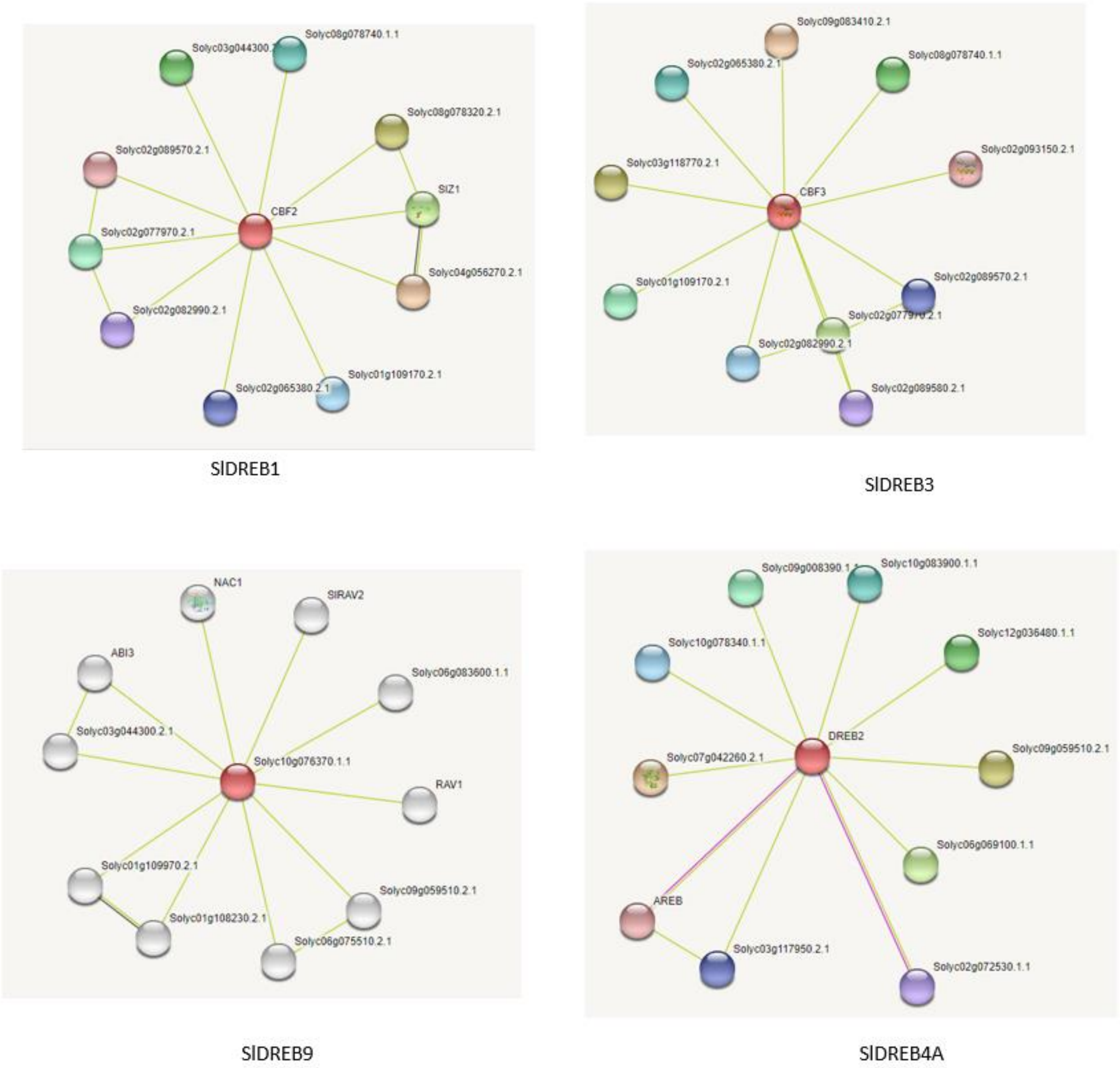
Protein Function SlDREB1

Protein Function SlDREB3

Protein Function SlDREB9A

Protein Function of SlDREB4A

Figure 4. Protein analysis of SlDREB1, SlDREB3, SlDREB4A, and SlDREB9A done through CASTp server.



SlDREB1

SlDREB3

SlDREB9

SlDREB4A

Figure 5 shows the different interactors of SlDRE1, SlDREB3, SlDREB4A and SlDREB9A done through STRING database.

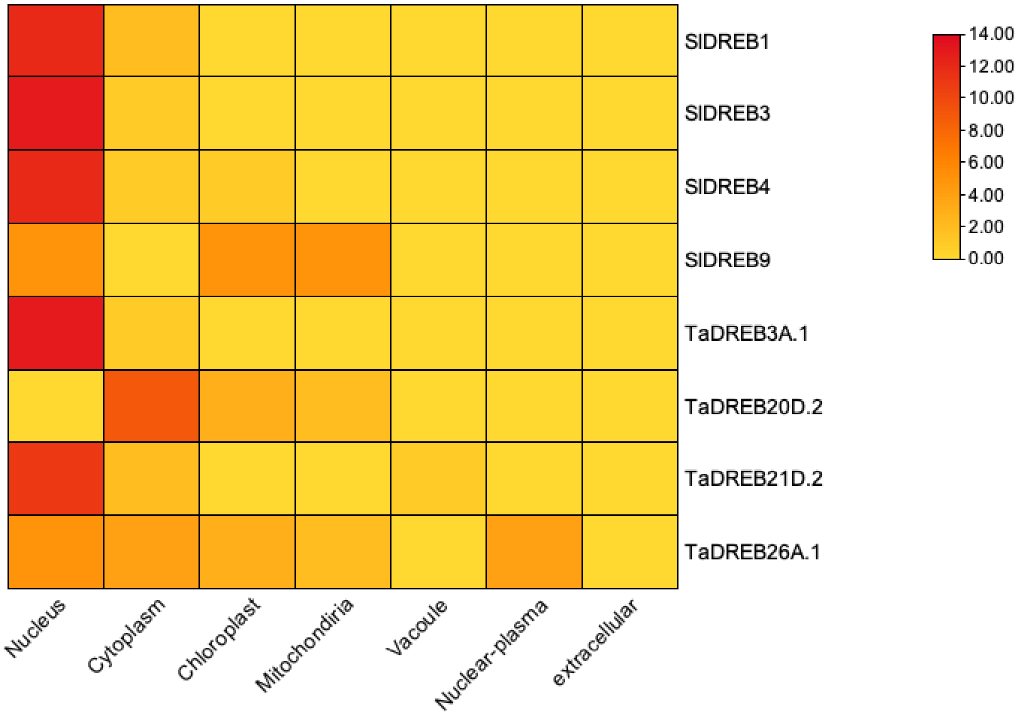


Figure 6 shows the heat map analysis of tomato genes i.e. SlDREB1, SlDREB3, SlDREB4A, SlDREB9A. and wheat TaDREB3A.1, TaDREB20D.1, TaDREB21D.2 and TaDREB26A.1.

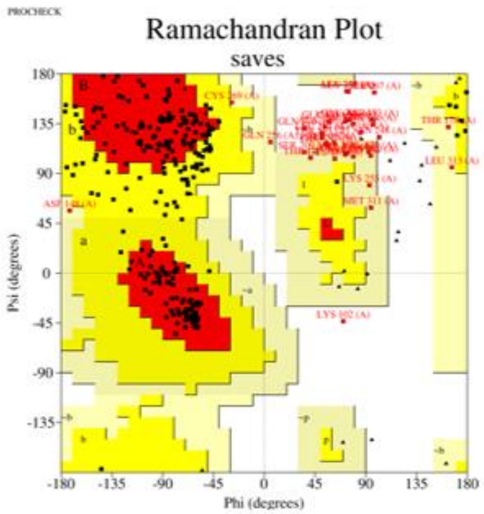
SlDREB1

SlDREB3

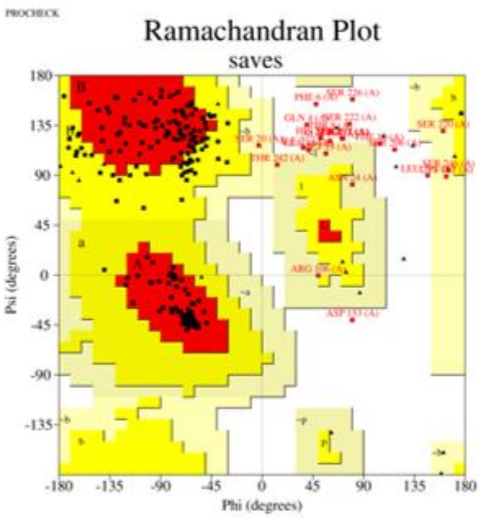
SlDREB4A

SlDREB9A

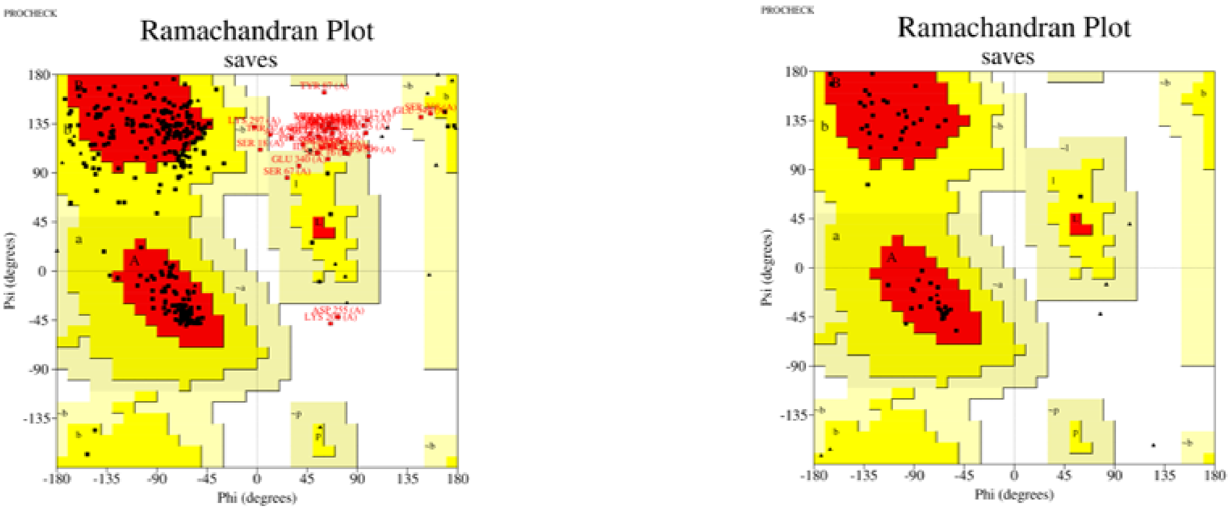
Figure 7 shows the protein models of SlDREB1, SlDREB3, SlDREB4A and SlDREB9A.



SlDREB1



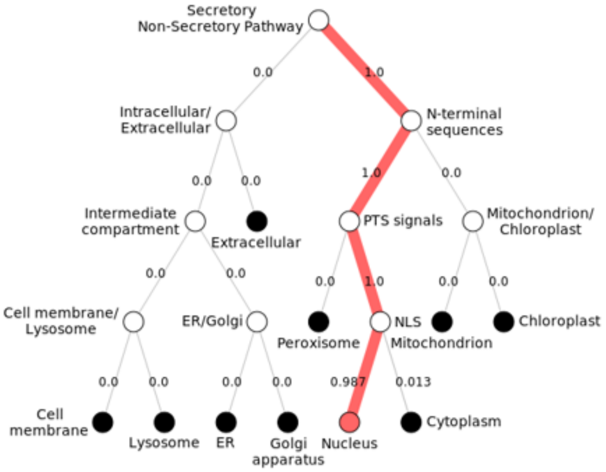
SlDREB3



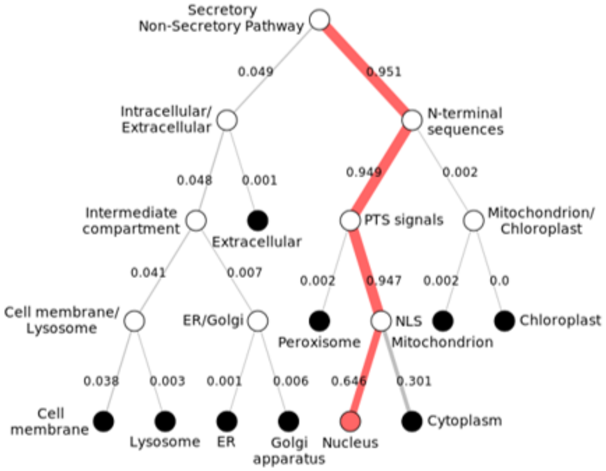
SlDREB4A

SlDREB9A

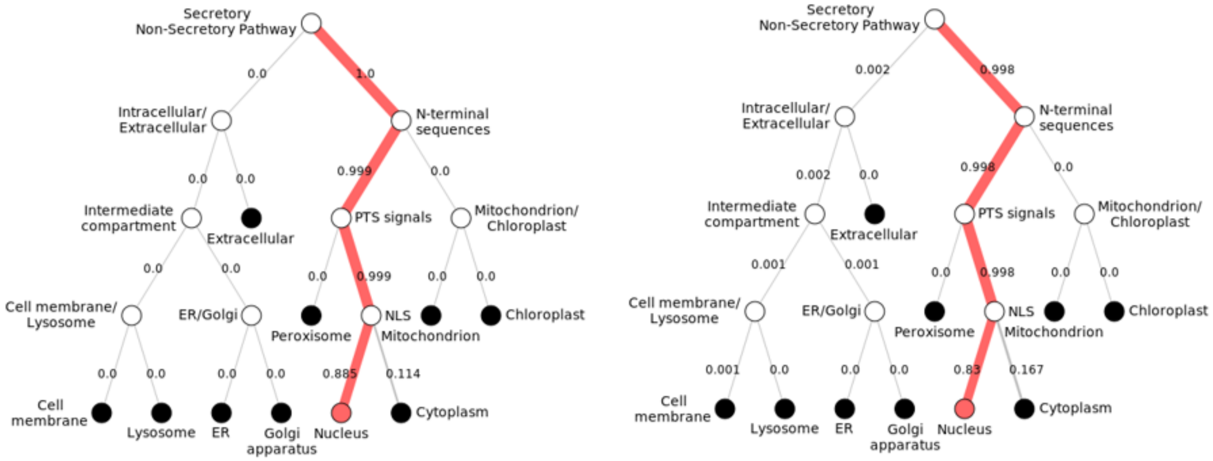
Figure 8 shows the Ramachandran plot for structure validation of genes SlDREB1, SlDREB3, SlDREB4A, and SlDREB9A.



Hierarchical Tree of TaDREB3A.1



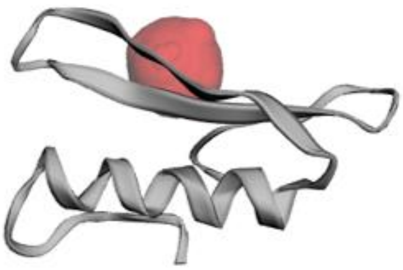
Hierarchical Tree of TaDREB20.1



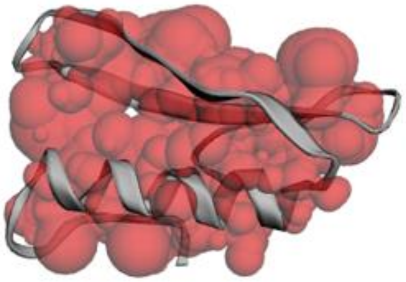
Hierarchical Tree of TaDREB21D.2

Hierarchical Tree of TaDREB26A.1

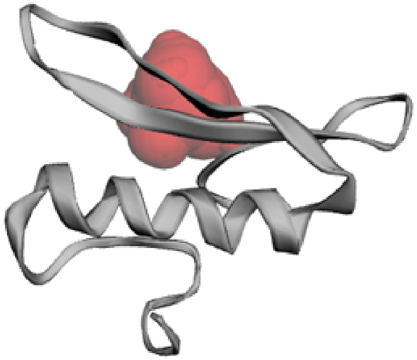
Figure 9 depicts the sub-cellular localization of TaDREB3A.1, TaDREB20D.1, TaDREB21D.2 and TaDREB26A.1.



TaDREB3A.1



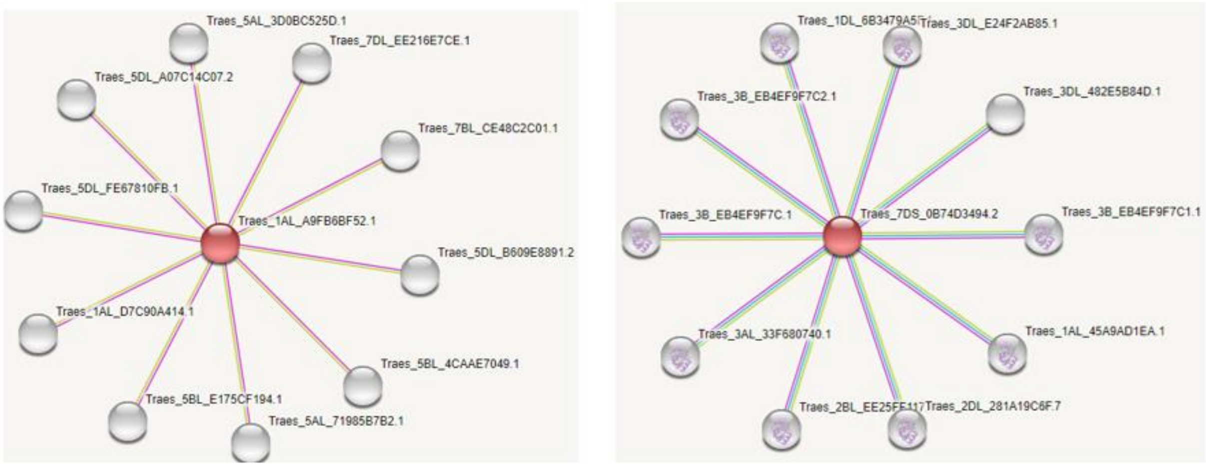
TaDREB20.1



TaDREB21.1

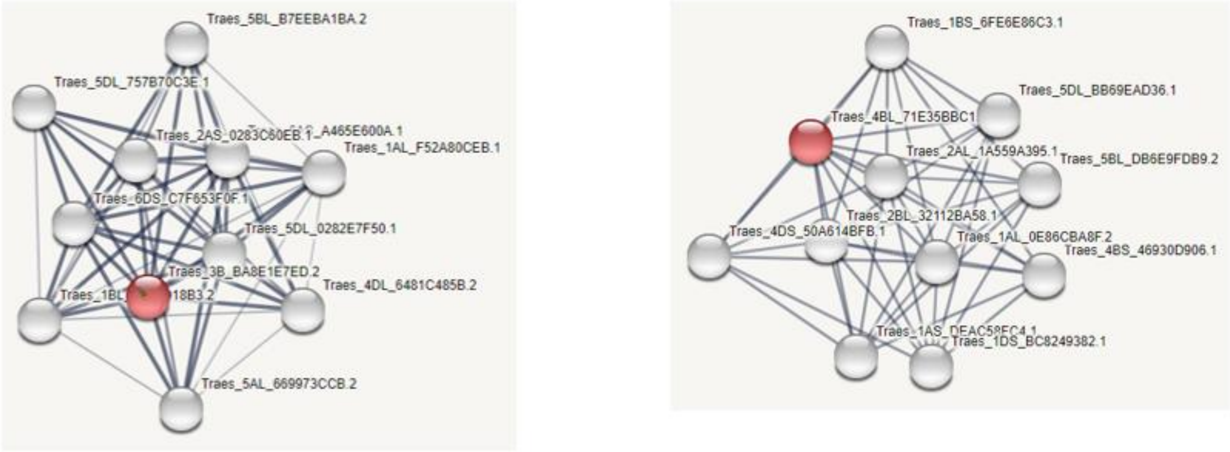
TaDREB26A.1

Figure 10 shows protein function analysis of wheat genes TaDREB3A.1, TaDREB20D.1, TaDREB21D.2 and TaDREB26A.



TaDREB3A.1

TaDREB20.1



TaDREB21.1

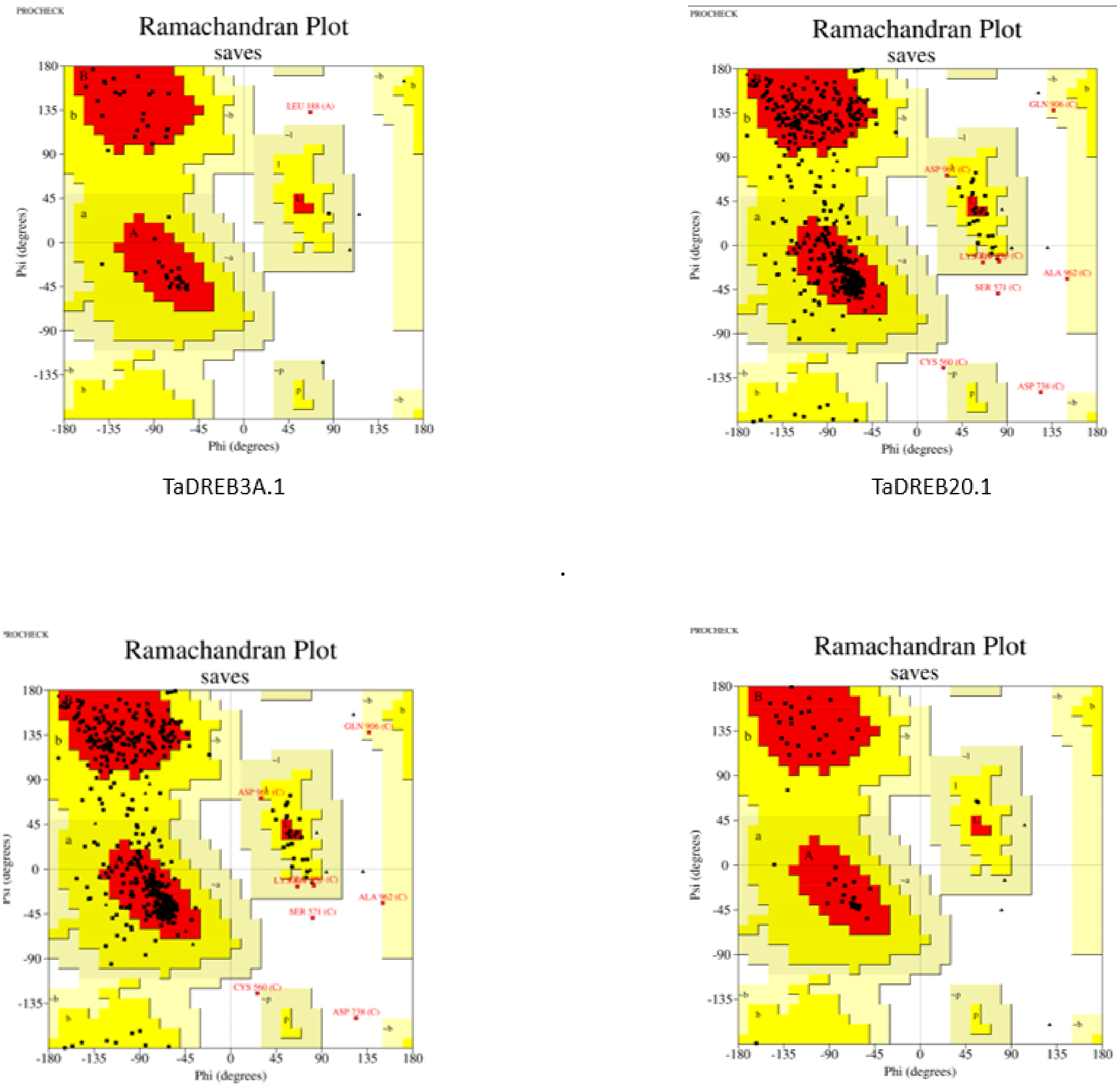
TaDREB26A.1

Figure 11 shows different protein interactors of TaDREB3A.1, TaDREB20D.1, TaDREB21D.2 and TaDREB26A.1

TaDREB3A.1 TaDREB26A.1

TaDREB26A.1 TaDREB26A.1

Figure 12 shows the protein modeling of genes TaDREB3A.1, TaDREB20D.1, TaDREB21D.2 and TaDREB26A.1 done through SWISS Modeling and further assessment was done through pyMOL



TaDREB3A.1

TaDREB20.1

TaDREB21.1

TaDREB26A.1

Figure 13 shows the Ramachandran Plots for structure validation of genes TaDREB3A.1, TaDREB20D.1, TaDREB21D.2 and TaDREB26A.1.

4.6.PCR Amplification and PCR Product purification

SlDREB3A and SlDREB3B genes were amplified using tomato DNA using primers sequences mentioned in the appendix. The condition of PCR is mentioned in material and methods section. After running the PCR, the bands were visualized by Gel electrophoresis followed by product purification. The results of Gel purification are presented in the picture form below. Wet lab experiment proved that, SlDREB3A has size of 795bp figure 15 shows and SlDREB3B has size of 510 bp figure 16 shows.

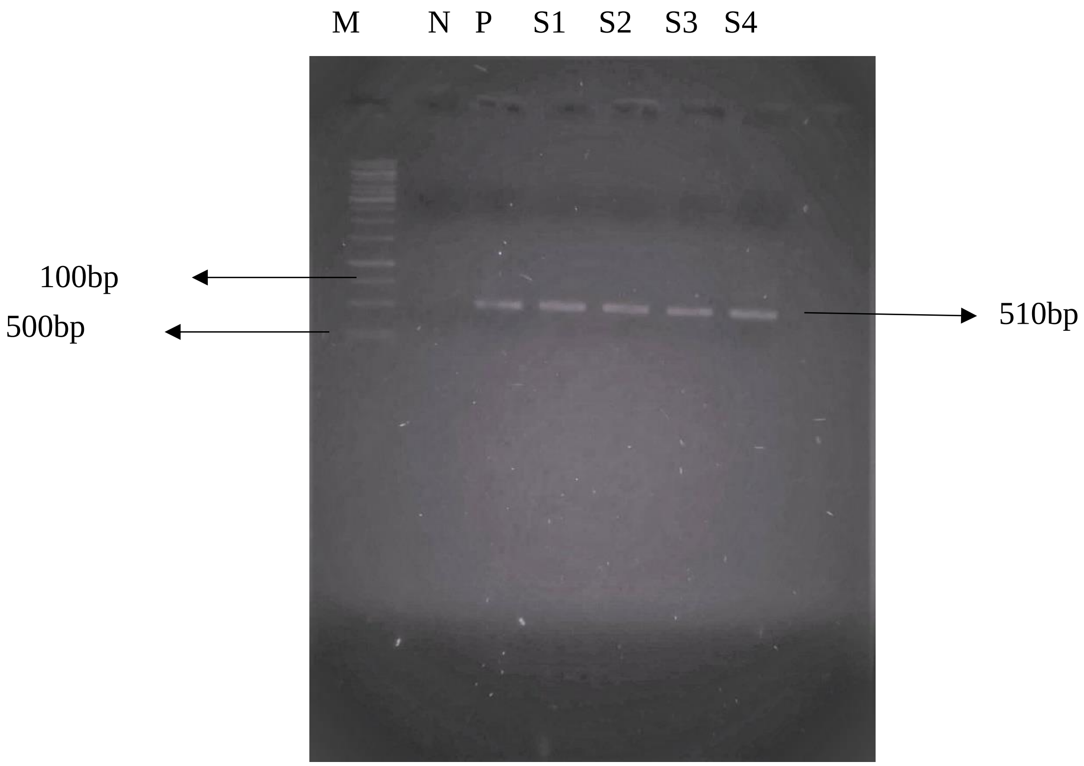


Figure 14. Gel picture of amplified and purified SLDREB3A gene. M (ladder), N (negative control), P(positive control), S1(Roma), S2(Pakit), S3 (Money Maker), S4 (Rio-Grande).

Figure 15 shows the amplified and purified StDREB3 gene. The M shows ladder of size 1kb, N represents the negative control, P shows positive control and the tomato sample products were loaded for example S1 represents the variety Roma, S2 represents the variety Pakit, S3 Money Maker and S4 shows the Rio-Grande variety. The StDREB3consists of 694 base pairs.

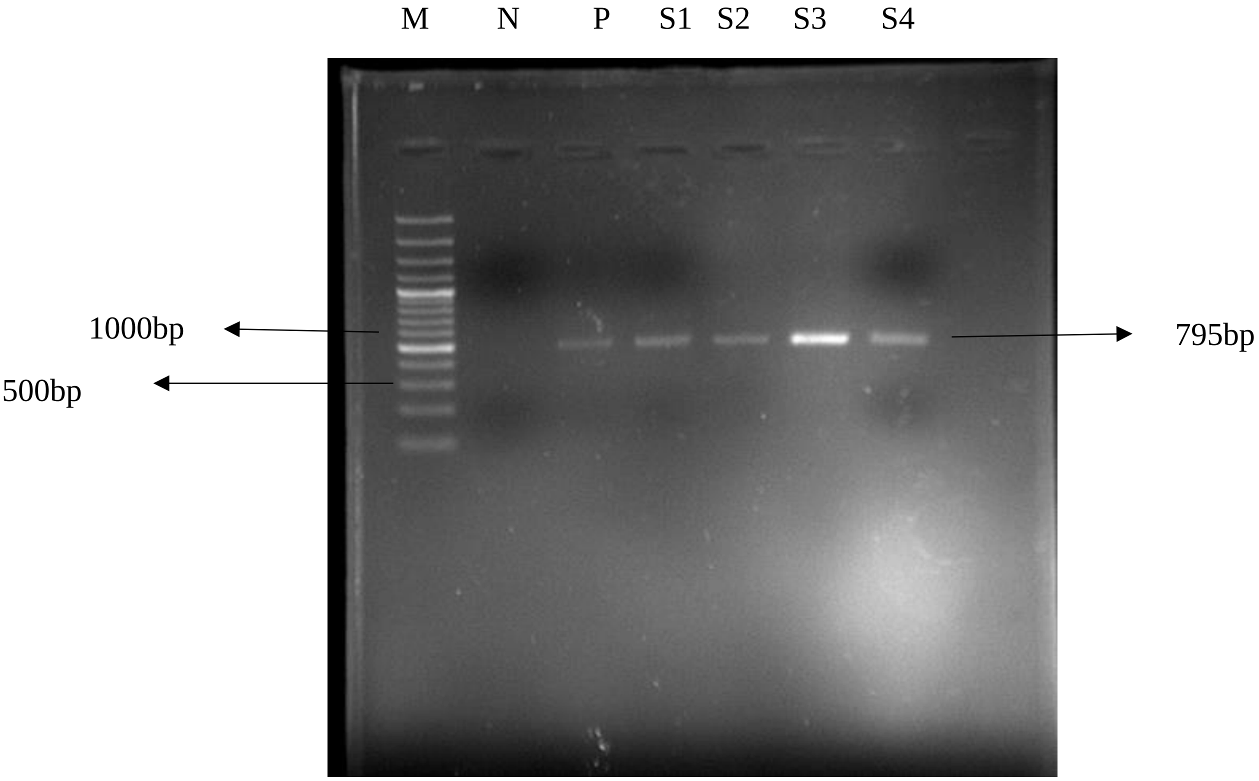


Figure 15. Gel picture of amplified and purified SlDREB3B gene. M (ladder), N (negative control), P (positive control), S1(Roma), S2(Pakit), S3 (Money Maker), S4 (Rio-Grande).

Figure 16 shows the amplified and purified LeDREB3 gene identified. The M shows ladder of size 1kb, N represents the negative control, P shows positive control and the tomato sample products were loaded for example S1 represents the variety Roma, S2 represents the variety Pakit, S3 Money Maker and S4 shows the Rio-Grande variety. The LeDREB3consists of 510 base pairs.

Discussion

Environmental circumstances such as dryness, excessive salt, and temperature change pose constant dangers to plants because they are sessile organisms. Changes in the environment generate growth constraints and stress, which reduces output and leads to substantial crop losses. More than 10% of the world's arable land is harmed by drought and salinity, reducing average yields of primary crops by more than 50%. Due to the fact that a single stress can affect multiple stages of plant growth and multiple stresses can operate on the plant at once, understanding how plants adapt to abiotic stresses is a very complicated process. Due to this, stress tolerance and adaptability mechanisms have been studied extensively for a long time (Lata et al., 2011).

Tomatoes are consumed globally for their nutritional value and health advantages. Tomatoes are good for nutritional improvement since they are rich in vitamins and minerals. Tomatoes are a common study subject for fruit development and composition. Antisense suppression of polygalacturonase in the fruit made the FLAVR SAVR tomato the first commercially available transgenic crop with an extended shelf life. Tomato plants are susceptible to various abiotic stressors that affect their development, growth, and output. In the scientific literature, the DREB gene family is recognized as a significant element in plant defense against abiotic stress. It is known that DREB genes have an AP2 domain that binds to the DRE/CRT cis-acting region, resulting in the production of stress-related genes. Members of the DREB gene family have been found in several plant species, although little is known about the tomato DREB gene family and monocot plants. No research to yet has revealed phylogenetic analyses between the tomato plant and other monocot plants. This study investigates the relationship between the tomato plant and other monocot plants (Mushtaq et al., 2021).

The tomato (Solanum lycopersicum L.) is a globally important vegetable fruit crop. Most tomato varieties are susceptible to abiotic stresses such as drought. Under drought conditions, tomato plant growth is inhibited and fruit output is drastically decreased. Consequently, discovering genetic elements of drought stress tolerance in tomato is essential for agricultural advancement. Tomato develops several transcription factors in response to drought, but only a handful have been functionally characterized to yet (Thirumalaikumar et al., 2018).

Droughts have been more widespread worldwide over the past few decades, and they are one of the most significant factors restricting agricultural productivity and sustainable agriculture.

Drought has drastically affected food and animal feed output, especially during the past two decades. Consequently, it is essential to create new cultivars with drought resilience for agricultural development. Transgenic technology has the ability to address this issue by producing drought-resistant genotypes with little or minimal influence on the morpho-biochemical and physiological performance of plants. A significant number of environmental stress-related genes were found and introduced into varied cultivated plants; the resulting transgenic plants exhibited enhanced drought resistance (Shinwari et al., 2020).

The alteration of transcription factors has shown to be an effective strategy for enhancing crop stress tolerance. Drought is one of the most harmful situations for the growth and health of plants. Numerous studies have demonstrated that manipulating drought-responsive transcription factors (TFs) can lead to drought-tolerant phenotypes in a variety of crop species. Tomatoes are one of the world's most significant vegetable crops. Few studies have investigated the molecular signaling pathways involved in this plant's response to water limitation, despite the fact that the majority of tomato cultivars are drought-sensitive. Transcriptome analyses have found a number of TFs in tomato that are drought sensitive. The functional analysis of these transcription factors (TFs) and the discovery of their signaling pathways are essential steps in elucidating drought response networks in tomato (Thirumalaikumar et al., 2018).

DREB (dehydration responsive element binding) is a subfamily of the APETALA2/ethylene transcription factor family. DREB transcription factors (TFs) utilize signal transduction pathways independent of ABA and contain conserved regions that bind to the dehydration responsive element (DRE) and the CRE (CRT). DREBs are often split into two subclasses, DREB1/CBF and DREB2, which are components of two different signal transduction pathways in response to low temperature and dehydration/high salinity, respectively (Erpen et al., 2018). The expression of SlDREB3 in tomato considerably affected stomatal conductance and transpiration, with transgenic plants displaying conductance and transpiration rates that were nearly twofold greater than those of the controls and much higher following ABA treatment. In response to variations in vapour pressure deficit, ABA controls stomatal closure to maintain water availability for plant development. The weaker responses to ABA in SlDREB3 over-expression lines leave the plants susceptible to water stress, making them unsuitable for usage in the field, particularly in rain-fed environments (Vishwakarma et al., 2017).

Reduced ABA levels appear to be at least one of the causes of early germination in various SlDREB3 over-expressing lines. SlDREB3 expression had no effect on ethylene-dependent activities, such as the triple response, nor on GA-related processes. In contrast, the majority of ABA activities except germination were altered in SlDREB3 lines, including transpiration/conduction/photosynthesis, leaf withering, and root growth (Upadhyay et al., 2017). It was discovered that SlDREB3 is implicated in plant responses to chilling stress, presumably through an ABA-independent signaling route. The overexpression of SlDREB3 enhanced chilling tolerance in transgenic tomatoes via boosting SlLEA expression, most likely (G. Wang et al., 2019).

The relevance of the DREB2 subfamily of DRE-binding proteins in stress-responsive gene expression is demonstrated by its activation by drought and high-salinity stress (Lata et al., 2011). The SlDREB2 gene of tomato is a typical transcription factor that identifies universal cis-DRE elements in target gene promoters as opposed to specific DRE patterns. Both silencing and overexpression of the SlDREB2 gene in tomato confirmed a regulatory role of the SlDREB2 gene in enhancing drought stress tolerance, indicating that the SlDREB2 gene is an essential transcriptional activator up-regulating stress-resistant genes and functional genes, thereby enriching the gene products of stress-resistant and enhancing drought stress tolerance in plants (Tao et al., 2022). Among the several DREBA4 family transcription factors found in tomatoes, SlDREBA4 has been found to have a pivotal regulatory function in the face of heat stress. Heat stress resistance is increased by the SlDREBA4 transcription factor via molecular interactions with osmolytes, stress hormones, antioxidant enzymes, heat shock proteins, and calcium-binding proteins (Mao et al., 2020).

The gene SlDREB3 encodes a member of the DREB family of transcription factors, which is likely involved in the central ABA pathway. The expression of this gene dampens ABA responses in several tissues. In addition to increasing seed production by at least 18% compared to controls, its expression also increases root development in an age-dependent manner and promotes plant growth under well-watered circumstances by increasing photosynthetic rates. SlDREB3 overexpression might increase crop production in micro-irrigated environments (Upadhyay et al., 2017). We may infer that SlDREB3 controls a component that is fundamental to ABA activities in all tissues and stages of development since it selectively attenuates ABA responses across tissues and processes as varied as germination, senescence, photosynthesis/transpiration/conductance. Seeds overexpressing SlDREB3 had lower amounts of ABA, suggesting that ABA biosynthesis/metabolism is a contributing factor. The fact that SlDREB3 has stronger effects when exogenous ABA is present than when it is absent, however, shows that ABA signaling itself may be disrupted (Upadhyay et al., 2017).

Stem elongation, leaf expansion, fruit development, and seed germination are just some of the many developmental processes that GAs regulate. GA and ABA are antagonistic in the regulation of numerous developmental processes: The growth-promoting effects of GA and the flowering-inhibiting effects of ABA are contrasted here. Our research showed that SlDREB expression was upregulated by GA therapy but downregulated by ABA, providing more evidence for the existence of an antagonistic relationship between the two. This differential connectivity between GA and ABA signaling pathways suggests that SlDREB serves as a regulator of equilibrium (J. Li et al., 2012). Root growth, which is prevalent in all transgenic lines, is significantly impacted by SlDREB3, with increases ranging from 34 to 67 percent over controls. This spike is mostly due to an increase in lateral root development, which may be partially attributed to ABA's ability to restrict lateral root growth (Upadhyay et al., 2017). Tomato ERFs and other identified ERF proteins from various plant species with the AP2/ERF domain were placed in a clade with genes known to be important for floral meristem development, organ identity, and abiotic stress response (Sharma et al., 2010).

It is feasible to draw the conclusion that DREB genes are the key controllers of abiotic stress responses and tolerance in plants exposed to severe conditions. Engineering DREBs would control the expression of numerous target genes that are induced by osmotic stress as well as up-regulate a collection of native stress-responsive pathways, resulting in physiological and biochemical changes in plants that would enable them to adapt and acclimate to osmotic pressures (Lata et al., 2011).

By changing metabolic pathways, new strategies for creating stress-tolerant plants may be developed as a result of a greater knowledge of the processes behind stress tolerance in plants provided by metabolomics. Modifying metabolic pathways is a challenging endeavor since the majority of the proteins in a route interact with multiple other proteins. Therefore, the only method to achieve effective metabolic engineering is to regulate several genes along the same or linked pathways. By integrating conventional and cutting-edge methods, multigene manipulation has also advanced to a high degree, creating the foundation for further development.(Krishna et al., 2019). Thus, DREBs would improve plant tolerance more than any other stress-inducible gene if genetically modified, making them ideal targets for genetic engineering and crop development (Lata et al., 2011).

Conclusion

In conclusion, an *in-silico* analysis was performed on the tomato plant, which included retrieval of tomato DREB sequences and other monocot plants from the kingdom plantae. Using the sequences, a phylogenetic analysis was performed to find the evolutionary relationship between the tomato plant and monocot plants. In addition, the various interactors of the selected tomato protein sequences were identified, and this was followed by the determination of the three-dimensional protein structure of the selected tomato protein sequences for protein validation. In addition to what has been discussed thus far, multiscale pocket binding structures of SlDREB have been uncovered. The study's findings uncovered patterns that are thought to be associated with biotic and abiotic stress responses, hormone induction, light regulation, cell development, and promoter binding sites. Phylogenetic comparison between Solanum lycopersicum and monocot plants showed DREB genes are classified into 6 families. DREB genes present in both dicots and monocots but dicots have a larger concentration of these genes than monocots, making them stronger drought-resistant plants. 3D structure modeling provides insight into secondary and tertiary structure of protein and further their structures are validated. Wet lab experiment proved that, SlDREB3A has size of 795bp and SlDREB3B has size of 510 bp.

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Assistant Professor
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