Characterization of Trihelix Genes in *Solanum tuberosum* Genotype(s) under Abiotic stresses

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

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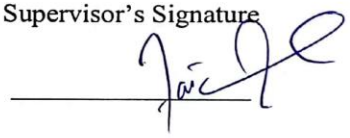
Supervisor's Signature



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Muhammad Umar

Master of Science in Plant Biotechnology

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Acknowledgment

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.

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Muhammad Umar

Dedication

This research project is dedicated to my Parents

List of Abbreviations

AP2	**APETALA2**
AREB/ABF	**ABA-responsive element binding protein/ ABRE binding factor**
bHLH	**Basic helix-loop-helix**
BLAST	**Basic Local Alignment Search Tool**
bZIP	**Basic leucine zipper**
BZR	**BRASSINAZOLE-RESISTANT**
CASTp	**Computed Atlas of Surface Topography of proteins**
CBF	**Core-binding factor**
CDP	**kinase Calcium dependent protein kinase**
Dof	**DNA binding with One Finger**
DRE/CRT	**Dehydration Responsive Element/ C-repeat**
ExPASy	**Expert Protein Analysis System**

FAO	**Food and Agriculture Organization**
GRAS	**Gibberellin-acid insensitive (GAI), Repressor of GA1**
GSK	**Glycogen synthase kinase**
MEGA X	**Molecular Evolutionary Genetics Analysis**
MEME	**Multiple Expectation maximizations for Motif Elicitation**
NADPH oxidase	**Nicotinamide Adenine Dinucleotide Phosphate oxidase**
PDB	**Protein Data Bank**
Pfam	**Protein families database**
Phyre2	**Protein Homology/ analogy Recognition Engine V 2.0**
SBP	**SQUAMOSA PROMOTERBINDING PROTEIN**
SMART	**Simple Modular Architecture Research Tool**
SOPMA	**Self-Optimized Prediction Method with Alignmen**

Table of Contents

Contents

List of figures

List of Table

Abstract

The Trihelix transcription factors are referred as a GT element because of their binding capacity with GT element. Trihelix transcription factors influence how plants grow and develop, as well as how they respond to abiotic stresses. Although Trihelix transcriptions factors has described and recognized in numerous plants, there is limited available knowledge regarding their presence in *Solanum tuberosum.* The current investigation involved the characterization of trihelix genes in *Solanum tuberosum.* Total of 27 StTH genes have been identified in *Solanum tuberosum*. All of these 27 StTH were divided into five subgroups as previously described in *Arabidopsis thaliana* (GT-2, GT-1, SH4, GT-y and SIP1). The genomic positioning and occurrences of gene duplication revealed the arbitrary dispersion of StTH genes across 12 chromosomes, with duplication events happening both in tandem and segmental patterns, involving 13 pairs of genes. An analysis of synteny unveiled 19 orthologs of StTH genes within Solanum tuberosum and 10 orthologs within *Arabidopsis.* Through in-silico promoter analysis, diverse arrays of cis-elements and functional variations were discovered, with light-responsive and hormone-responsive elements, along with development-related, abiotic stress-responsive, and promoter-associated elements, prevailing in St Trihelix genes. In various biological and molecular pathways, gene ontology unveiled the involvement of StTrihelix proteins, including defense response, regulation of transcription, nucleotide transmembrane processes, ATP binding, and phosphorylation. Two genes StTH 22 and StTH 25 were checked for drought stress. Both of these gene's show expression in stem, root and leaves, hence the more expression was analyzed in leaves. This is the first study to look into how potato trihelix genes respond to drought stress. The insights from our findings will be valuable for forthcoming research on the comparative and molecular mechanisms of Trihelix genes in different plant species. To sum up, our discoveries indicate the potential significance of StTH22 and StTH25 in contributing to essential roles under drought stress conditions in potatoes.

Keywords: Trihelix, Solanum tuberosum, Gene ontology, MYB/SANT, Gene duplication and Cis-elements**.**

Chapter 1

Introduction

1.1 *Solanum tuberosum*

Potatoes rank as the world's third-largest food crop in terms of production volume, trailing only wheat and rice (Handayani et al., 2019). The potato which originated in Bolivia and Peru's Andean. It is rightfully referred to as the "king of vegetables" because it is the only crop that is grown in more than 100 nations throughout the world. Potato is very nutritious contains 80% water, and 205 solid ingredients. About 85% of this mass is made up of starch, and the remaining 15% is protein. Niacin, riboflavin, thiamin, and vitamin C are among the vitamins also present in potatoes. Minerals including calcium, iron, magnesium, phosphorus, sodium, ,and potassium (Noonari et al., 2016). Approximately 20 million hectares of farmland are used to grow potatoes globally, and in each year about 366 million tons of potatoes are produced. As cash crops that are largely farmed in these regions, potatoes provide a sizable source of income. It is a crucial crop in tropical regions such as the Rift Valley in Africa, Andes and Southeast Asia where grown for money and basic necessities (Devaux et al., 2021). In acknowledgment of the potato's significance as the leading non-cereal crop globally, as well as its contributions to ensuring food security and reducing poverty, the United Nations General Assembly (UN 2006) designated 2008 as the International Year of the Potato (FAO 2009).

In 2022, 7 million more people in the Asia-Pacific region faced extreme food insecurity, owing primarily to the ongoing Ukrainian crisis, the Global Food Crisis, as well as conflict and climatic shocks. By the end of 2022, the region would have 69.1 million people experiencing acute food insecurity, an increase of 41.5 million from prior to the pandemic. Arable land quality has diminished as a result of excessive intensification through mono-cropping and inadequate irrigation methods (Nasir et al., 2022). Abiotic stresses are a recurring problem for potato farmers in addition to diseases and pests (He et al., 2018). Droughts and flooding have reduced soil quality and increased salinity as a result. As cities continue to grow in population, a substantial amount of valuable farmland will be lost for highways and buildings over the next few decades (Sun et al., 2015) .

1.2 Status of potato in Pakistan

Potatoes are an important agricultural crop in Pakistan and are widely grown in various parts of the country. According to the Pakistan Bureau of Statistics, potato cultivation covers an area of around 160,000 hectares in the country, an overall production of approximately 4 million metric tons per year (Ali et al., 2015). Punjab province is the largest potato producing region in Pakistan, accounting for about 70% of total potato production of country. Other major potato producing areas include Sindh, Khyber Pakhtunkhwa, and Baluchistan provinces. Potatoes are an important cash crop for farmers in Pakistan and contribute significantly to the country's agricultural economy (Nawaz et al., 2020).

They are widely consumed in Pakistan, both as a main ingredient in various dishes and as a snack food (Ijaz-ul-hassan & Khan, 2022).However, despite being a major producer of potatoes, Pakistan still faces challenges in terms of quality control, storage, and transportation of the crop. There is a need for improved infrastructure and technology to ensure that the crop is properly stored and transported, as well as for better quality control measures to ensure that the potatoes meet international standards (Pakistan Economic Survey, 2021).Punjab accounts for 83% of total potato agricultural acreage, with KPK accounting for 10%, and Baluchistan accounting for 6%. Due to its spring and autumn crops, Punjab holds the distinction of making the largest contribution in both area and productivity. Important potato-producing regions in Punjab include Gujranwala, Narowal, Okara, Gujranwala, Kasur Pakpattan, Sheikhupura, Toba Tek Singh and Narowal. Dir, Mansehra, and Nowshera are important potato-producing areas in KPK. Kalat, Kila Saifullah, and Pishin are important potato-producing areas in Baluchistan (Pakistan Agriculture Research Council).

Table 1. *List of important potato varieties grown commercially in Pakistan.*

Red Skinned Potato Variety	White Skinned Potato Variety
Asterix	Ajax
Cardinal	Diammat
Desiree	Hermes
Kuroda	Multa
Lady Rosita	Santana
Lal-e-Faisal	Sante
Oscar	Patrons
Rodeo	

1.3 Potato farming in Pakistan

The potato, a Solanaceae family member, is recognized as a crop with a short growth season. Depending on the variety, the herbaceous plant known as the potato grows to a height of 60 cm (Nasir & Toth, 2022). The fact that potatoes can be vegetatively reproduced is a major factor in their popularity around the world. Farmers can use this approach to save a percentage of their produce for seed potatoes, improving food availability and protecting preferred kinds. Potatoes yield the greatest food energy per acre. The potato is mostly a crop for temperate regions. Due to their thermosensitivity, potatoes can only be grown in temperate climates with lengthy days (Khalil et al., 2021).

Worldwide, there are about 5,000 different potato varieties. Numbering around 3000, they are predominantly found in the Andes region, spanning countries such as Chile, Ecuador, Bolivia, Peru, and Colombia. *Solanum tuberosum* is categorized into two subspecies: andigena, referred to as Andean, and tuberosum, also recognized as Chilean. Potatoes produce more nutrient-dense food more quickly than other significant crops, require less land, and can withstand the hard climate (Bettoni & Mouillé, 2019). The optimal temperature ranges for various stages of potato development are as follows: tuber formation thrives at 25°C, vegetative growth prefers 20°C, and

sprouting occurs best within the range of 16–24°C. Low (below 10 °C) and high (over 30 °C) temperatures both inhibit tuber growth. whereas the best yield is obtained at daily temperatures ranging from 18 to 20 °C. almost every form of soil, with the exception of alkaline and saline soils, are ideal for growing potatoes at their best. The least amount of resistance to tuber enlargement is provided by physically loose soils, hence they are favored. Additionally, sandy and loamy soils that are organically rich and have good drainage and aeration are suitable.

The recommended pH range for potato production is 5.2- 6.4 (Pakistan Agriculture Research Council). There are around 5000 different potato varieties found globally. Pakistan produces both red and white skin types, but customers prefer the red skin types. Afghanistan, Indonesia, Kuwait, Sri Lanka, and Malaysia are among the countries that import locally grown potatoes. In 2015-2016, 402,434 tons of potatoes were shipped to the aforementioned nations, resulting in agricultural exports of approximately Rs. 8.42 billion (Ahloowalia et al., 2004). Russia is another prospective market for Pakistani potatoes (Khan, 2019) .

1.4 Importance of potato

The potato (Solanum tuberosum L.), which has a very high yield potential and great nutritional value, is a key crop in agricultural production systems. The potato is an excellent source of nutrients such as minerals, proteins, vitamins, and fats. Additionally, they are increasingly being used as industrial product feedstock (Koch et al., 2020). In 79% of countries around the world, potatoes are farmed, making them the most common vegetable crop. In terms of the number of countries that grow it, it ranks fourth globally in terms of tonnes produced, after rice, wheat, and maize.Potato is the most affordable as well as accessible protein and carbohydrate food sources and it also included significant quantities of vitamins and a few amounts of minerals (T. Hussain, 2016). Pakistan's potato farming systems are rather complicated and diversified due to the country's fluctuating climatic, social, cultural, and agronomic factors.

In Pakistan, potatoes are grown as three different crops: in the autumn, in the spring and in the summer. Autumn and spring crops are typically grown twice a year in Punjab and Khyber Pakhtunkhwa's plains, while a third crop is grown in the summer in KPK's mountainous regions (Qasim et al., 2013).The employment of new seed varieties and contemporary technology has enhanced potato production (Buckseth et al., 2022). It's becoming a significant source of

international hunger relief and food security are interdependent. It is anticipated that one day it would replace all of the world's hunger as a major commodity Foods. This innate potential is not shared by any other food crop produce as many different processed goods as the potato, which is loved by people of all ages. They are abundant in minerals like calcium, copper, iron, magnesium, manganese, and phosphorous, as well as vitamins like A, B1, B2, B6, and C, folate, riboflavin, and pantothenic acid (Ali et al., 2015). According to FAO Director General Jacques Diouf, the potato is an important ally in the fight against world hunger and poverty.

The potato is a major export in developing nations because it can produce considerable yields on small plots of land and be marketed as a cash crop to supplement the earnings of small farmers (Jansky et al., 2019). All forms of potatoes raw, boiling, peeled, or mashed have therapeutic and restorative effects. Even the boiling water that you used to prepare them can be utilized. Potato skin is a good source of potassium, iron, zinc, fiber, and calcium. Because it is wholesome, nutritive, and energizing, potatoes are advised by modern medicine for those who are prone to weight gain. It promotes healthy bowel function, prevents ulcers, and aids in the healing of scars (Umadevi et al., 2013). Potato has emollient, antispasmodic, and diuretic effects. The thickening agent potato is utilized in the cosmetic business. A great source of biomass is potatoes. When potato starch is boiled with weak sulfuric acid, glucose is produced, which can then be fermented to produce alcohol. Additionally, Potatoes are fed to animals (Smerilli et al., 2015).

1.5 Significant barriers to potato productivity

There will be 9.1 billion people on the earth by 2050, a 34% increase. The majority of this population growth will occur in developing countries. Despite having the highest output, climate change is having a negative influence on potato yield and productivity. Abiotic stressors (drought, heat, humidity, etc.) and other interacting variables cause significant crop loss. biotic stresses (insects, pests, and illnesses) and abiotic stresses (salinity and excessive rainfall) (Majeed & Muhammad, 2018).Abiotic stressors are the main causes of production limitation. Key abiotic stimuli that impact on a number of physiological, morphological, and transcriptional modifications singly or in combination include heat, cold, salinity, and drought (Dahal et al., 2019). Poor soil, incorrect fertilizer application, and temperature extremes (heat) are the main abiotic factors limiting potato productivity. The vast majority of Pakistani potato farmers are

illiterate, and they are not aware of the characteristics of soil and how they affect crop growth (Fahad et al., 2017).

Monoculture and intense crop farming are particularly widespread due to ignorance, which degrades the physical quality of soil and its nutritional makeup. Furthermore, due to budgetary limitations, the timely application of adequate fertilizers appears to be beyond the means of the majority of farmers (Qasim et al., 2013) Potatoes grown in soils deficient in nutrients and with poor physical characteristics were unable to produce the required tubers.Now productivity of potato seriously threatened by drought, especially in dry and semiarid areas. Because potato plants are susceptible to soil moisture stress, a lack of water results in a discernible alteration in their development and yield characteristics. The intensity of water scarcity is influenced by growth phases as well as intensity and duration (Wang et al., n.d.). Water constraint hinders root growth and nutrient uptake while delaying emergence. Additionally, there is a decrease in tuber size and quantity, which reduces yield.

In crop plants, drought decreases gaseous exchange characteristics and biomass production. ROS (reactive oxygen species) are more commonly formed; they oxidize lipids and damage DNA. As a result, irrigation is constantly required to grow crops with high yields and desirable qualities (Bahar et al., 2021). During the reproductive and vegetative growth of a plant, temperature variations are a natural occurrence. However, during the hot summer months, severe temperature variations may damage the intermolecular connections necessary for ideal growth, impacting the maturation and tuber set of potatoes. Because of the increased growth rate and high respiration level caused by high temperature stress, yield may be lowered. Temperature affects tuber growth because higher temperatures inhibit the tuberization signal (Rana et al., 2020). Global warming may cause the growing season to be extended and temperatures to be near optimal for assimilation in low-temperature stress situations. Potatoes are a crop that is vulnerable to frost. Potatoes can suffer considerable damage at temperatures below 0 °C.

Potato crops, like other crops, are affected both individually and together by variations in atmospheric carbon dioxide, drought, and temperature (Huang et al., 2019). ROS signaling molecules influence both plant development and responses to biotic and abiotic stress. Excessive oxidative stress, on the other hand, causes an exceedingly destructive oxidative burst that reacts with lipids, proteins, and nucleic acids, finally resulting in cell death (Cruz De Carvalho, 2008).

In addition to directly affecting potatoes, climate change has an impact on the populations and geographic ranges of a number of potato pests and diseases. Numerous pathogens (bacteria, fungus, viruses, and nematodes) attack potatoes, drastically lowering its production. Biotic restrictions include bacterial illnesses like bacterial wilt, brown rot, and common scab as well as fungal diseases like dry rot, powdery scab, Fusarium wilt, early blight, and late blight (Pereira, 2016).

1.6 Trihelix transcriptions factor

Trihelix transcription factors, which are commonly referred to as GT factors (for GT-1-like), are a type of transcription factor discovered in plants. It ranks fourth in the world in terms of tones produced, after maize, wheat, and rice, in terms of the number of nations that produce it. (Völz et al., 2018).Plants are the only ones that have DNA-binding proteins with the trihelix motif. They became known in the 1990s as one of the earliest identified transcription factor gene families (Levy et al., 2012). Because of their binding capabilities with GT elements, they are known as GT factors in plants (Xiao et al., 2019).Originally thought to be involved in the regulation of light-responsive genes, subsequent research revealed their significance in growth, tissue development, growth of embryos, petal loss, plant organ development, and biotic and abiotic stresses (Wang et al., 2016).Trihelix member were initially assumed to involved in the regulation of light-responsive genes, but they were later discovered to be involved in embryo development,, petal loss, tissue development, plant organ development, biotic and abiotic stressors, and growth (Yasmeen et al., 2016).

1.7 Research objectives

Trihelix genes are important in abiotic stress responses, therefore the following research objectives were established:

` 1. To identify Trihelix gene family and Insilco characterization of conserved domains, gene ontology (GO), conserved domains, structure, evolutionary relationships and cis regulatory element of Potato.

2. To perform expression profiling of StTH22 and StTH25 genes in *Solanum tuberosum* under drought stress.

Chapter 2

Review of Literature

2.1 Abiotic stresses

Abiotic stress threats to plant growth and development are obviously one of the rising ecological implications of climate change, and food security issues are worsened by increasing numbers of people fighting for natural resources (Fahad et al., 2015) . Environmental change are expected to have greatest impact on agricultural production due to the negative impacts of rising carbon dioxide and high temperatures, particularly in low latitudes inhabited by poor countries, involve the development of adaptation methods (Pereira, 2016).Abiotic stress impairs numerous biochemical, morphological and biological processes that are closely linked to vegetative growth and production. Abiotic stress has a negative impact on a variety of morphological, biochemical, and biological functions related to plant growth and productivity (Cheng et al., 2016).

Drought, cold, heat, and salinity, are among the major abiotic factors threatening global food security. These factors have the potential to reduce biomass output, plant survival, and agricultural yields by up to 70% (Ahmad et al., 2013). Abiotic stress develops when plants are subjected to adverse growing conditions, and it impedes their growth and yield. Plant metabolism is interrupted under the majority of abiotic stress circumstances due to the inhibition of metabolic enzymes, a lack of substrate, an excessive demand for particular compounds, or a combination of these and other variables. Therefore, the metabolic network restructured in order to maintain vital metabolism and adapt to current stress conditions by adopting a new steady state. Furthermore, metabolic reprogramming is required to supply stress-responsive proteins, suitable solutes, and other anti-stress compounds (Obata & Fernie, 2012).

2.2.1 Drought stress

Drought (also known as "water stress") is one of the most dangerous environmental conditions. It can occur due to a variety of factors, including insufficient rainfall, salt, extremely hot or cold weather, and excessive light intensity. Drought may impact several physiological, morphological, biochemical, and molecular aspects of plants.

2.2.1 Effects of drought stress

One of the main things limiting the world's food supply is drought. Crop growth projections anticipate that this problem will only get worse. Drought hinders normal growth, and disrupts the water cycle. This phenomenon is more challenging to understand in plants since they exhibit a vast variety of physiological and biochemical processes at the cellular and organismal levels. Stomatal closure, membrane damage, and a halt in the activity of numerous enzymes, particularly those involved in ATP generation, are the principal causes decreasing photosynthetic rate (Farooq et al., 2012). Stress due to drought lowers plant water relations, stem extension, root proliferation, and leaf size.

Drought conditions on a global scale have resulted in a 21% decline in Triticum aestivum L. production and a 40% decrease in Zea mays L. production in recent years (Farooq et al., 2009). Drought can also reduce the rate of photosynthesis, which is the process by which plants produce energy from sunlight. This can result in a decrease in the amount of energy available to the plant, which could happen to reduced growth (Wang et al., n.d.). In extreme cases, drought can cause plants to shed their leaves prematurely. This can be a survival mechanism for the plant, as it reduces the amount of water lost through transpiration (Vyver & Peters, 2017). Drought-stressed plants are more susceptible to disease and pest infestations, which can further damage the plant's health. All things considered, drought can have a substantial impact on plant health and growth, which can result in poorer crop yields and ecosystem productivity (Seleiman et al., 2021).

2.2.2 Salinity stress

"Soil salinity refers to the presence of water-soluble salts, including sodium (Na^+), potassium (K^+), chloride (Cl^-), and sulphate (SO_4^{2-}). While sodium (Na^+), and chloride (Cl^-) are not considered plant nutrients, other ions such as (K^+) and (SO_4^{2-}) (Stavi et al., 2021).The second most severe global issue that negatively impacts agricultural productivity is soil salinity. Salt deposition, which can originate from primary (natural) or secondary (anthropogenic) processes, makes soil salty (Uri, 2018). The three main processes are atmospheric precipitation, marine deposition, and source rock weathering Secondary processes include insufficient groundwater drainage, insufficient water management, long-term continuous irrigation, irrigation with brackish groundwater, and cultural irrigation practices. With an expected annual growth of

0.3-1.5 million acres of agriculture, soil salinity is rising quickly, reducing crop output by up to 20% (Evelin et al., 2019).

2.2.2 Salinity effects on plants

Salinity, a term used to describe how much salt is present in the soil or water, can have a variety of consequences on plants. Here are a few examples of how salinity affects plants. Through their roots, plants take up water, but when the soil or water is salty, a strong osmotic potential is produced that hinders the plants' ability to do so. As a result, the plant may experience water stress, which can cause stunted growth or even death (Kolomeichuk et al., 2021). Salinity can prevent plants from absorbing vital elements like potassium, calcium, and magnesium, which can lead to nutrient shortages. Plant quality, yield, and growth may be impacted by this (Hu & Schmidhalter, 2005). Plants may be more sensitive to osmotic stress if there are high salt concentrations in the soil or water. This stress may have an effect on a plant's cells and tissues, affecting its growth and output (Munns, 2002).

Normal conditions result in a higher osmotic pressure in plant cells than in soil solution. This increased osmotic pressure is used by plant cells to transport water and other minerals from the soil solution into their root cells. Because the osmotic pressure in the soil solution is greater than that in plant cells under salt stress, the presence of excess salt limits the plant's capacity to store water and minerals like Ca^{+2} or K^{+}. Sodium and chloride ions can infiltrate cells and directly injure cell membranes while also stimulating cytoplasmic metabolic activity. Secondary effects of salt stress include reduced cytosolic metabolism, membrane function, and cell growth as a result of these basic effects. Due to the formation of ROS, oxidative stress typically follows salinity stress (Isayenkov & Maathuis, 2019). In summary, salinity can have various negative effects on plants, including reduced water uptake, nutrient uptake, and photosynthesis, as well as ion toxicity and osmotic stress (Manavalan et al., 2009) .

2.2.3 Heat stress

Heat stress is a condition that occurs when an individual, animal, or plant is exposed to high temperatures that exceed their physiological limits, leading to a range of physical and physiological responses. In plants, heat stress can lead to reduced growth, decreased photosynthesis, wilting, leaf senescence, and even death. High temperatures, in addition to

hurting plant cells and tissues, can have an impact on agricultural productivity and quality (Hasanuzzaman et al., 2013).

2.2.3 Heat effects on plant

Heat stress is defined as a rise in temperature that lasts long enough to permanently impede plant growth and development. Heat shock or heat stress is generally believed to be a brief increase in temperature, often 10-15C over ambient. Heat stress, however, is a complex function of temperature (in degrees), duration, and rate of increase (Wahid et al., 2007). Heat stress is commonly used to describe a rise in temperature that lasts long enough to impair plant growth and development permanently. Events associated with HS may have varying effects on plant physiological processes and community structure due to inter-annual differences in the timing and length of hot days. As a result, the season and stage of growth at which HS events occur will determine how plants respond to HS in terms of their photosynthetic activity (Wang et al., 2016).

About 15% of the world's wheat is produced in the Indian lowlands, but it is predicted that climate change would make this region a short-season, heat-stressed producing environment. Similar to this, a 3–4°C rise in temperature might result in a 15–35% drop in agricultural production in Africa and Asia and a 25–35% drop in the Middle East (Bita & Gerats, 2013). Some crops' yields can be decreased by prolonged exposure to high temperatures because it affects pollination, the growth of flowers and fruits, and nutrient intake. Fruits and vegetables can suffer from heat stress, which can affect their flavor, colour, and texture. Generally speaking, heat stress has a limit beyond which it can harm a plant's growth and productivity. This limit varies between species and cultivars, but generally speaking, prolonged exposure to temperatures exceeding 90°F (32°C) can be harmful to most plants. Because of this, it's crucial for farmers and gardeners to be aware of the possible impacts of heat stress on their crops and to take precautions to lessen those effects, such as providing shade, increasing watering, and selecting heat-tolerant plant kinds (Parker et al., 2020).

2.2.4 Cold stress

Abiotic stress, also known as cold stress (temperatures ranging from 0 to 15 degrees Celsius) has a deleterious impact on plant growth and agricultural productivity. Plants under cold stress experience phenotypic alterations such reduced leaf development, wilting, and chlorosis, which might ultimately result in necrosis (Wani et al., 2016). Plant plasma membrane is significantly harmed by cold stress, and this has been connected to the dehydration that cold stress causes. Membrane rigidification, which is adversely affected by chilling stress, is the main process that triggers subsequent cold stress responses in 16 plant cells (Liu et al., 2018) . Cooling stress lowers the activity of numerous enzymes, including those that detoxify ROS, and degrades protein complex stability. These occurrences cause severe membrane damage, photoinhibition, and reduced photosynthesis. In addition, freezing stress affects protein synthesis and gene expression by boosting the formation of secondary RNA structures. Different levels of freezing stress can be tolerated by different plants.

Tropical and subtropical plants like rice, maize, and tobacco cannot withstand cold temperatures, however Arabidopsis and a few overwintering cereals can (Hussain et al., 2018). Cold stress can have a substantial impact on plant growth, development, and output. Plants that are subjected to cold stress may have decreased photosynthetic rates. This decline is due to both lower activity of photosynthesis-related enzymes and damage to the photosynthetic system of plants, particularly the chloroplasts. The uptake and transportation of nutrients by plants can be impacted by cold stress. This could lead to nutritional deficits and have an impact on the plant's overall growth and development (Thakur et al., 2010).

2.2.4 Abiotic stress tolerance in plants

Abiotic stressors, such as UV radiation, excessive salt, heavy metals, and low or high temperatures, are harmful to plant growth and development, having a huge negative impact on agricultural productivity worldwide (Rodziewicz et al., 2014). Five general plant defense’s against abiotic stresses will be discussed: the cuticle, which serves as the plant's outermost shield; unsaturated fatty acids (UFAs), which act as membrane modulators and oxylipin precursors; RS scavengers, which control RS homeostasis; and molecular chaperones, which maintain proteins and subcellular structures (He et al., 2018).

Stress avoidance and stress adaptation are two different categories of stress tolerance mechanisms. The most well-known method of stress avoidance is the growth of vegetative organs like as seeds, bulbs, tubers, or other organs that may survive in difficult ecological conditions. After the tolerance mechanism is activated, plants generate distress metabolic pathways that comprise reversible activities with lower energy expenditure and lower enthalpy volatility (Mickelbart et al., 2015). Plants adapt to different environments and interact easily to changes in physiological processes and cellular metabolism as a reaction to distinct abiotic stressors (Hasanuzzaman et al., 2020).

2.2.5 Drought tolerance

Due to the complexities of the water-limited environment and changing climate, drought stress is one of the greatest impediments to worldwide agricultural productivity. To deal with water stress or drought, plants have evolved a variety of morphological, physiological, biochemical, cellular, and molecular defense mechanisms. Drought can be resisted by plants in four ways: avoiding it, enduring it, escaping it, and recovering from it (Fang & Xiong, 2015). Drought tolerance and drought avoidance are the two main plant defenses against water scarcity stress. Drought tolerance refers to a plant's ability to tolerate a dry environment via various physiological mechanisms such as osmotic adjustment using osmoprotectants (Ilyas et al., 2021).

Drought avoidance entails maintaining physiological processes like as stomata regulation, root system expansion, and other functions when water is scarce. The capacity of a plant to resume growth after being exposed to intense drought stress is known as drought recovery (Manavalan et al., 2009). Furthermore, ABA causes stomatal closure as well as the activation of numerous stress-related genes. According to one study, a regulatory system independent of ABA regulates the expression of genes induced by drought (Hossain et al., 2016). A plant responds to a lack of water by changing the expression of numerous genes, which it controls via complicated transcriptional networks. It is critical to investigate stress regulatory networks and identify significant regulators that can be used to develop stress tolerance in plants (Osakabe et al., 2014).

2.2.6 Salinity tolerance

The ability to withstand salt stress is a complicated phenomenon that is influenced by the interactions of substances used in numerous biochemical and physiological processes. There are two primary phases to plants' responses to salinity (Gupta & Huang, 2014). Stomatal closure and cell growth inhibition are brought on by an ion-independent growth reduction, which mostly affects the shoot and occurs from minutes to days. A second stage involves the accumulation of cytotoxic ions over the course of days or even weeks, which slows down metabolic processes, triggers premature senescence, and ultimately results in cell death (Isayenkov & Maathuis, 2019).

2.2.7 Cold tolerance

Freezing tolerance and resilience to cold stress are closely related aspects of plant adaptation. Some theories suggest that low-weight solutes like proline and soluble sugars play a role in shielding plants from the harmful effects of cold (Yadav, 2010). Cold stress inflicts particular damage to the plant's plasma membranes, and this damage is associated with dehydration that comes along with the cold conditions. In the realm of cold resistance, CBFs (-Repeat Binding Factors) take the center stage. Ordinarily, these are governed by the circadian clock and photoperiod, but during cold spells, they kick in to modulate genes that help the plant cope (Nurhasanah Ritonga & Chen, 2020). A general signal transduction mechanism of transcriptional regulation under various abiotic stresses is illustrated in **(figure 1).**

2.3. Role of transcriptions factor in abiotic stress

Aside from cold stress, plants face a broad range of abiotic pressures, from nutrient deficits and salinity to drought and extreme temperature variations (Waqas et al., 2019). Plant development, growth, and production may be negatively impacted by these conditions. These conditions can negatively skew plant development and yield. Plants, however, are not helpless. Over time, they've crafted intricate molecular networks to handle these stressors (Hoang et al., 2017). Transcription factors regulate gene expression by binding to certain DNA sequences found in the promoter regions of target genes. A variety of physiologic, biochemical, and molecular alterations in plants are brought on by abiotic stressors. These reactions entail the

activation or repression of many genes that provide plants the ability to handle stress and preserve cellular homeostasis. The primary regulators in organizing these intricate reactions are transcription factors. In response to stress signals, TFs bind to specific DNA regions known as cis-regulatory elements to activate or repress target genes. With the aid of this dynamic regulation, plants may modify their metabolism, signaling pathways, and defense systems to increase their ability to withstand stress (Wang et al., 2016). A variety of transcription factor families have been linked to plant responses to abiotic stress. Some of the TF families that have been thoroughly studied are AP2/EREBP, NAC, MYB, MYC, WRKY, bZIP, and bHLH. These transcription factor families engage with multiple signaling pathways that are responsive to stress and have a variety of activities (Sun et al., 2018).

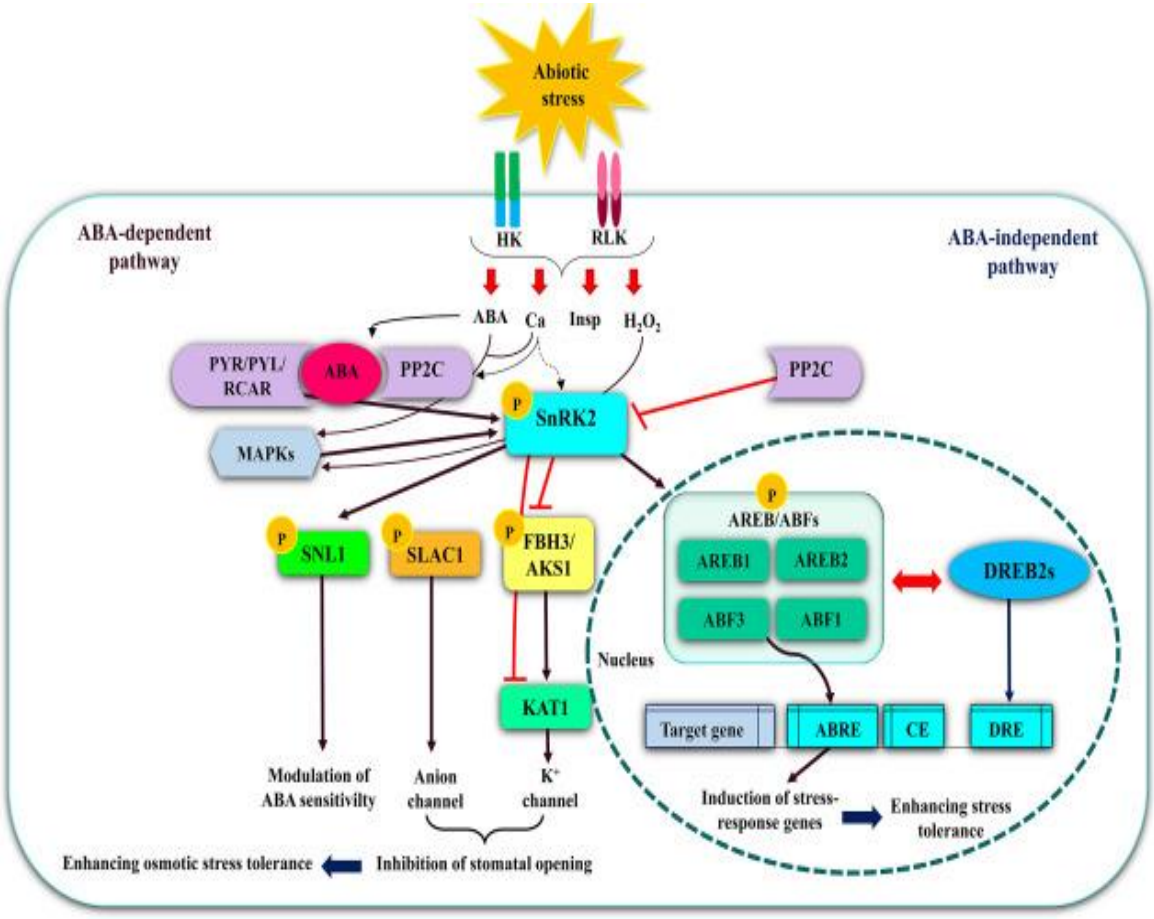


Figure.1. Defense signaling in plants against diverse abiotic/biotic external Stress.

2.3.1. Trihelix transcription factor

DNA binding protein Trihelix only present in plants. These transcription factor first discovered in 1990, These are the first protein which discovered in plants, They are sometimes referred to as "GT factors" because of their capacity to bind to GT elements (Levy et al., 2012).Trihelix was first discovered in pea (*Pisum sativum)* and soybean nuclei (*Glycine max Merr.),* then in those of *Arabidopsis*, tobacco *(Nicotiana tabacum L*.), and rice *(Oryza sativa),* (Yasmeen et al., 2016). Because they all have an a-helix-turn-a-helix shape and are assumed to be closely related to the Myb DNA-binding domain, the trihelix DNA-binding domain is classified with Myb/SANT-like domains in pfam (Wang et al., 2016).

2.3.2 Role of Trihelix genes under abiotic Stress

Trihelix TFs control the expression of light-dependent genes, respond to biotic and abiotic stresses, and participate in a variety of developmental processes, including morphogenesis (the development of many flower organs and leaves) and the development of trichomes and embryos (Wang et al., 2016).OsGT-2 is activated by salt, drought, ABA, and oxidative stress, with salinity stress being the most potent inducer. This proves that OsGT-2 is a stress-responsive factor. OsGT-2's critical function in the regulation of salt stress was further shown by investigations of its gain- and loss-of-function (Noonari et al., 2016). A number of abiotic stimuli and phytohormone treatments have been shown to strongly influence two SlGT genes, SlGT-27 and SlGT-34, indicating that these proteins may serve as essential regulators in stress and hormone responses. Furthermore, the majority of SlGT genes were altered by hormone therapies and abiotic stimuli, particularly in response to heat stress and oxidative damage. Furthermore, approximately half of the SlGT genes' expression was significantly up- and down-regulated (Yu et al., 2015).

2.3.3 Trihelix genes in potato

We were able to identify each possible Trihelix gene encoded in the *Solanum tuberosum* genome thanks to the completion of whole genome sequencing. Additionally, the sequenced potato genome allowed us to perform a thorough bioinformatics study to investigate the genome organization and structure of the Trihelix gene family in potato as well as important facets of evolution and functional diversity. Furthermore, a complete understanding of the three-dimensional (3D) structures of five group StTrihelix proteins (GT-1, G-T2, SH4, GT, and SIP1),

as well as possible cis-regulatory elements (CREs), is now plausible. Between plants, there are large differences in the distribution of Trihelix genes. Gene duplication is regarded as an important element in plant evolution. Gene duplication events, which provide the novel genes required for plant evolution, considerably enhance plant variety (Panchy et al., 2016).The Trihelix gene family has grown dramatically, allowing for deeper and more complex functional distinctions. A variety of abiotic stresses and phytohormone treatments significantly influenced the expression of several SlGT genes, including SlGT-27 and SlGT-34. 36 Trihelix genes, for instance, have been studied in tomato (Yu et al., 2015).94 trihelix genes present in wheat, many of which have been regulated in salts and cold stress (Xiao et al., 2019).

The trihelix family is represented by 41 members in the rice genome. Aridity and severe salt stress were identified as detrimental abiotic variables by OsMSLs, as were stress signaling molecules like ABA (abscisic acid) and hydrogen peroxide. OsMSL28 expressed strongly in response to treatments such as hydrogen peroxide, drought, and high salt levels, whereas OsMSL39 expressed synchronously in all treatments, whereas OsMSL28 displayed modest expression. Furthermore, OsMSL16/27/33 expressed significantly in response to ABA and drought treatments (Luo, 2010). A simple, economical, and environmentally friendly approach is to create more effectively genotypes with significantly higher stress tolerance and adaptation (Thiry et al., 2016). In order to identify and use of stress-responsive genes, plant scientists can generate better crops with increased stress tolerance with the use of plant genomics and bioinformatics.

Chapter 3

Material and Methods

3.1 Identification and sequence retrieval of Trihelix genes in *Solanum tuberosum*

To find the Trihelix gene family within the S. tuberosum genome, amino acid sequences from *Arabidopsis thaliana* Trihelix proteins were sourced from TAIR (https://www.arabidopsis.org/). Employing these sequences as query material, a BLASTp search was conducted against *Solanum tuberosum* genome v4.0 via the Sol Genomics Networks (SGN) (https://solgenomics.net/), with a set homology search criterion of 1x10^-5. Further analysis of the DNA-binding domains, particularly the Myb/SANT-like structures, in the potato amino acid sequences was executed via InterProScan (https://www.ebi.ac.uk/interpro/search/sequence/). MEGA X software was applied to align the filtered potato Trihelix proteins as a step in amino acid conservation evaluation. A telltale marker, the presence of tryptophan in candidate sequences, served as an additional criterion for Trihelix gene selection. ExPASY's ProtParam tool (https://web.expasy.org/protparam/) was an option to compute the physical characteristics of the Trihelix proteins, such as molecular weight in kDa, protein length, and isoelectric point (pI).

3.2 Phylogenetic analysis of St Trihelix genes

To study the evolutionary links between AtTrihelix and StTrihelix proteins, we aligned their full amino acid sequences using the MUSCLE algorithm with its standard settings. Post-alignment, we constructed phylogenetic trees via the NG Phylogeny platform (https://ngphylogeny.fr/). We used a maximum likelihood approach rooted in the Poisson substitution model and included 1000 bootstrap samples for statistical robustness. StTrihelix proteins were classified into the same five subgroups previously established for AtTrihelix proteins.

3.3. Gene structure and protein

To examine exon-intron architecture, the genomes and whole coding sequences (CDS) of each StTrihelix gene were acquired from Phytozome v12. By comparing the CDS sequences of the StTrihelix genes with their respective genomic sequences, the Gene Structure Display Server was utilized to generate a detailed graphical representation of the exon-intron organization (GSDS 2.0) (http://gsds.gao-lab.org/). With the following parameters: (i) an ideal motif width of

6–50 amino acids, (ii) each sequence, occurrence 0-1 (iii) a maximum of ten motifs. MEME (https://memesuite.org/meme/tools/meme) version 5.3.2 was used to find the conserved motifs in S. tuberosum Trihelix proteins.

3.4. Chromosomal mapping and gene duplication

Each StTrihelix gene's chromosomal location was identified using data from the Phytozome v12.1 database. The TB tools (ToolKit Biologists Tools) programme was used to map the physical positions and relative spacing of the StTrihelix genes on the respective potato chromosomes. Two gene expansion mechanisms were considered while analyzing gene duplication, these are tandem and segmental duplications.The Ka (non-synonymous) and Ks (synonymous) values were estimated using the ToolKit Biologists Tools (TB tools) programme (https://github.com/CJ-Chen/TBtools) to analyze the influence of selective pressure and divergence time on StTrihelix genes. The method T=Ks/2x*MYA was used to estimate the approximate divergence time, where 34 x=6.56 x 10-9 and MYA=10.

3.5 Cis-regulatory elements analysis in the StTrihelix gene promoters

To scrutinize the cis-regulatory elements in the StTrihelix genes, 2.5 kbp sequences upstream from the ATG translational start site for each of the 27 identified genes were obtained via Phytozome v12.1 (https://phytozome.jgi.doe.gov/pz/portal.html/). These promoter sequences were later processed through the Plant CARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

3.6 Gene ontology (GO) annotation and subcellular localization prediction

For gene ontology analysis of the StTrihelix genes, Blast2Go (https://www.blast2go.com/) was implemented. This application mapped and annotated the transcript sequence of each gene to unravel its biological processes (BP), cellular compartments (CC), and molecular functions (MF), utilizing default settings for BLASTx search and InterPro Scan. Lastly, Wolf PSORT (https://wolfpsort.hgc.jp/) was employed to determine the intracellular locations of these StTrihelix genes.

Wet Lab Methodology

3.7 Tuber collections and plant growth

Potato tubers of Kuroda variety were collected form National agriculture research center Islamabad (NARC). Potato tubers were grown in a controlled condition at ASAB glasshouse. The temperature was kept between 18 and 25 degrees Celsius and plants were watered on alternate days. Potato tubers were grown in well-drained, sandy soil.

3.8 Extractions of RNA by trizol method

Total RNA was isolated from leaves Shoot and Stem of Potato plant by using Tirzol method for RNA extraction described by with some changes. To prevent sample melting, frozen leaves Roots and shoots samples from a -80°C freezer were moved to the extraction site in a flask filled with liquid nitrogen and held there until further processing. Using an autoclaved mortar and pestle, frozen leaf samples were ground into a powder. The samples were pulverized into a fine powder one by one. 1ml of the Tirzol reagent was directly added to the powder in the mortar to create the slurry. Then, the slurry was poured into a 1.5 ml Eppendorf tube, homogenized using inversion mixing for 10 seconds, and incubated for 10 minutes on ice. After incubation, the material was centrifuged (14,000 rpm for 10 minutes at 4°C). The material was centrifuged, and the supernatant was pipette-transferred into a clean Eppendorf tube for five minutes on ice.

The supernatant was combined gently with 200ml of chloroform before being incubated for 5 minutes on ice. Once more, the mixture was centrifuged for 15 min. at 14,000 rpm and 4C. A yellowish protein layer in the midst of the two phases that formed was observed. Into a 1.5 ml Eppendorf tube was put the upper aqueous phase. After adding 500 ul of cold isopropanol, samples were incubated at -20°C for two hours. Following incubation, samples must be centrifuged for 10 minutes at 14,000 rpm. The centrifugation process produced the whitish pallet. Supernatant was carefully discarded, but the pallet was kept. To clean it, the pallet was resuspended in 1 cc of 75% ethanol. After washing, the sample was centrifuged to recover the pallet for 5 minutes at 4oC and 9500 rpm. The pallet was air dried to remove any remaining ethanol droplets after the supernatant was discarded. Following that, the pallet was dissolved in 25 l of RNase-free water. Nanodrop was used to assess the purity of the RNA. The RNA was kept in a freezer at -80C for later usage.

3.9 Primer designing

To design primers for qRT-PCR CDS sequences of StTH25 and StTH22 retrieved from the Phytozome. From CDS sequence the primer were design manually. OligoCalc was used to determine parameters such as GC concentration, melting temperature, and primer size after primers were designed.

For amplification of StTH25 following primer set was designed and used

StTH25 (F) ATGAGGCTTGGAAGATGAAG

StTH25 (R) CAGTTACCAACGGATTTACC

For amplification of StTH22 following primer set was designed and used

StTH22 (F) TAATCGTAGCCCGGAACAGT

StTH22 (R) ATGGATAGAGGCAGAAGGTG

3.10 Complementary DNA synthesis

The mRNA was converted into single stranded complementary DNA following RNA extraction. In a 0.2 ml PCR tube, oligo dT, Nuclease Free water, and RNA sample were added for cDNA synthesis. The reagents were mixed while the tube was spun down. In the thermal cycler, the tube containing the reagents was incubated for 5 min at 65C. The tube was immediately put on ice after incubation too cold for at least one to two minutes. The same 0.2 ml PCR tube was then filled with 5X buffer, 10mM dNTPs, RiboLock 38 RNase inhibitor, and reverse transcriptase enzyme before being spun down and put back into the thermal cycler at 42°C for another 60 minutes. The termination phase was performed after this one for 5 min. at 70C. Following cDNA synthesis, the tubes were placed in a freezer and kept at 20°C (**Table 2).**

3.11 Real time PCR

The reverse transcriptase polymerase chain reaction (RT-PCR) was used to confirm the presence of cDNA using housekeeping gene primers (Actin). A 0.2 ml PCR tube was filled with deionized water, 10X reaction buffer, MgCl2, 2.5 mM dNTPs, Actin forward and reverse primers, cDNA, and Taq DNA Polymerase for the PCR method. The reagents were mixed by spinning down the tubes. Thermal cycler was used to perform 35 cycles of denaturation at 95°C

for 3 minutes, annealing at 95C for 30 seconds, 60°C for 1 minute, and extension at 72°C for 45 seconds. The final extension was performed at 72C for 7 minutes. Both a positive and a negative control were used in the reaction the former with all PCR chemicals and the latter without cDNA. Gel electrophoresis was carried out when the PCR reaction was finished. The chemical reagents and quantity given in (**Table 3).**

3.12 Gel electrophoresis

PCR products were used in gel electrophoresis. To make a 1% agarose gel, 0.5g of agarose was combined in 50mL of 1X TAE buffer. After heating the mixture in the oven for at least one minute, or until it became transparent, 5 uL of ethidium bromide was added. The casting tray with the comb fastened in it received the gel. After removing the comb and adding 1X TAE buffer to the gel tank, samples are deposited into the tray's wells. For 40 minutes, 80 volts of electric potential was provided for gel electrophoresis. A gel documentation system was used to analyze the gel under UV light after that.

3.13 Quantitative real time polymerase chain reaction (qRT-PCR)

StTH25 and StTH22 gene primers were used in Quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR) for expression analysis (**Table 4).**

Table.2. *Reagents used in cDNA synthesis and their quantities.*

S. No.	Reagents	Quantity
1	Nuclease free water	4.25 ul
2	Oligo dT primer	1 ul
3	RNA sample	1 ul
4	5X reaction buffer	2 ul
5	RiboLock	0.25 ul
6	10mM dNTPs	1 ul
7	Reverse Transcriptase	0.5 ul
	Total Volume	10 ul

Table.3. *Chemicals and their quantity used in PCR reactions.*

S.No.	Chemicals	Quantity
1	Deionized water	15 µl
2	10X reaction buffer	2 µl
3	MgCl2	2 µl
4	2.5mM dNTPs	2.5 µl
5	Actin forward primer	1 µl
6	Actin reverse primer	1 µl
7	cDNA	1 µl
8	Taq DNA Polymerase	1 µl
	Total Volume	25ul

Table.4. *Reagents and their quantity used in qRT-PCR.*

S.No.	Reagents	Quantity of reagents
1	Nuclease free water 9.5µL	9.5µL
2	SYBR Green	12.5µL
3	Forward primer	1µL
4	Reverse primer	1µL
5	cDNA	1µL
	Reaction volume	25 µL

Chapter 4

Results

4.1. Identification and sequence retrieval of Trihelix genes in *Solanum tuberosum*

The amino acid sequences of Trihelix members in *Arabidopsis thaliana* were utilized as query sequences against the *S. tubersoume* genome v4.0 in a BLASTp homology search to find the Trihelix genes in Potato. After deleting additional redundant hits with insignificant e-value and identity %, a total of 27 Trihelix genes, named as StTrihelix1 to StTrihelix27, were found in potato. Using InterProScan, it was discovered that each StTrihelix contained a conserved Tryptophan, which is a feature of this gene family, as well as a Myb/Sant-like domain. Physical characteristics include the protein's length, isoelectric point, molecular weight (Mw), chromosomal location, and length of the conserved domain is mentioned in (**Table 5).**

4.2. Phylogenetic analysis of StTrihelix genes

A phylogenetic tree for Trihelix proteins was created using a maximum likelihood phylogenetic technique with 1000 bootstrap replicates to investigate the evolutionary relationship and sequence homology between *Arabidopsis* and *S. tuberosum* Trihelix protein sequences. The 27 members of the Trihelix gene family from *Solanum tuberosum* were grouped into five subgroups using the labels GT-1, GT-2, SH4, GT-Y, and SIP1 **(Figure 4.0).** The subgroups GT-1 has only three members it is the smallest subgroups while SIP1 subgroups has 11 member it is largest subgroups, GT-2 and GT-Y both has a 4 member and SH4 has 5 members. All StTrihelix genes contain Tryptophan which is highly conserved amino acid and Features of this gene family. This is confirmed by aligning all the 27 sequences of StTrihelix using mega 11, and after confirming this tree is constructed.

4.3. Stress application and sampling of plant

Plants were watered regularly every day before subjecting to drought stress. To impose drought, plants grown were well watered for 28 days after emerging. For drought stress application water was withheld for 14 day and the control plants were watered throughout the

experiment. After 14 days of drought plants were rewatered for 3 days and sampled **(figure 4.1)**. For expression analysis of genes, the leaf, root and shoot sample were collected from Potato plants of all applied treatments. For the extraction of RNA, all of the leaves, branches, and roots from each plant were cut with sterile blades and put in correctly labelled aluminum sheets that were immediately placed in liquid nitrogen and kept in -80°C freezers.

4.4. Gene structure (Introns exons) analysis

The arrangement of introns and exons plays a crucial role in the development of gene families. For the graphical representation of the organization of gene structure, the genomic DNA of StTrihelix genes and c-DNA aligned and compare. Gene structure results showing that one exon is present in 11 StTrihelix genes (1, 10,12,14,19,2,21,3,4,6,26), While 9 StTrihelix genes contain two exons and one intron (11,16,17,18,20,5,7,24,25), one intron and upstream region is present in 3 StTrihelix gens (3, 15,18) While StTrihelix 27 genes contain two exons and one upstream regions, other three have more than two introns and exons like three exons and two introns are present in StTrihelix 22, StTrihelix 9 contain the six exons and five introns and StTrihelix 23 contain 7- exons and 6 introns **(figure 4.2).**

4.5. Motif analysis

The MEME software is used for motif analysis 10 conserved motifs were discovered with their length ranges from 28 to 50. Motif 5 was small motif with 28 amino acids residues (GWGGTRGWRGTGKTGGWGGHGGVGRKRR) while Motif 8, 7,4, 1 these are largest motif with 50 amino acid residues. All the subgroups have different motif compositions shown in **(Figure 4.3).** Motif 1,2,3 and 6 presents in all 27 sequences of StTrihelix genes while Motif 9, 10 and 5 presents in 25 sequences of StTrihelix genes and Motif 4 and 8 presents in 11 sequences of StTrihelix genes. The motif seven present only in 14 Genes of StTrihelix. Width of Motif, E-Value, Sites, sequence of motif and length is shown in**.**

4.6. Gene duplication, chromosomal mapping, and synteny analysis

The chromosomal location of 27 Trihelix genes was determined using Phytozome v.13 at its whole length. All StTrihelix genes were physically mapped and distributed throughout the Potato genome using the TB Tools Software. Maps showed that the distribution of genes across 12 chromosomes varied genetically.6 StTrihelix genes were present on chromosome 1(chr01)

Table.5. Showing Transcript ID, Gene name, Chr ID, Start and End site, Molecular weight, Pi value and Orf length of StTrihelix genes.

Gene name	Chr ID	Start Site	End Site	Molecular Weight	Pi-Value	Orf-length	Group
StTH1	1	81528746	81529925	74	6.88	392	GT-Y
StTH2	1	11102593	11103520	34767.38	9.18	308	SH4
StTH3	1	10996840	10997602	28928.51	8.78	253	SH4
StTH4	1	68692348	68693338	37097.35	9.16	329	SIP1
StTH5	1	74192190	74194173	37677.4	9.35	343	SIP1
StTH6	1	11192312	11193239	34721.36	9.41	308	SH4
StTH7	2	14693362	14695368	38311.26	8.75	338	SH4
StTH8	2	32148114	32151164	33361.81	5.86	289	SIP1
StTH9	3	50954292	50956821	40378.88	7.66	362	GT-1
StTH10	3	61349032	61350154	41270	9.32	373	SIP1
StTH11	4	59881350	59884089	69388.46	6	628	GT-2
StTH12	5	18966388	18967447	40568.39	4.69	352	SIP1
StTH13	6	29588759	29597022	29189.72	9.33	256	SIP1
StTH14	6	58346886	58347867	37859.9	6	326	SIP1
StTH15	7	50241011	50243544	44403.6	9.78	397	SIP1
StTH16	8	33973251	33974764	54437.69	6.11	466	GT-Y
StTH17	8	2907023	2915907	37376.58	9.02	320	GT-1
StTH18	8	44913462	44919614	36271.53	5.25	315	SH4
StTH19	8	1041976	1043221	46317.6	9.17	414	SIP1
StTH20	9	3631853	3634922	62435.27	6.44	542	GT-2
StTH21	9	4116298	4117237	35984.79	5.74	312	GT-Y
StTH22	9	8020167	8021634	34972.43	7.17	298	GT-1
StTH23	9	4094434	4101705	57982.96	6.25	533	SIP1
StTH24	11	233040	235097	44073.62	5.1	389	GT-2
StTH25	11	7626318	7628007	57413.52	7.99	503	GT-2
StTH26	12	60706116	60707496	51483.42	5.61	459	GT-Y
StTH27	12	1498039	1501995	42529.73	8.59	383	SIP1

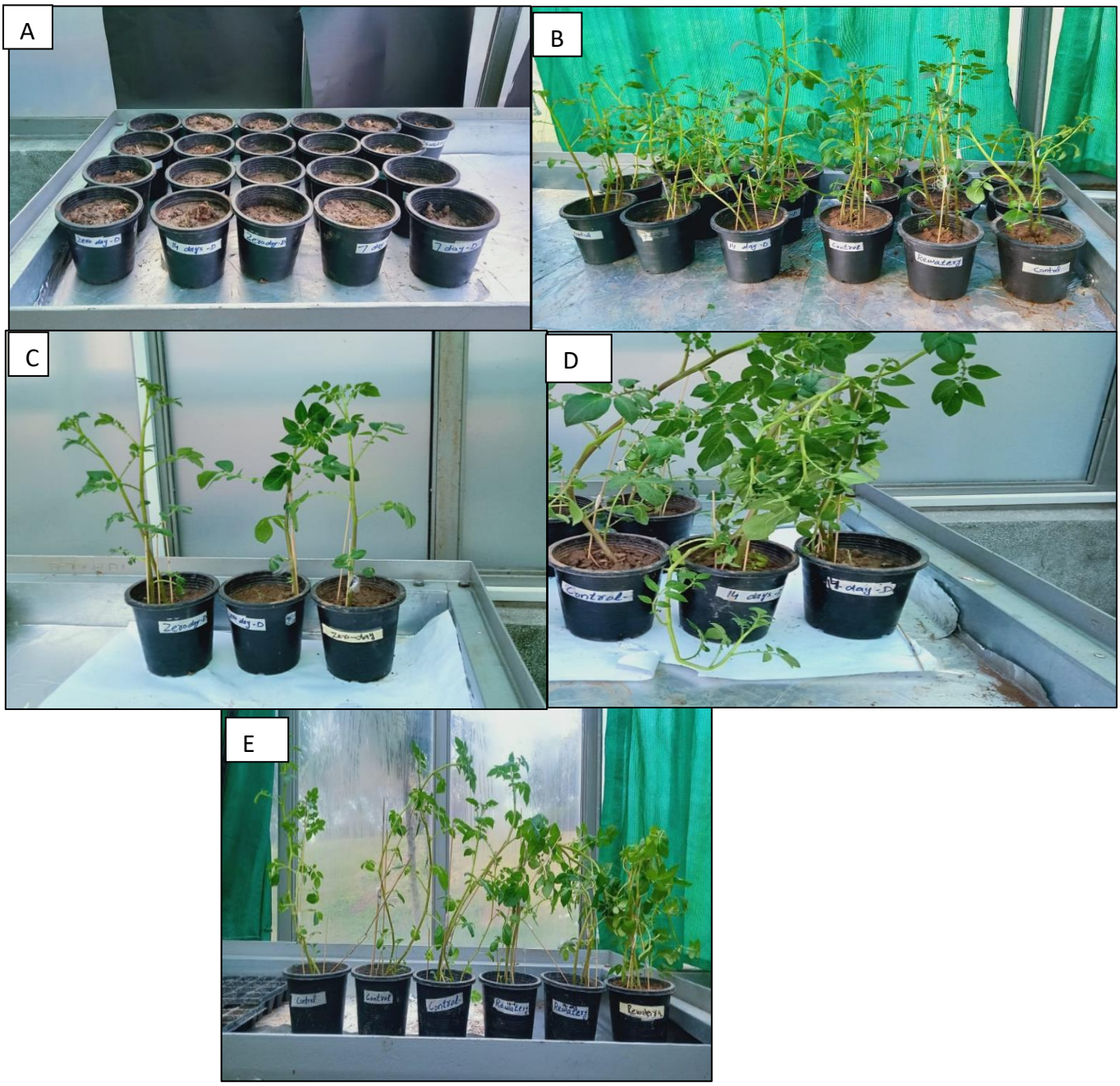


Figure.4.1. *Plant grown in ASAB glass house Under controlled condition. (**4.1 A)** showing the sowing of plant. (**4.1 B)** showing the growth of plant **(4.1 C)** showing the first day sampling of plants (4.1 D) represents the 14-days control, stress plants while **(4.1 E)** represents the rewatering plant.*

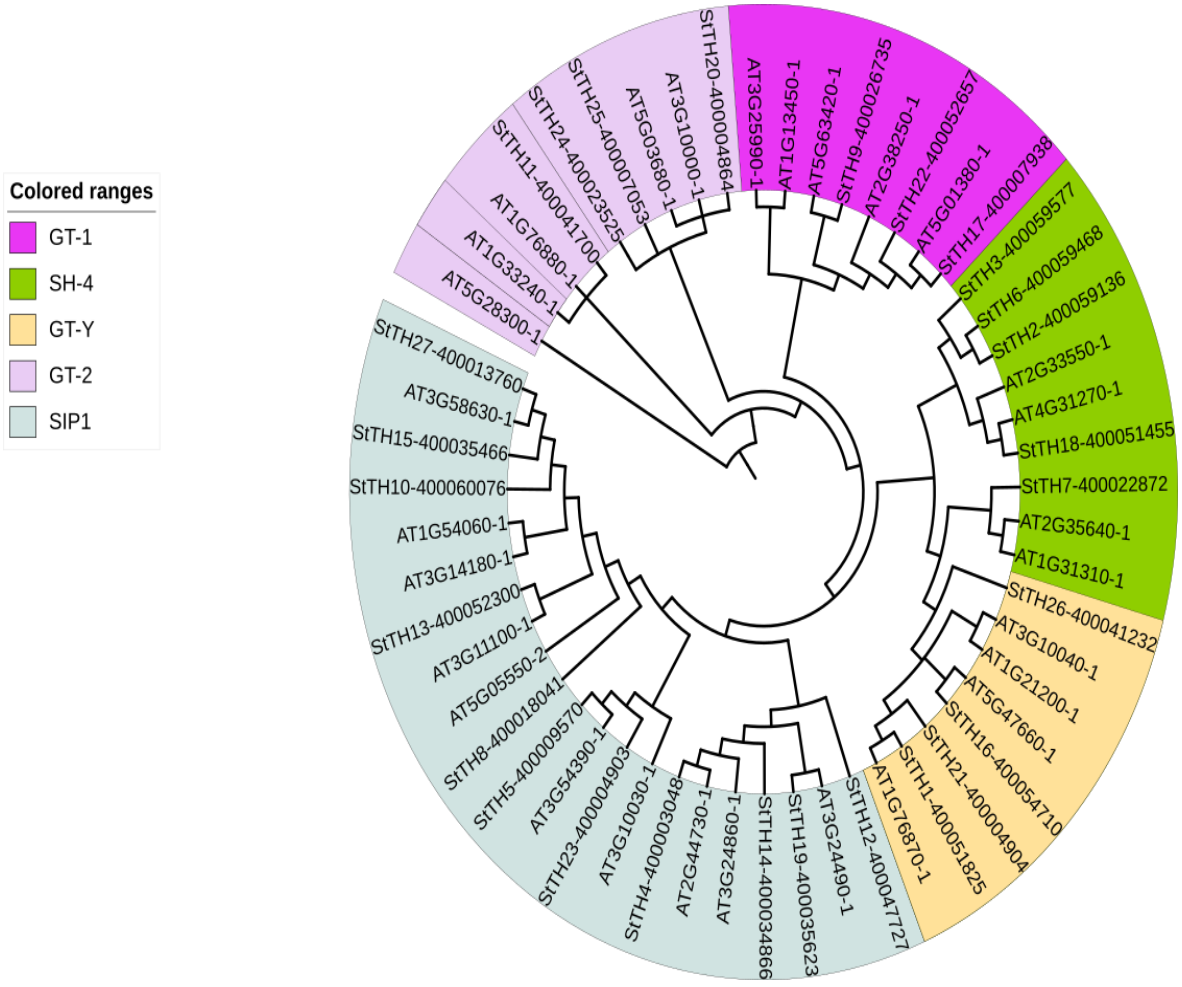


Figure 4.0. *Phylogenetic tree between A. thaliana and Solanum tuberosum, Utilizing the NG Phylogeny software, maximum likelihood method and 1000 bootstraps were used. Each colour represents a specific class.*

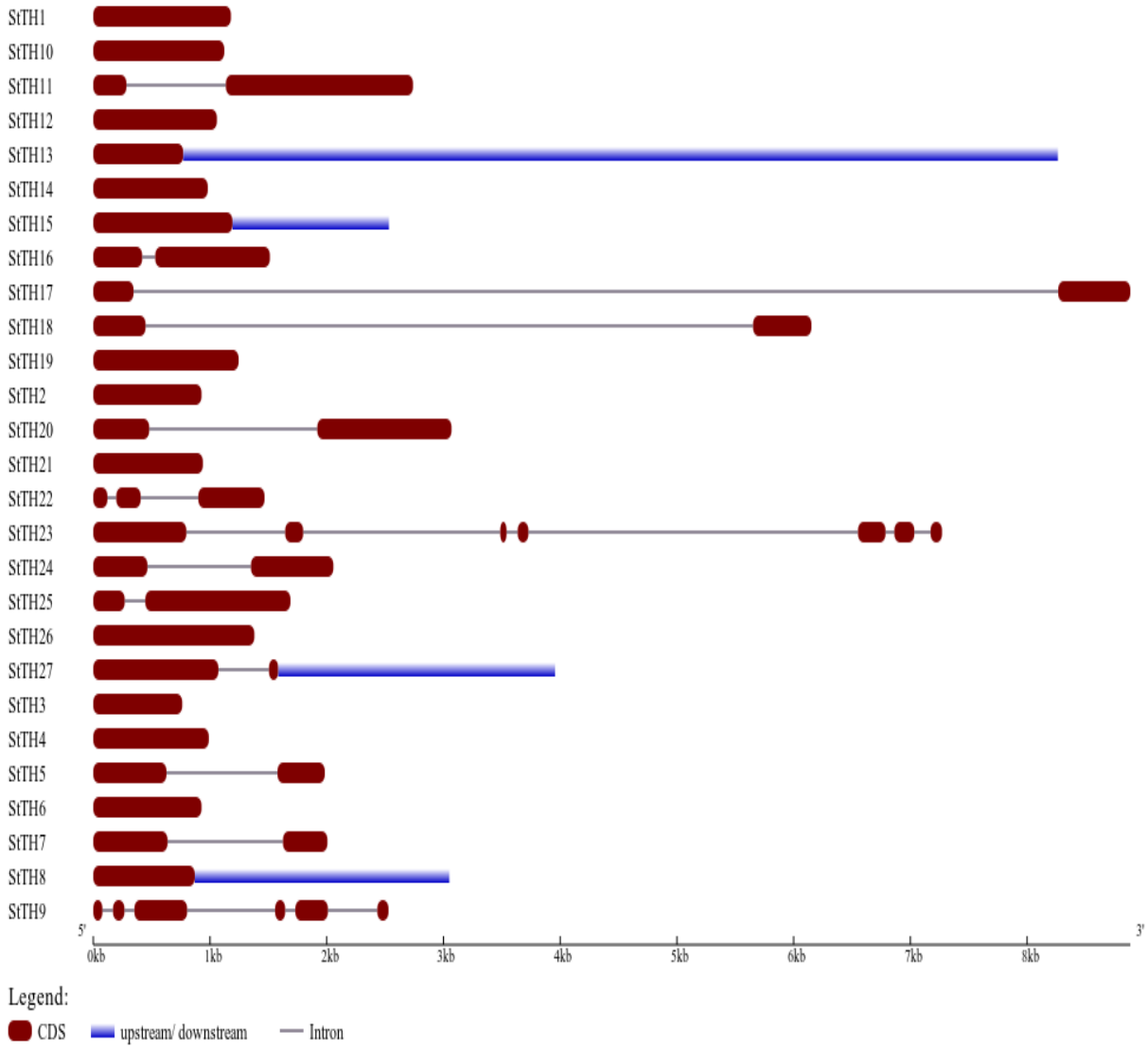


Figure 4.2. *StTrihelix genes Intro-exon organizations. Gene Structure Display Server (GSDS) is used for analyzed Gene structure. Maroon and Black line colors are representing exons and introns respectively. Corresponding sizes of StTrihelix genes.*

Figure 4.3. *By using MEME software Ten motifs predicted within StTrihelix protein sequences. Motifs were represented by unique coloured boxes, whereas non-conserved areas were designated by grey lines.*

which have highest number of genes as analyze to other chromosomes. However, three chromosomes (05,04,07) had only one gene which considered as the least number of genes present on chromosomes. Chromosomes number (02,03,06,11,12,) had only two genes. Chromosome number 8(08) contain the 4 genes and five genes are presents on chromosomes number 9 (09) shown in **(figure 4.4)**. The source of genomic evolution in plants is gene expansion events that build new linkages throughout the genome and allow the maintenance of specialized functionalities. Both types of duplications, Tandem and segmental duplication events were discovered in the Potato genome. According to gene duplication data, chr04, chr05, chr07, and chr12 each have one pair of segmentally duplicated genes, while 2, 3, and 6 and 11 each have two such genes. Three segmental duplicated gene pair were present on chromosomes 8 and 9.

Tandem duplication genes are found in two pairs on Chromosome 1, and one pair segmental duplication genes (**Figure 4.5)**. Ka/Ks values were measured for these 13 gene pairs to estimate the divergence period and kind of selection in each pair. If the Ka/KS ratio is equal to one, the gene pair is subject to neutral selection. If the ratio is less than one, the gene pair is subject to purifying selection. If the ratio is greater than one, the gene pair is under positive selection. Purifying selection caused StTrihelix duplication in 13 gene pairs, and the results also revealed that their divergence happened between 1 and 39 years ago shown in (**Table 6).** By comparing their genomes to those of S. tuberosum, synteny analysis was used to establish the evolutionary relationship that revealed the collinearity of the StTrihelix genes in A. thaliana and S. lycopersicum **(Figure.4.6 A. B).**

4.6. Subcellular localization and gene ontology (GO)

Gene ontology annotation was done by a blast go software on 27 StTrihelix genes followed by gene mapping, Blast hits and inter pro scan **(Figure 4.7.A).** StTrihelix genes' molecular function biological, and cellular compartment roles were estimated. Molecular function **(Figure 4.7.B)**. results revealed that StTrihelix genes were involved in Transcription cis regulatory, ATP membrane transporter, Kinase activity, Exonuclease activity, UMP kinase activity, ATP binding and RNA binding activity.Biological Process **(Figure 4.7.C)** shows that these are involved in Phosphorylation, nucleotide Transmembrane, glutamine family amino acids, negative regulation of genes, mitochondrial transmembrane, and Purine tans membrane transporter. Gene ontology

results also show that their cellular compartments **(Figure 4.7 D)** of StTrihelix genes occur in Nucleus, membrane and cytoplasm. Two software programs confirmed the StTirhelix genes' subcellular localization, and the results, which were shown in the shape of a heat map, revealed that the StTirhelix genes were highly concentrated in the nucleus after the other Fourteen organelles **(Figure 4.8)**.

4.7. Cis regulatory element analysis

The Plant CARE Software was used to analyses putative cis regulatory regions involved in transcriptional activity and response to biotic and abiotic stimuli in potato using a 1500 bp upstream region of the 27 StTrihelix gene **(Figure 4.9**). Based on their functions, the cis-elements classified into seven major groups. These classes were (i) hormone related, (ii) development related, (iii) biotic stress responsive, (iv) promotor related, (v) light sensitive, (vi) unidentified cis regulatory components, (vii) and (viii) abiotic stress responsive, as established by statical analysis. Promotor-related cis elements occupied the most space (62%), followed by abiotic stress resistance (14%), hormone-related (7%), unknown cis elements (6%), light-responsive elements (5%), biotic stress responsive elements (2%), and development-related cis elements (4%) **(figure 4.10)**. The presence of TATA-box, CAAT-box, AT-TATA box, A-box, and TATA elements that are involved in promotor binding site was examined in StTrihelix proteins. All 27 StTrihelix genes had several TATA- and CAAT-boxes, which serve as binding sites for transcription factors to initiate transcription **(figure 4.10.A)**.

Light responsive were G-Box, CT-motif-box, GATA-motif,Sp1, GT1-motif I-box, LAMP-element,ACE,ATC-motif,ATCT-motif,chs-CMA1a,chs-CMA2a,C-box,Gap-box,TCCC-motif,Pc-CMA2c,GA-motif,chs-CMA2b,sbp-CMA1c,AAAC-motif,AT1-motif,GRA,L-box,and Box II, The vast region's Box4 and G-box motifs regulated the transcription rate of light-differentiation, blood vessel development, and macromolecule regulation.

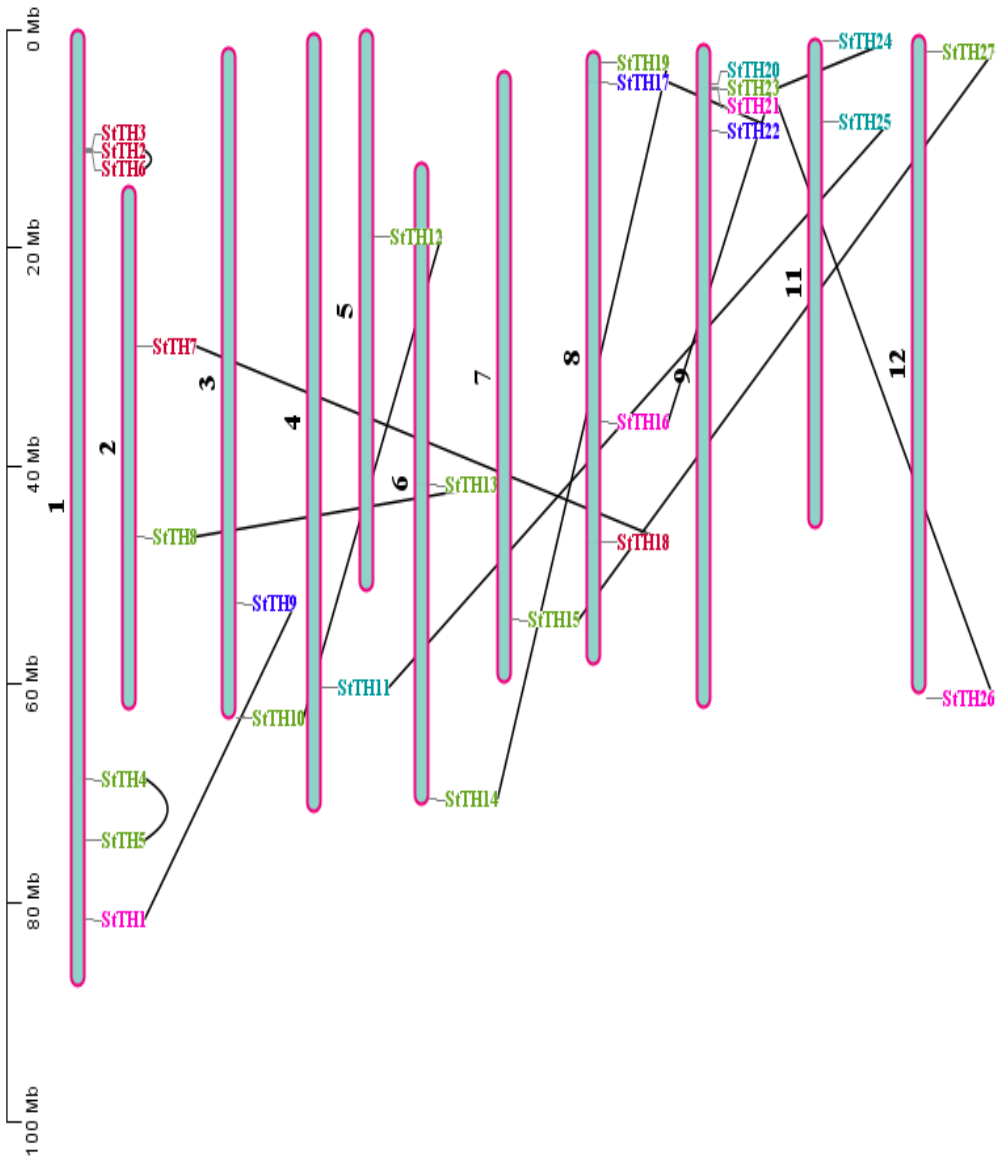


Figure 4.4. *The chromosomal distribution of the 27 StTrihelix genes within the genome was mapped using TB methods. The length of each chromosome shown by scale.*

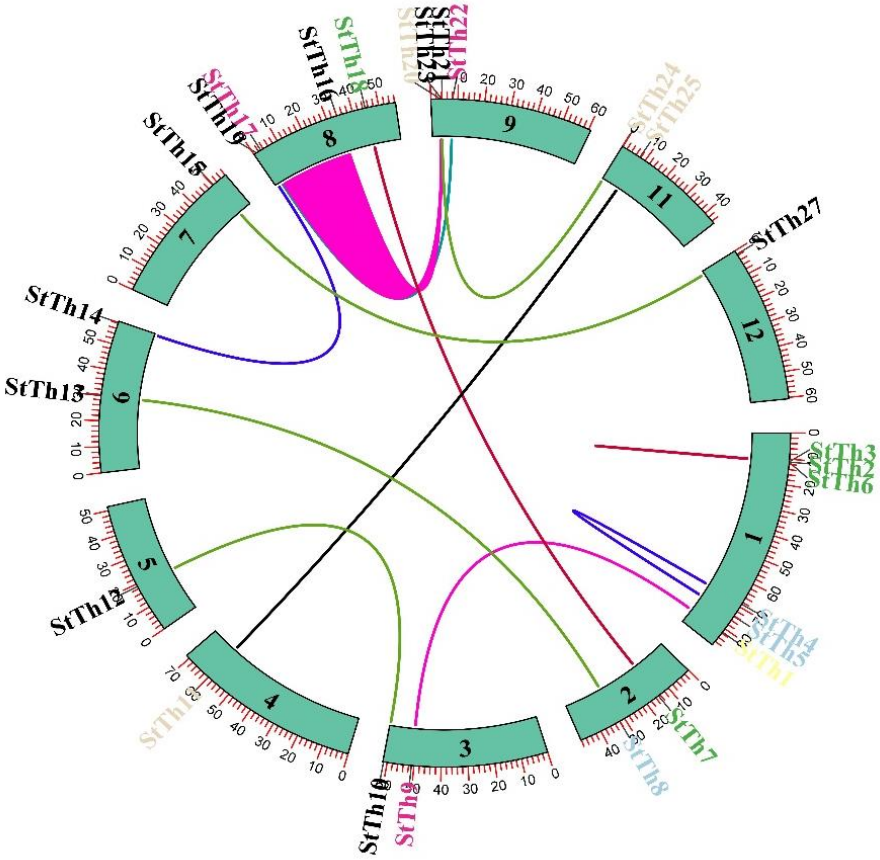


Figure 4.5. *Tb tool software was used to analyze circos.Circos showing Segmental and Tandem duplications. Chromosome one showing the tandem duplications while other chromosomes showing the segmental duplications.*

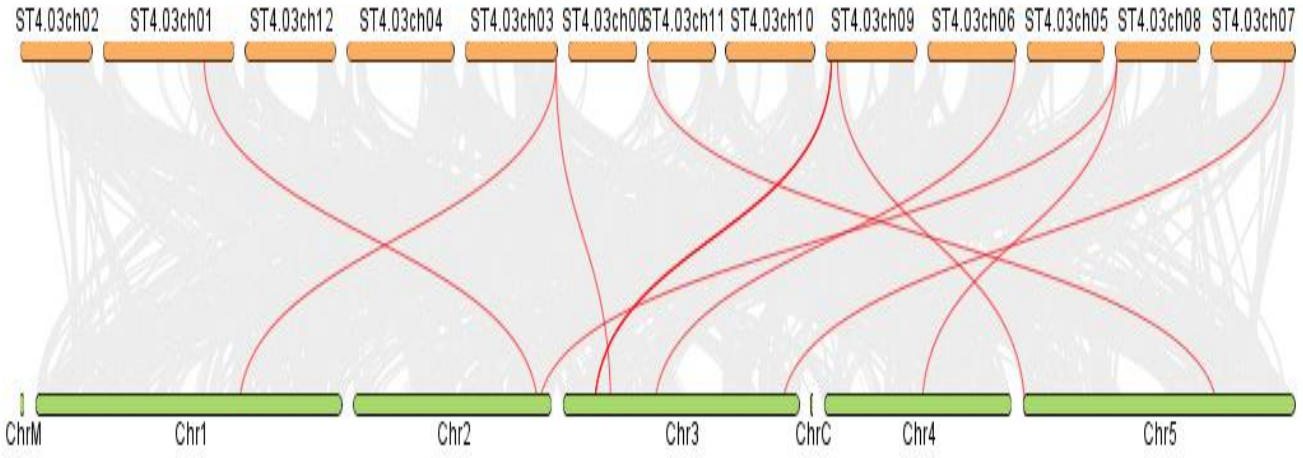


Figure.4.6. A. *Synteny analysis was done between S. tubersoume and A. thaliana for homology and evolutionary relationship. Green and Red bars denote chromosomes of S. tubersoume and A. thaliana. Grey lines represent collinear block within both genomes.*

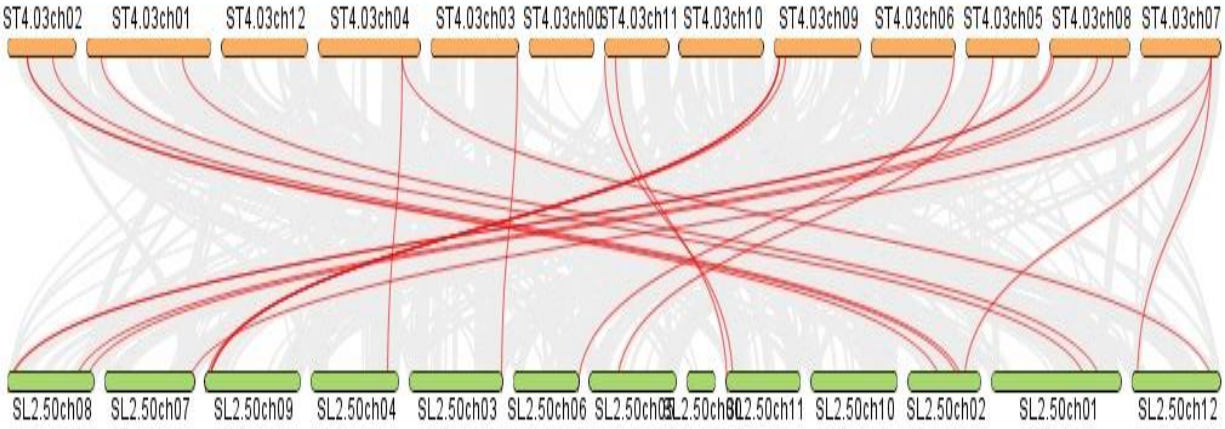


Figure.4.6. B. *for Knowing homology and evolutionary relationship Synteny analysis was conducted between S. lycopersicum and S. tubersoume. Orange and green bars denote chromosomes of S. tubersoume and S. lycopersicum. Light Grey lines indicate collinear block within both genomes. Red lines designated to respective homolog Trihelix genes present between both genomes.*

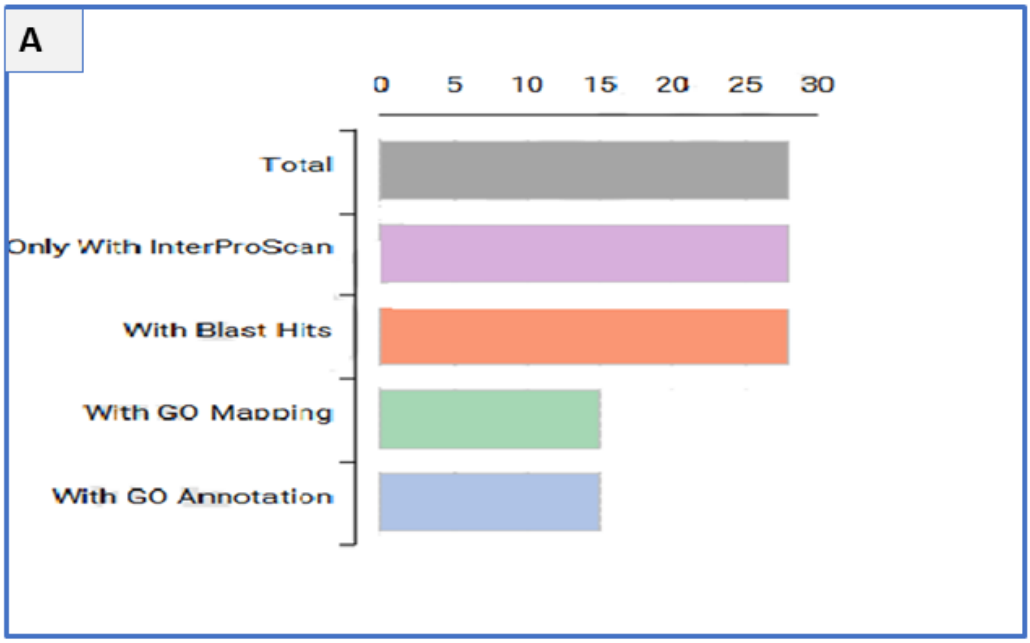
Table. 6. *Showing the Ka KS value, ratio ka/KS ratio, Time MYA duplications and Selections.*

Gene 1	Gene 2	Ka	Ks	Ka/ks Ratio	Time MYA	Duplications	Selection
StTH7	StTH18	0.4739	0.5045	0.939345887	38.4527439	Segemental	Purification Selection
StTH23	StTH24	0.50035	0.51595	0.969764512	39.32545732	Segemental	Purification Selection
StTH16	StTH 20	0.4432	0.4582	0.967263204	34.92378049	Segemental	Purification Selection
StTH13	StTH 8	0.18215	0.4217	0.431942139	32.14176829	Segemental	Purification Selection
StTH12	StTH10	0.2513	0.3789	0.663235682	28.87957317	Segemental	Purification Selection
StTH15	StTH27	0.0821917	0.29625	0.277440338	22.58003049	Segemental	Purification Selection
StTH9	StTH1	0.40585	0.43655	0.929675868	33.27362805	Segemental	Purification Selection
StTH25	StTH 11	0.16555	0.4632	0.357405009	35.30487805	Segemental	Purification Selection
StTH6	StTH 2	0.01470546	0.015780535	0.931873032	1.20278468	Tendem	Purification Selection
StTH14	StTH19	0.2786	0.45605	0.610897928	34.75990854	Segemental	Purification Selection
StTH4	StTH5	0.32315	0.4555	0.709440176	34.7179878	Tendem	Purification Selection
StTH21	StTH 26	0.1873	0.36935	0.50710708	28.15167683	Segemental	Purification Selection
StTH 22	StTH17	0.1921	0.43605	0.378815456	33.23551829	Segemental	Purification Selection

The AACA-motif and GCN4-motif are vital in endosperm expression, the circadian rhythm regulates it, the O2 site regulates zein metabolism, the RY element aids in seed-specific regulation, the E2Fb and F-box regulate the cell cycle, and the MSA-like is a mitosis-specific activator as **.** Unnamed__2 Unnamed__4 Unnamed__6 Unnamed these considered as the motif with **(Figure 4.10.C.).** box S, W box, WRE3, WUN-motif these motifs were biotic stress cis regulatory element **(Figure 4.10.D.)**.

Abiotic stress sensitive cis elements contained the motifs AT-rich sequence, ARE, CCAAT-box,regulates zein metabolism, the RY element aids in seed-specific regulation, the E2Fb and F-box regulate the cell cycle, and the MSA-like is a mitosis-specific activator as. Unnamed__2 Unnamed__4 Unnamed__6 Unnamed these considered as the motif with **(Figure 4.11.E.).** box S, W box, WRE3, WUN-motif these motifs were biotic stress cis regulatory element . Abiotic stress sensitive cis elements contained the motifs AT-rich sequence, ARE, CCAAT-box,Abiotic stress sensitive cis elements contained the motifs AT-rich sequence, ARE, CCAAT-box, DRE core, DRE 1, GC-motif, LTR, MBS, MBS 1, STRE, TC rich repetitions, MYB, MYC, MYB recognition site, MYB binding site, MYB like site, and AT-rich Element **(Figure 4.13.F.).** MBS and MBS1 are MYB binding sites involved in drought inducibility and gene regulators of flavonoid biosynthesis, whereas MYB and MYC are dehydration sensitive motifs found largely in StTrihelix gene promoters.

AT-rich Element and AT-rich sequence act as binding site for AT-rich binding proteins and act as maximum activation mediator upon perception of elicitors Some of the hormone-related cis regulatory elements discovered in StTrihelix genes are ABRE, ABRE3a, ABRE4, AuxRR-core, CGTCA-motif, ERE, GARE-motif, TATC-box, TCA, TCA-element, TGACG-motif, TGA-element, 3-AF1, binding site, AuxRE, ABRE2, RY-element,P-box, and TGA-box (**Figure 4.10.G.).**It has been demonstrated that the response to methyl-jasmonate (MeJA) is mediated by two motifs (CGTCA and TGACG), but the response to gibberellic acid is mediated by three motifs (P-box, GARE-motif, and TATC-box). Ethylene activates the anti-oxidative defense


A
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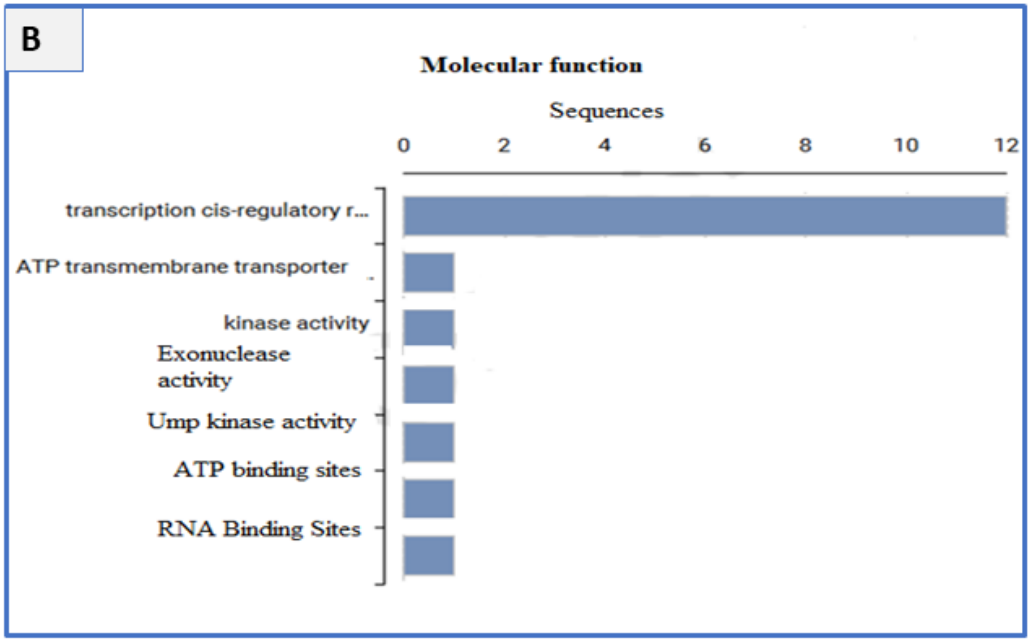
B
Molecular function
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ATP transmembrane transporter
kinase activity
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Ump kinase activity
ATP binding sites
RNA Binding Sites

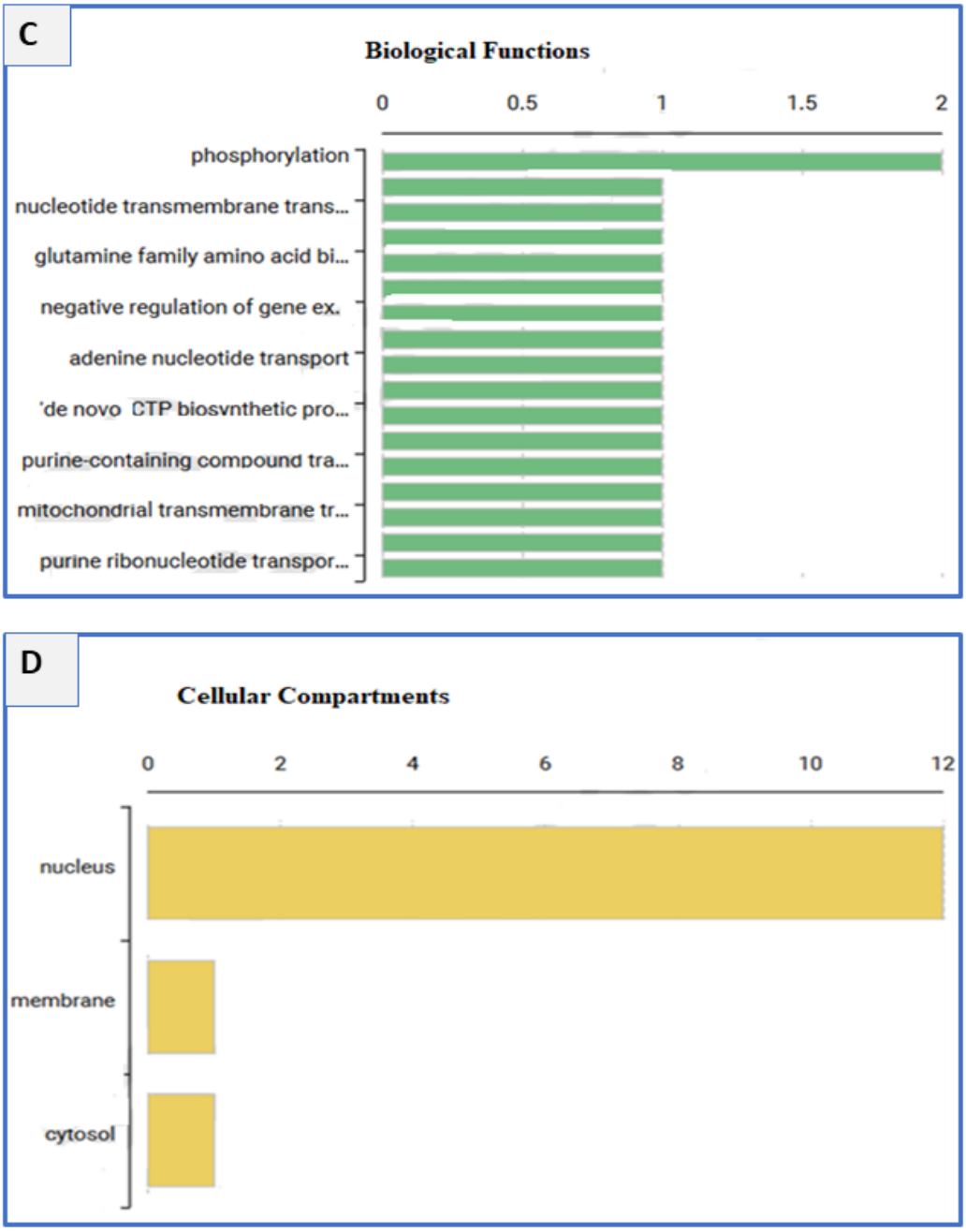

Figure 4.7. *Blast go software were used for analyzing the Gene ontology of StTrihelix genes. 4.7 (A) showing the Total Blast, (B) showing the Molecular functions, (C) showing the Biological Functions and (D) representing Cellular Compartment.*

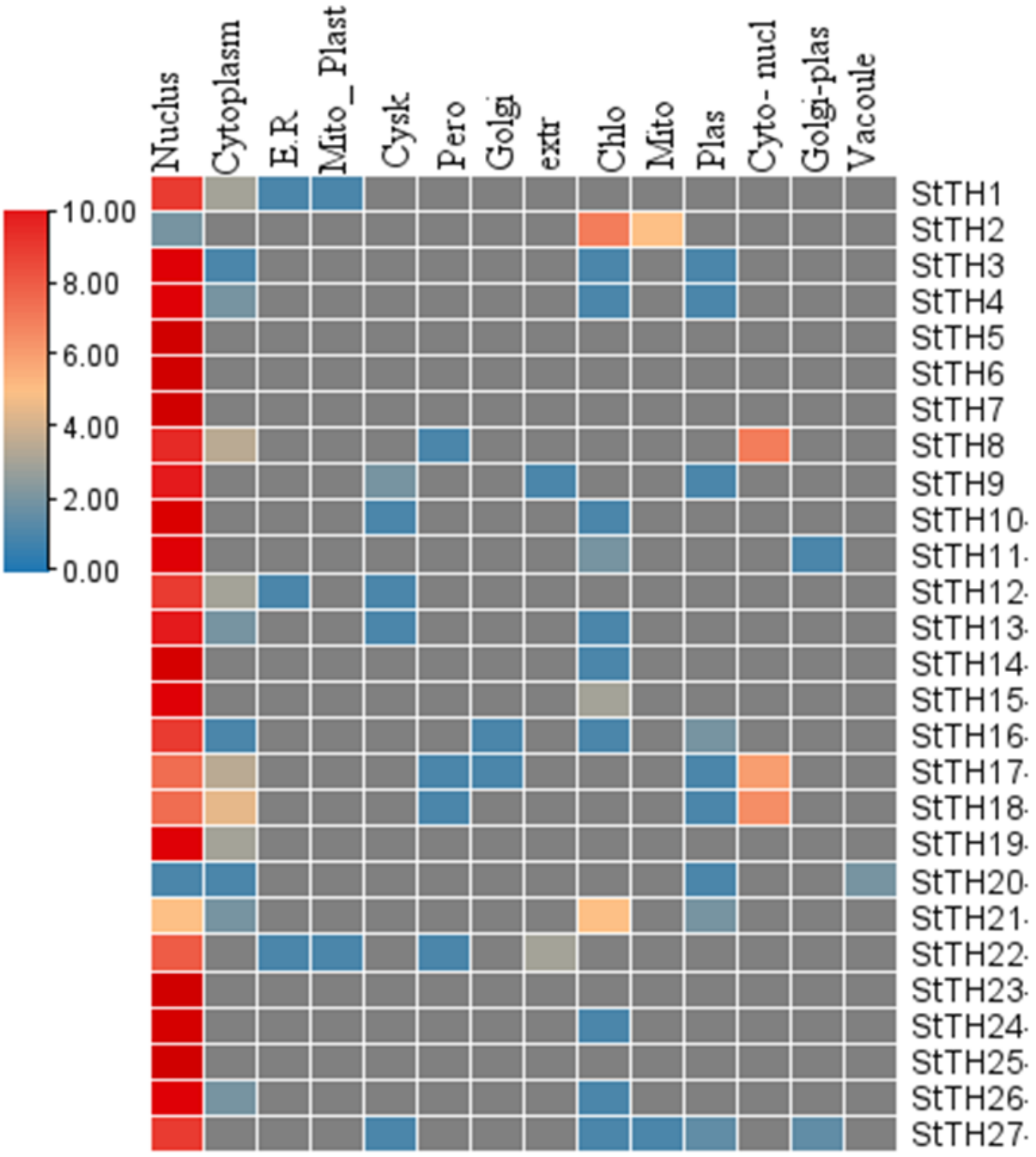

Figure 4.8. *Illustration of cellular localization prediction of 27 StTrihelix proteins.*

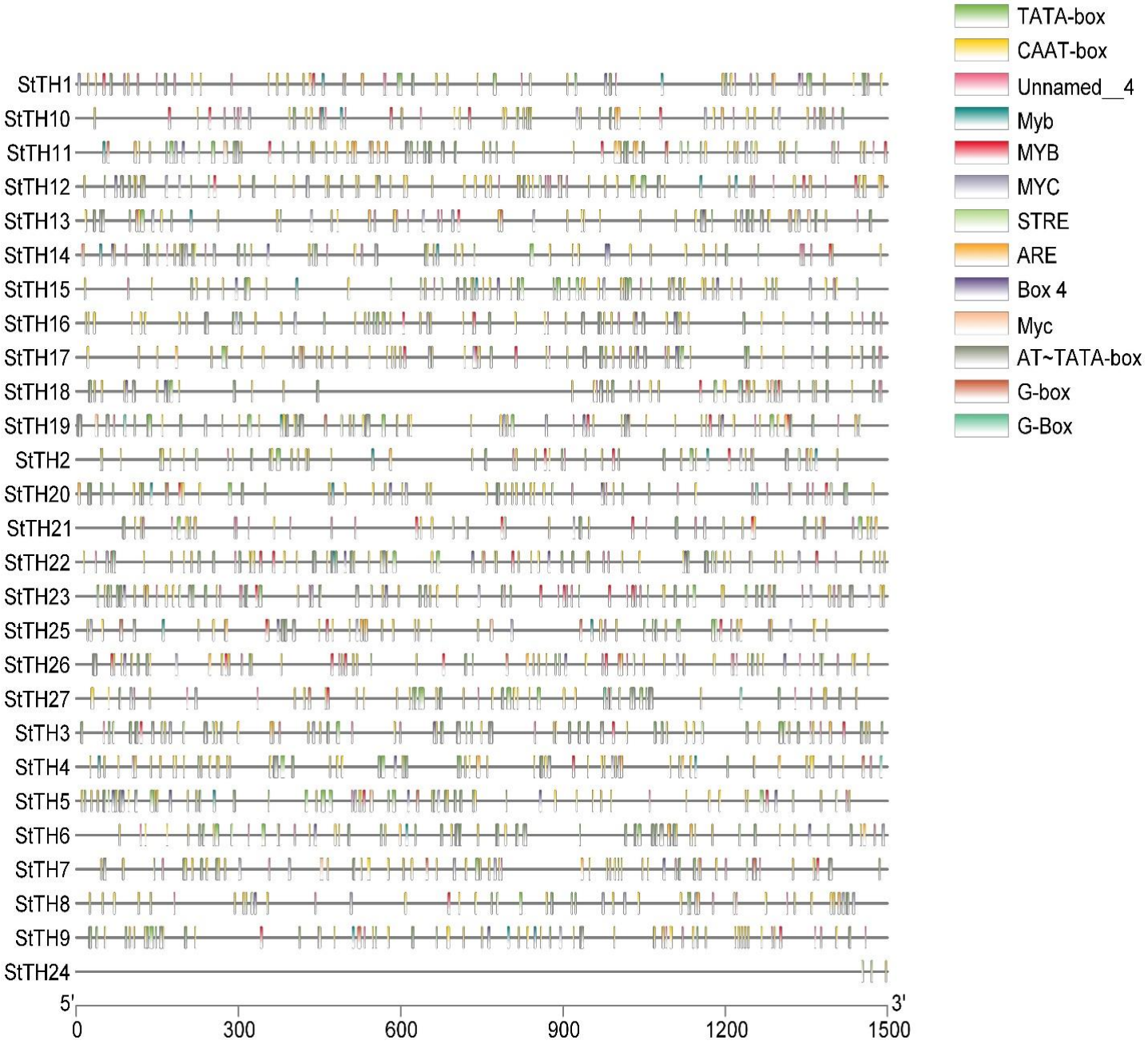

Figure 4.9. *Cis regulatory elements (CREs) analysis in 27 StTrihelix sequences. Each bar represents specific CRE which is designated with specific color.*

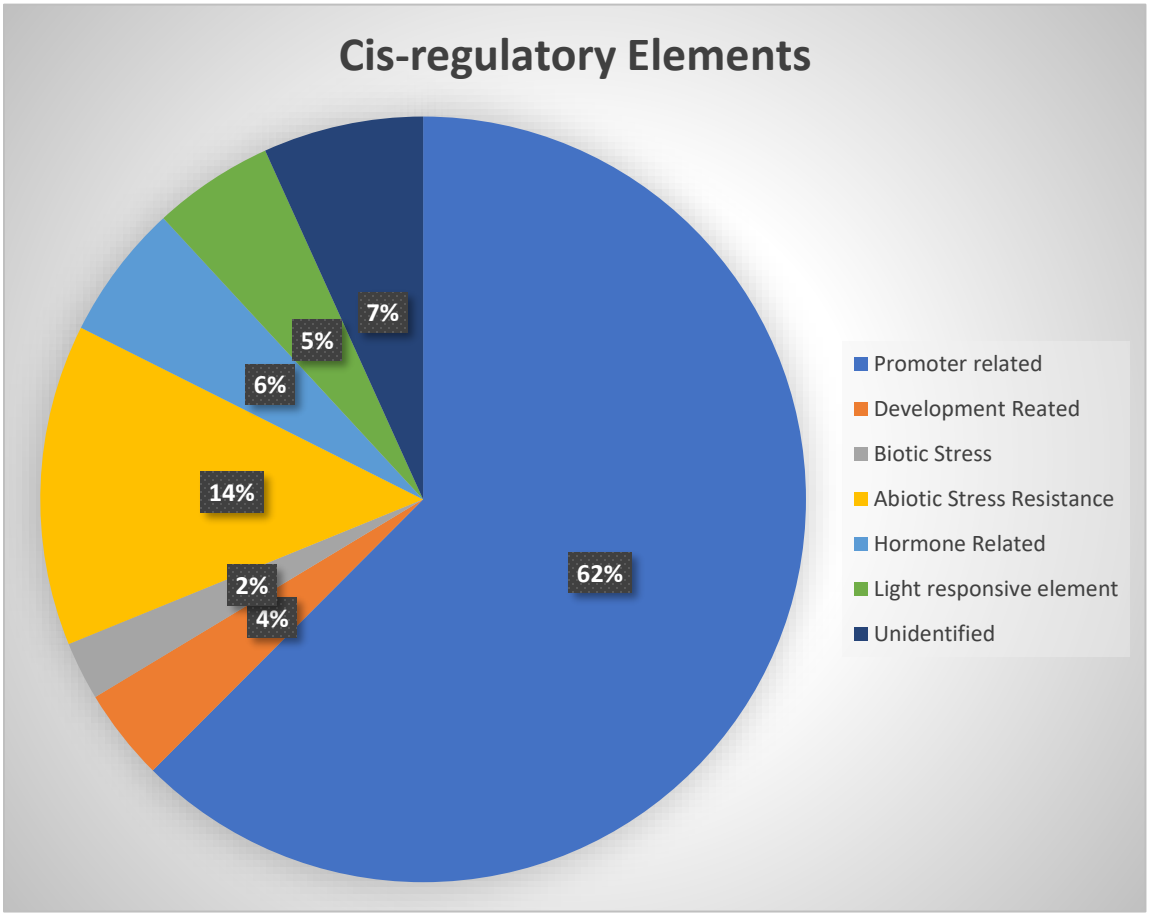

Figure 4.10. *Cis-regulatory element representation on pie chart of seven categories elements. Pie chart also representing percentage of Cis element.*

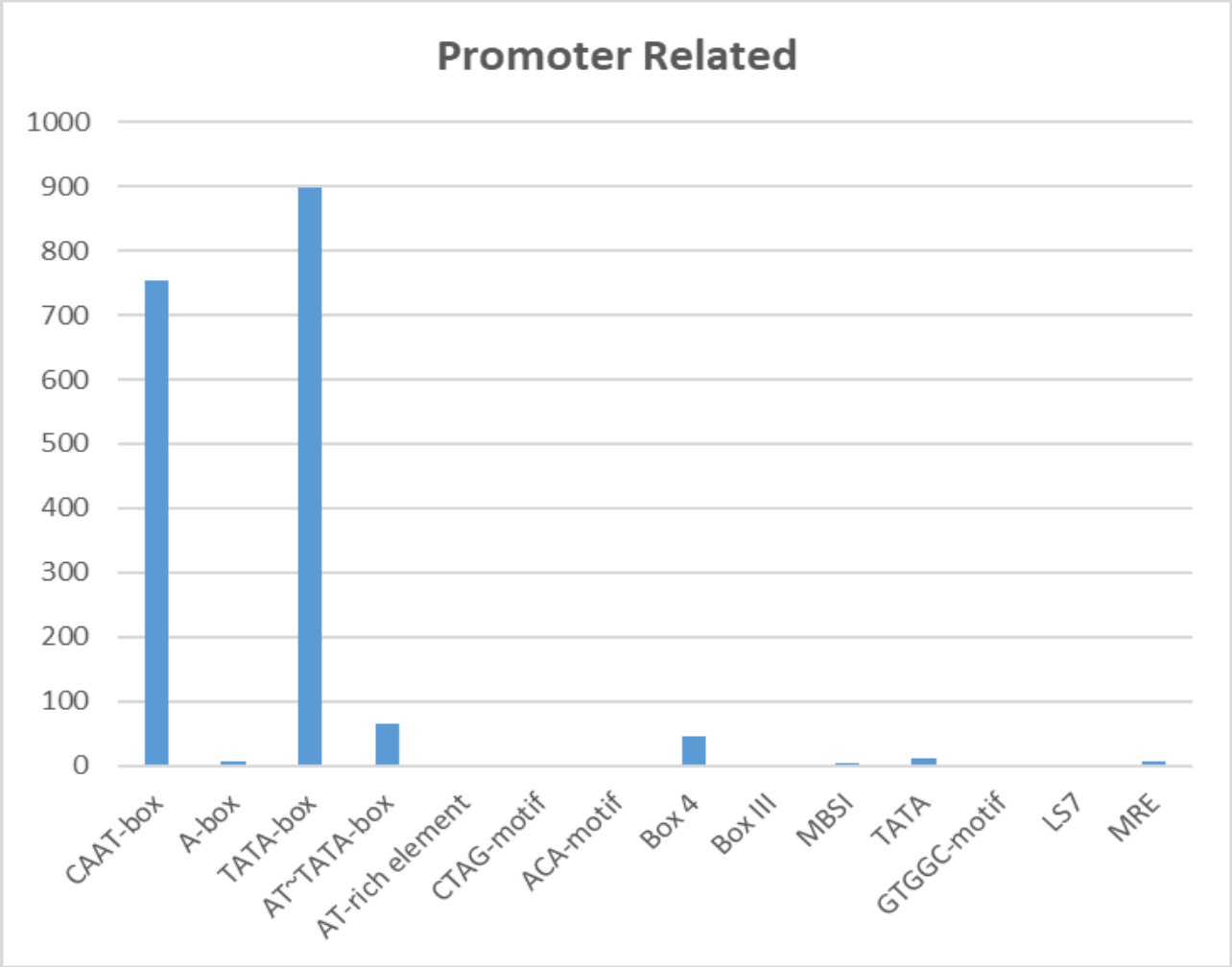

Figure 4. 10. A. *Promotor related cis regulatory elements*

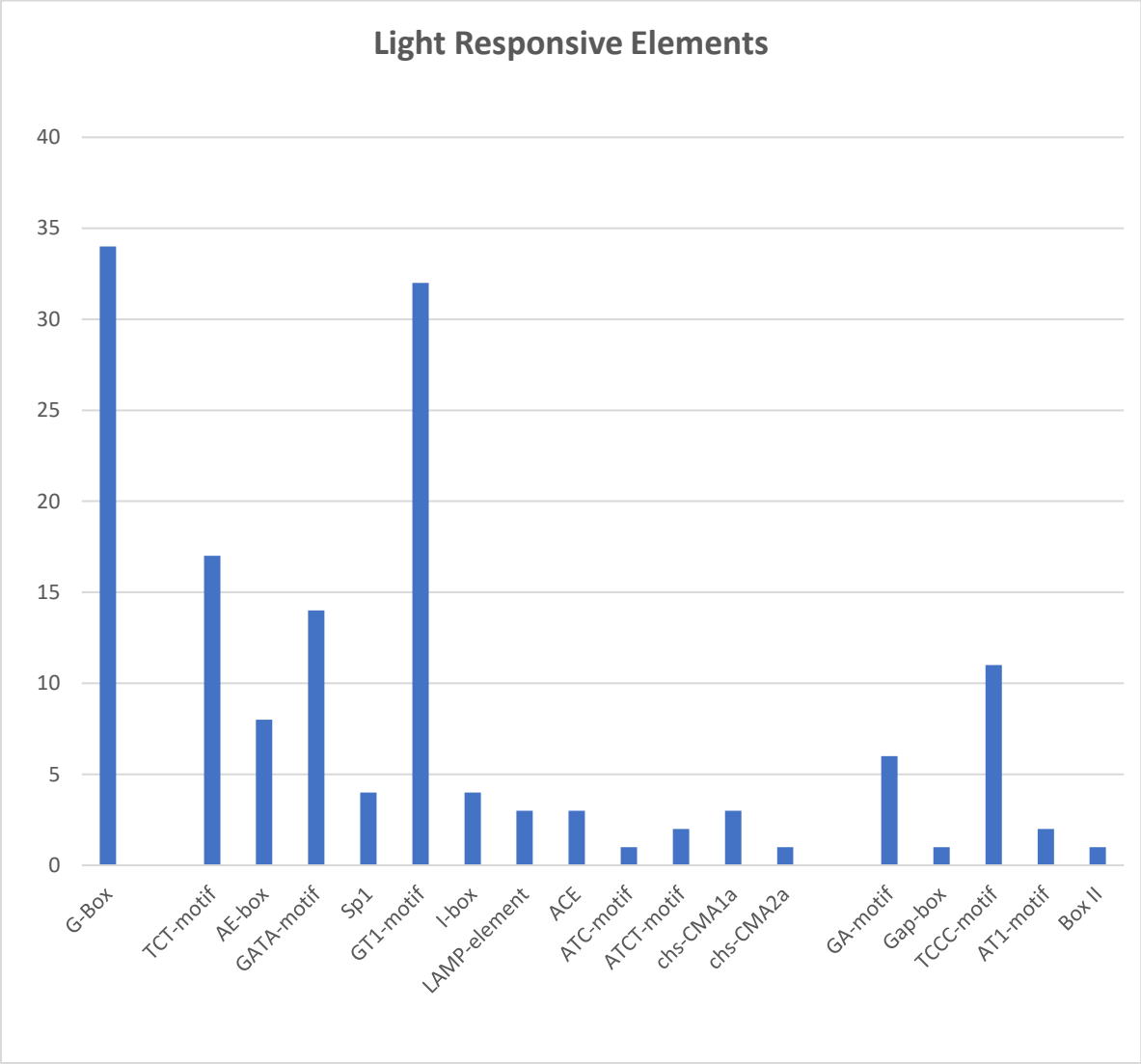

Figure 4. 10. B. *Light stress responsive cis elements.*

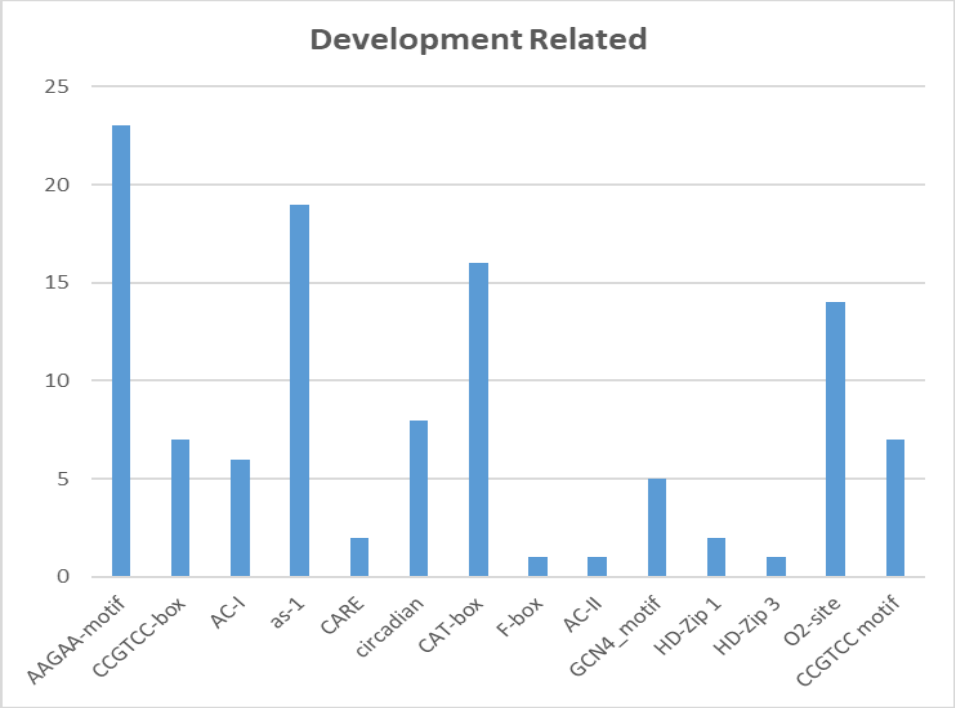

Figure 4. 10.C. *Development related cis regulatory elements.*

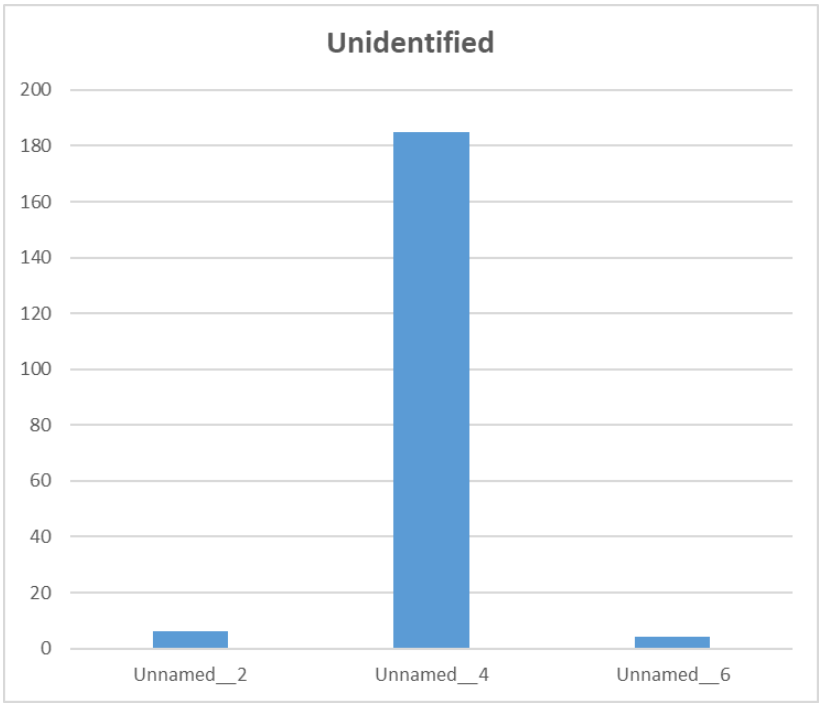

Figure 4. 10.D. *UnIdentified cis regulatory elements.*

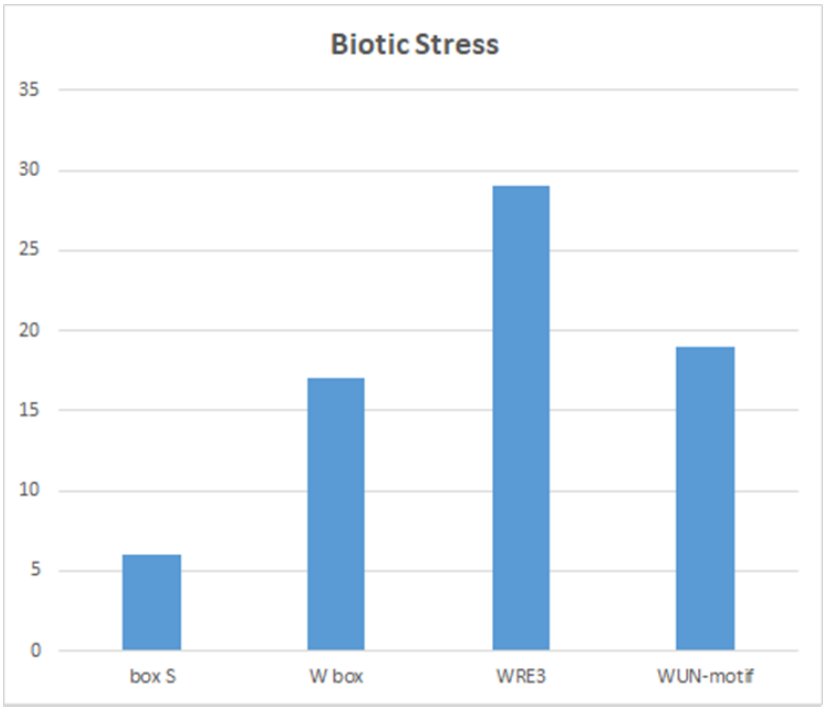

Fig.4.10. E. *Biotic stress responsive cis element.*

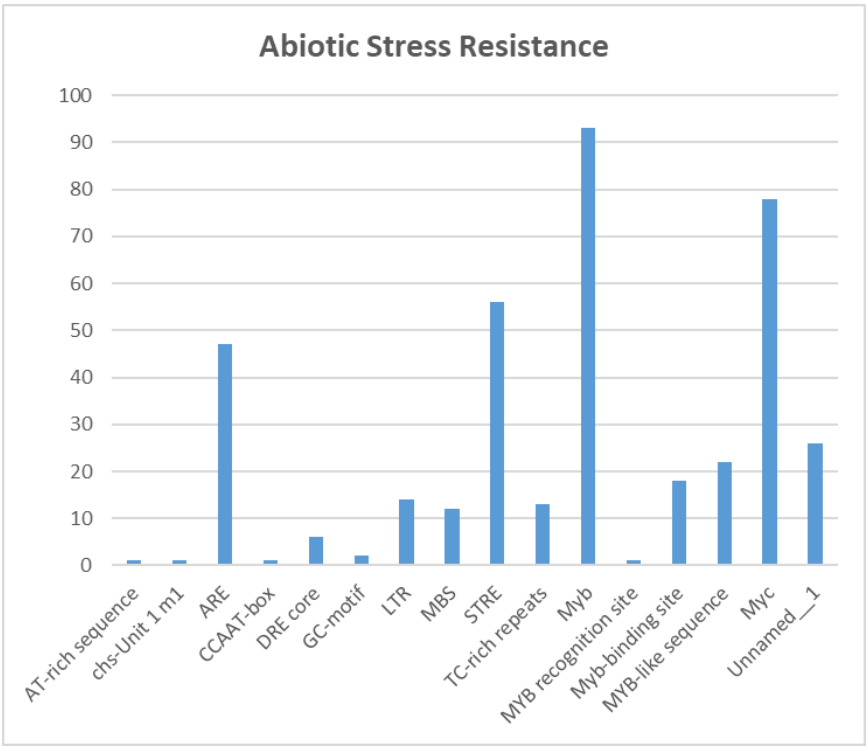

Figure 4.10.f. *Cis regulatory elements that are responsive to abiotic stress.*

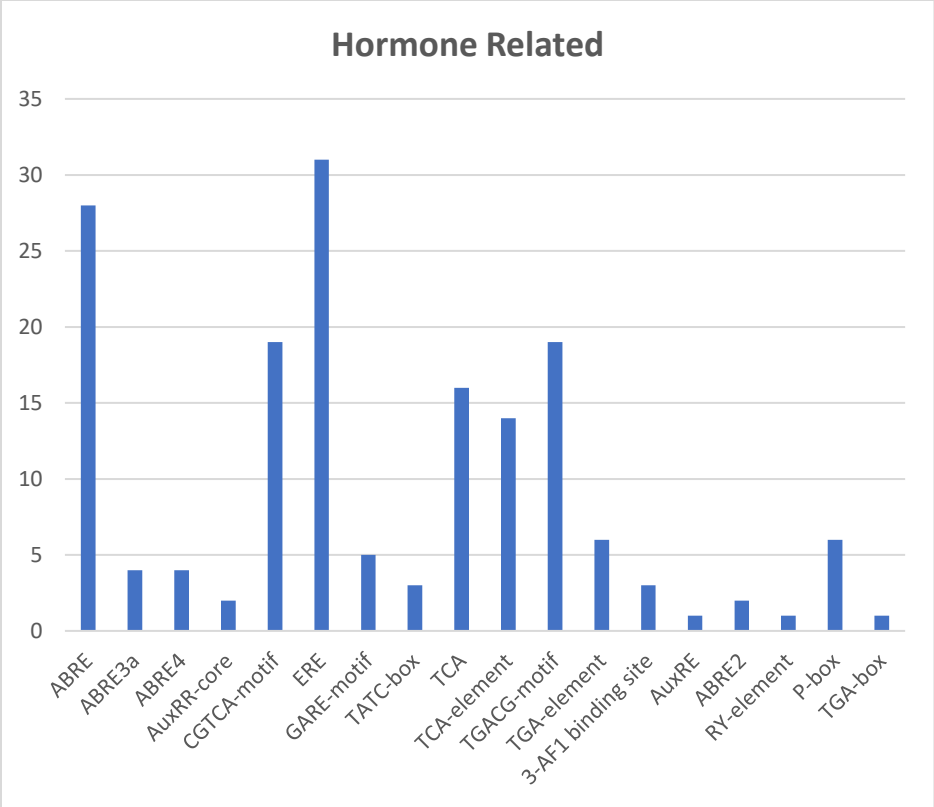

Figure 4.10.G. *Hormone related cis elements.*

mechanism in plants, which lowers oxidative stress and improves plant growth and photosynthetic efficiency (Iqbal et al., 2017). A powerful antioxidant, salicylic acid (SA) plays crucial functional roles in abiotic stressors as freezing stress, salt, and drought (Lefevere et al., 2020).

4.8. Patterns of Trihelix gene expression in different tissues

Two Trihelix (*StTH*25, *StTH22)* genes were selected randomly from 27StTH Trihelix for analyzing the expression level of trihelix genes under Drought stress. Their relative expression patterns under drought by RT-PCR in leaves, root and shoot was analyzed. Our results showed that both of these genes show high expression in leaves as compare to root and shoot under drought stress.

4.9. Relative gene expression of *StTH 25* under drought

Expression analysis of StTH 25 genes showed up regulations in root stem and leaves in treated group. It has shown high expression at 14 days with relative fold value 3.3, while expression become downregulated in 17 day with relative fold value 2.2 when plants regularly watered after 14 days of stress. In leaves its showed high expression as compare to control group, while after three days rewatering its expressions becomes low but still expressions was more as compared to control group (**figure 4.11).** In root and shoot tissue it showed high expression at 14 days with relative fold value 1.8, 2.1, but after rewatering its expression downregulated as compared to control group and their relative values given as 0.26, 0.16 (**Figure 4.12, 4.13)**.

4.10. Relative gene expression of *StTH 22* under drought

Real time PCR analysis of StTH 22 trihelix were also upregulated under drought conditions. This gene was highly regulated at 14 days with leaves as compare to shoot and root **(figure 4.14)**. The relative fold change value of leaves, shoot and root for 14day stress plants were 4.1, 1.9 ,1.8. Our results also showed that this gene is upregulated at 14 days in root stem and leaves, while in leaves after rewatering it also show low expression but in root and shoot its show downregulations as compared to control group **(figure 4.15, 4.16)**. The relative fold change value of leaves, shoot and root for rewatering plants were 2.5, 0.65 and 0.26.

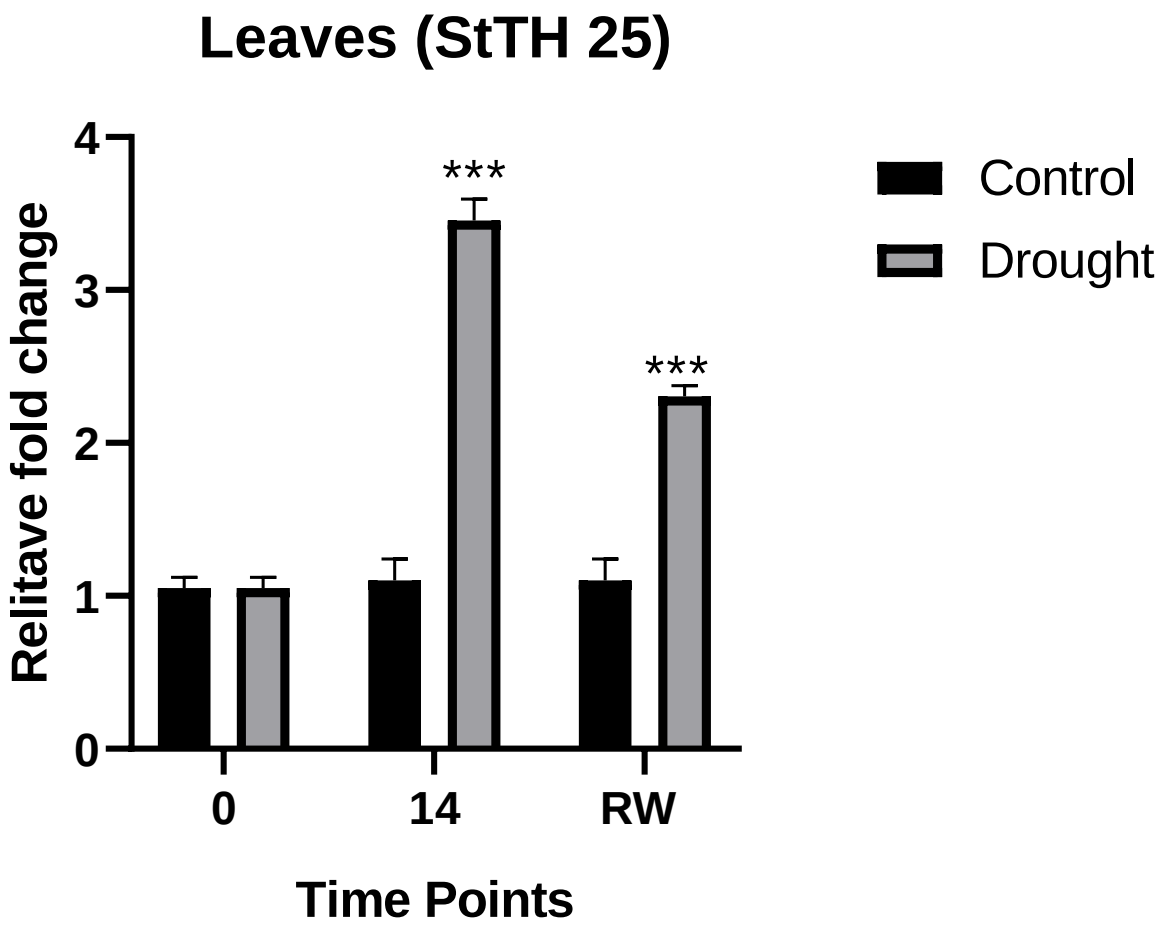

Figure 4.11. *Expression analysis of StTH 25 Trihelix genes in leaves under drought stress*

Expression analysis for each group is given as Zero day shows Control group, 14 days show treated group and 17-day show rewatering. p-value smaller than 0.05 will considered as significant. _*‘is depicting p-value less than 0.05, _**‘p-value less than 0.01, _***‘p-value less than 0.001.

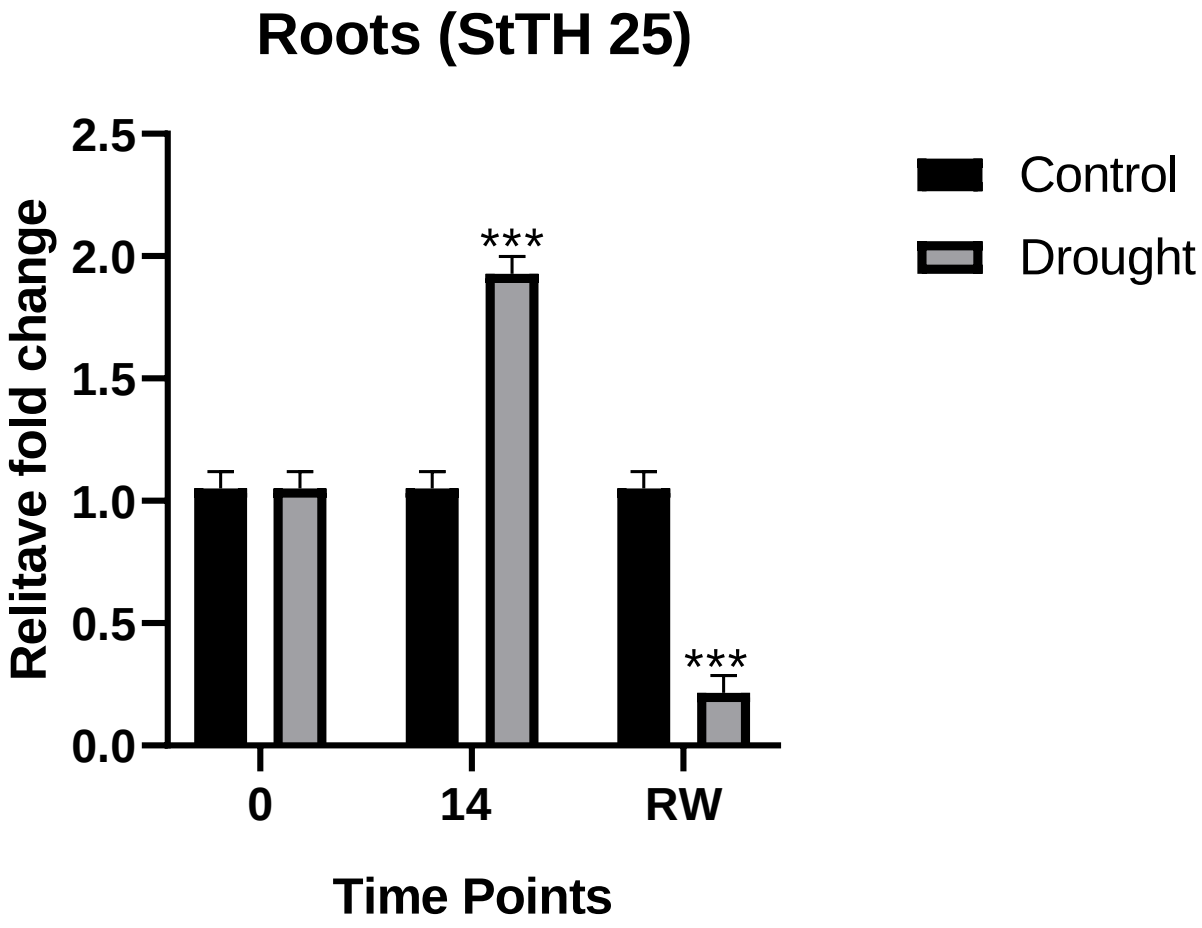

Figure 4.12. *Expression analysis of StTH 25 Trihelix genes in Root under drought stress.*

Expression analysis for each group is given as Zero day shows Control group, 14 days show treated group and 17-day show rewatering. p-value smaller than 0.05 will considered as significant. _*‘is depicting p-value less than 0.05, _**‘p-value less than 0.01, _***‘p-value less than 0.001.

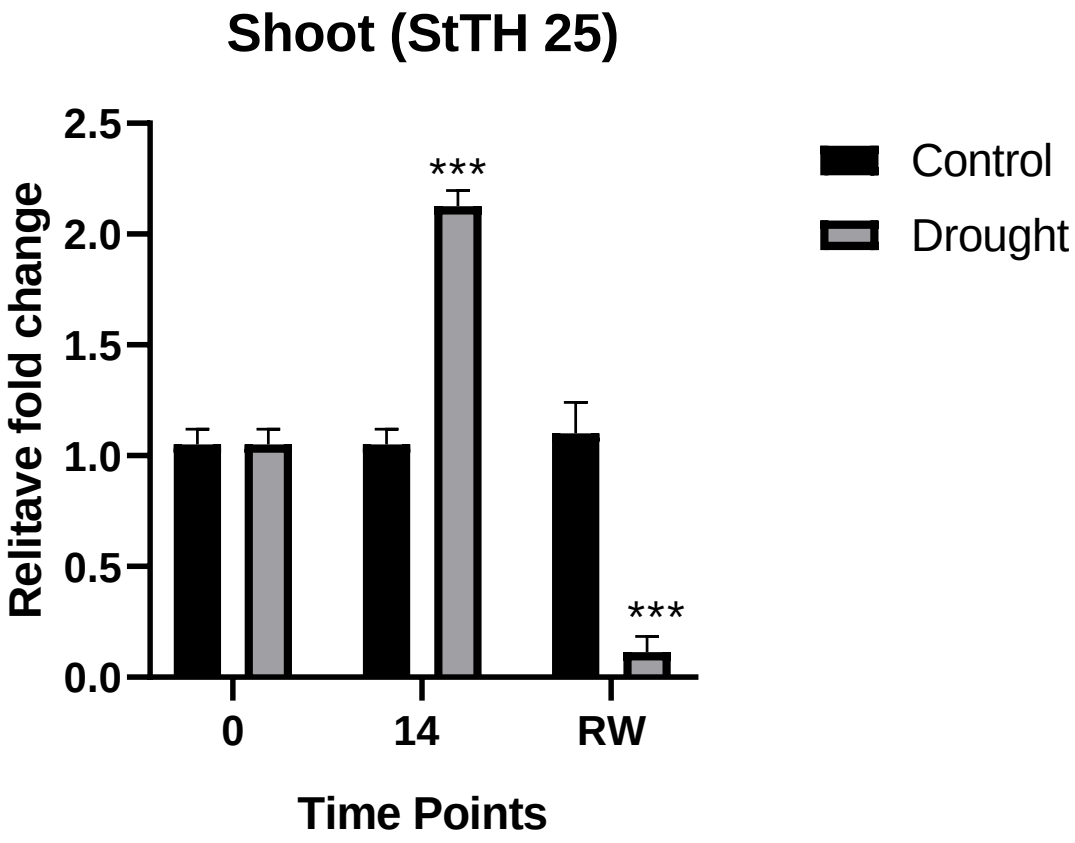

Figure 4.13. *Expression analysis of StTH 25 Trihelix genes in shoot under drought stress.*

Expression analysis for each group is given as Zero day shows Control group, 14 days show treated group and 17-day show rewatering. p-value smaller than 0.05 will considered as significant. _*‘is depicting p-value less than 0.05, _**‘p-value less than 0.01, _***‘p-value less than 0.001.

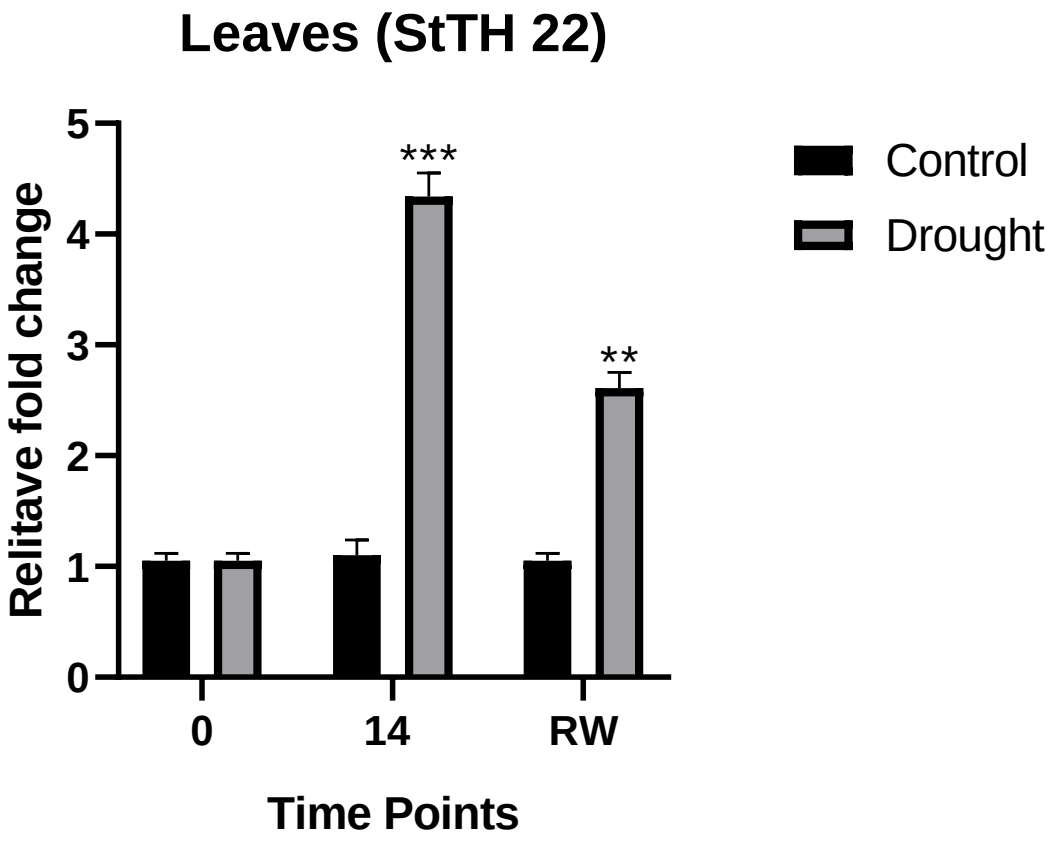

Figure 4.14*. Expression analysis of StTH 22 Trihelix genes in leaves under drought stress.*

Expression analysis for each group is given as Zero day shows Control group, 14 days show treated group and 17-day show rewatering. p-value smaller than 0.05 will considered as significant. _*‘is depicting p-value less than 0.05, _**‘p-value less than 0.01, _***‘p-value less than 0.001.

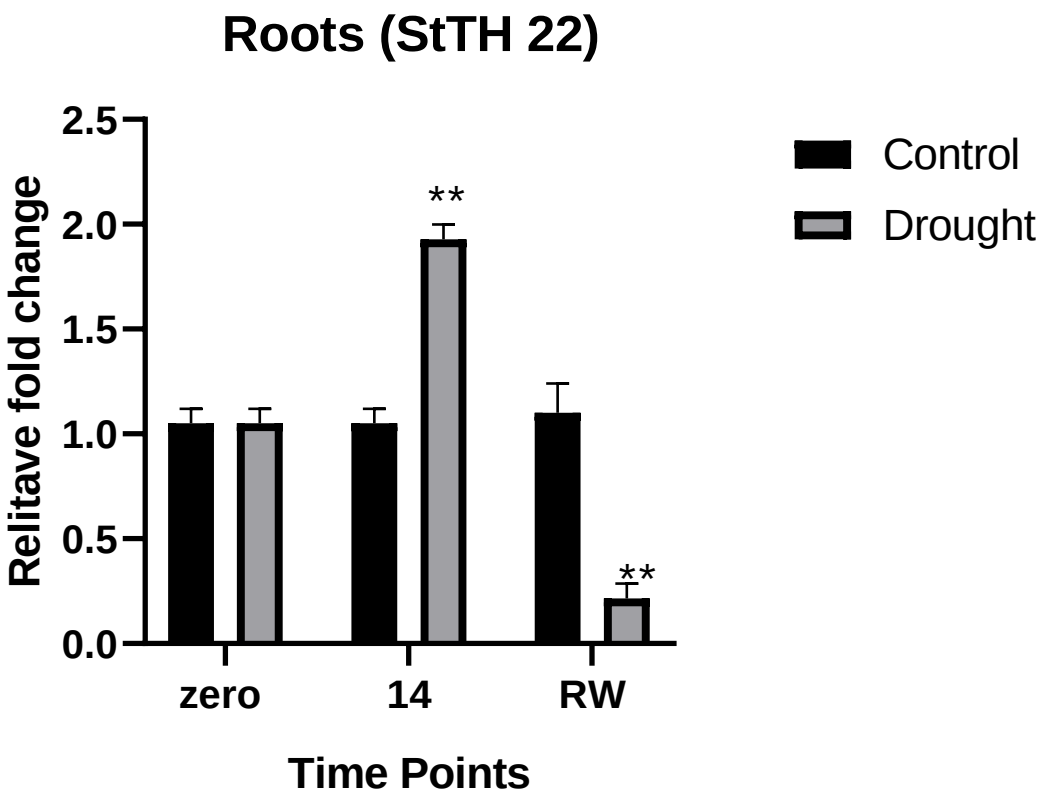

Figure 4.15. *Expression analysis of StTH 22 Trihelix genes in root under drought stress.*

Expression analysis for each group is given as Zero day shows Control group, 14 days show treated group and 17-day show rewatering. p-value smaller than 0.05 will considered as significant. _*‘is depicting p-value less than 0.05, _**‘p-value less than 0.01, _***‘p-value less than 0.001.

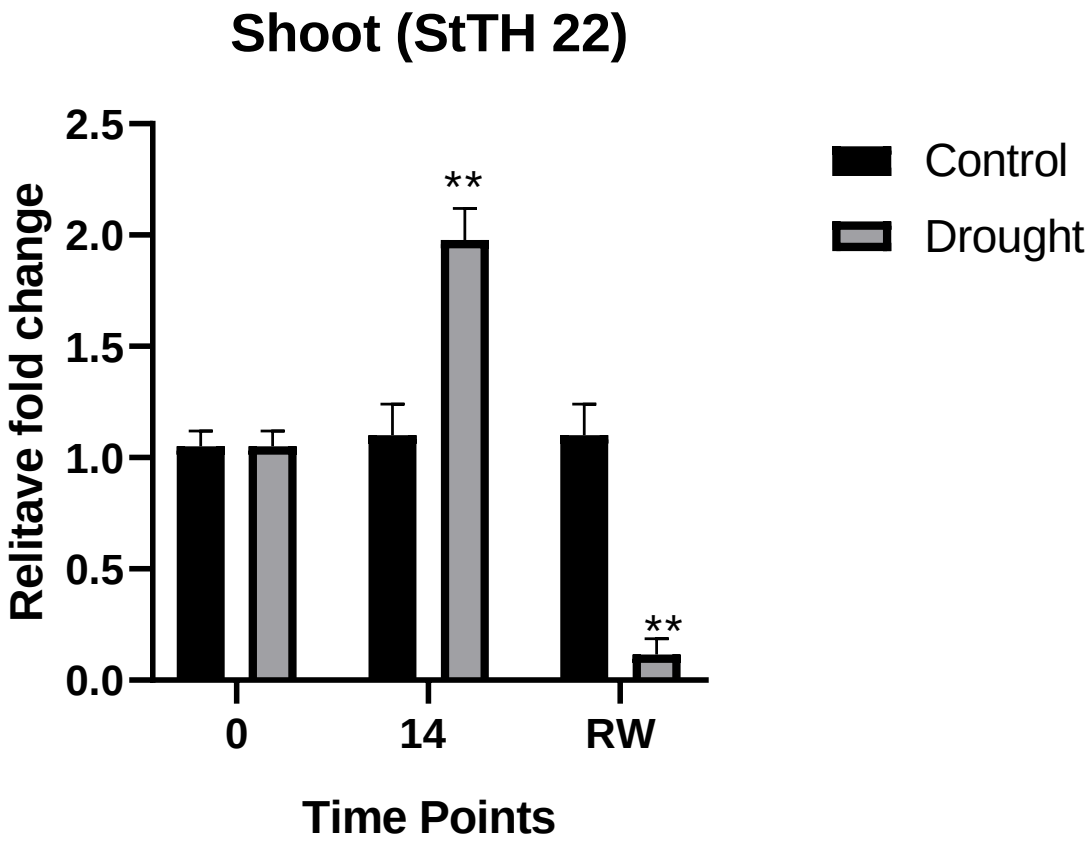

Figure 4.16*. Expression analysis of StTH 22 Trihelix genes in root under drought stress.*

Expression analysis for each group is given as Zero day shows Control group, 14 days show treated group and 17-day show rewatering. p-value smaller than 0.05 will considered as significant. _*‘is depicting p-value less than 0.05, _**‘p-value less than 0.01, _***‘p-value less than 0.001.

Chapter 5

Discussion

More than 100 nations rely on potatoes as one of the most significant crops in the world. The potato plant is susceptible to a number of abiotic stresses, and recurring stress outbreaks endanger its continued growth. Members of the trihelix family perform critical roles in a variety of growth and development processes affecting flowers, stomata, trichomes, embryos, and seeds, as well as functions in response to abiotic and biotic environmental stressors. The trihelix family is often referred to the GT factors due to its shared DNA binding domain, which consists of three tandem helices (helix-loop-helix-loop-helix) (Levy et al., 2012). The Trihelix genes have been discovered in a variety of plants due to the availability of the whole genome sequence. Untill now Trihelix gene family is not discovered in potato. Therefore, the current work provided comprehensive information on the Trihelix genes' genome-wide characterization in *Solanum tuberosum.* Using an *Arabidopsis* Trihelix gene as a query sequence, 27 putative Trihelix genes in *S. tuberosum* were identified and classified into five subgroups that corresponded to A. thaliana Trihelix orthologs.

The genome-wide analysis demonstrated the difference between *S. tuberosum and A. thaliana* in terms of the number of genes and genome size. Calculating the physiochemical properties of plant protein families is important to understand the probable roles of proteins. In previous studies more Trihelix genes were reported in *Chenopodium quinoa* (47) (Li et al., 2022), *Sorghum* (40) (K. Li et al., 2022) *Brassica* (455) (Xiao et al., 2019) *Oryza sativa* (41) (Li et al., 2019) *S. Lycopersicum* (36) (Yu et al., 2015) *Populus trichocarpa* (56) (Wang et al., 2016) wheat (80) and 27 B. distachyon (Wang et al., 2019). Calculating the physiochemical properties of plant protein families is necessary in order to understand the probable roles of proteins.

The Trihelix proteins in potatoes displayed a variety of physiochemical properties, indicating that these genes may be involved in a variety of aspects of plant development, including light response, growth developmental processes, and abiotic stress responses in plants. The Trihelix protein length from 253 to 628 amino acids residues, their molecular mass ranges from 74 to 69388.46 Dalton and their isoelectric point ranges from 4.69 to 9.78 (**Table 2).** Furthermore, the five previously discovered *Arabidopsis* subgroups (GT-1, GT-2, SH4, SIP1, GT-Y) were

revealed through phylogenetic analysis of Trihelix proteins from *S. tuberosum* and *A. thaliana* (Yasmeen et al., 2016). The subgroups GT-1 has only three members it is the smallest subgroups while SIP1 subgroups has 11 member it is largest subgroups, GT-2 and GT-Y both has a 4 member and SH4 has 5 members **(Figure 4.0).** The gene structures of the StTrihelix genes were examined to find out more about their structural diversity. Gene structure analysis show that one exon is present in 11 StTrihelix genes (1, 10,12,14,19,2,21,3,4,6,26) These 11 Trihelix genes has no introns these only contain exons.),While 9 StTrihelix genes contain two exons and one intron (11,16,17,18,20,5,7,24,25), While StTrihelix 27 genes contain two exons and one upstream regions, other three have more than two introns and exons like three exons and two introns are present in StTrihelix 22, StTrihelix 9 contain the six exons and five introns and StTrihelix 23 contain 7- exons and 6 introns **(figure 4.2).** According to several studies, having a compact gene structure with few or no introns improved plants' ability to respond quickly to a variety of abiotic stimuli (Jeffares et al., 2008). StTrihelix gene structural variation reveals functional diversity that may have been brought on by environmental or evolutionary alterations in the plant genome profile.27 Trihelix genes were revealed to be unevenly distributed across 12 potato chromosomes based on chromosomal positions.

It has been proposed that the asymmetrical arrangement of genes contains clues about their evolutionary history. Gene duplication events that occur as a result of adaptive evolution are the fundamental cause of plant diversity and the formation of adaptive specialization in genes (Flagel & Wendel, 2009). Polyploidization or whole genome duplications (WGD) are thought to be the main drivers of evolution that give rise to novel characteristics and new transcriptional regulatory sites that can change expression patterns (Panchy et al., 2016). Tandem duplications resulted in two homologous pairings, whereas segmental duplications resulted in 11 orthologous pairs (**Figure 4.3)**. Gene pair showing Tandem duplication were present on same chromosome. The most recent tandem duplication events for two SH4 subgroup genes were thought to have occurred one million years ago. Tandem duplications are also well recognized to contribute significantly to adaptation in the growth and operation of abiotic stress responsive genes. Two pairs of genes from the SH4 subgroup were considered to have undergone tandem duplication most recently around 1 million years ago (**Table 4).** *S. lycopersicum*, *Triticum aestivum*, *Oryza sativa*, these also undergone both segmental and tandem duplications. According to earlier research, tandem repeats frequently share same cis-acting components and possibly have related

purposes (Flagel & Wendel, 2009). The current study suggests that the promoter regions of StTrihelix tandem gene duplication pairs shared comparable regulatory components and functions. To further know the potentially evolutionary process of StTrihelix genes interspecies synteny was done. Due to the tight evolutionary link between *S. tuberosum* and *S. lycopersicum*, there were more StTirhelix gene orthologous occurrences with SlTrihelix genes than with AtTrihelix genes (**Figure 4.6 A, B).** 3103 cis-regulatory elements (CREs) were found and classified into seven functional kinds in this study. The cis regulatory element revealed the participation of several components in promotor binding sites, light responsive elements, hormonal controls, and developmental concerns **(Figure 4.10).**

The occurrence of Promotor related cis element were significantly high followed by abiotic stress resistance, hormone related, light responsive element and unidentified, respectively. TATA-box, CAAT-box, AT-TATA box, A-box, and TATA components involved in promotor binding site were examined in StTrihelix proteins**.** Light responsive were G-Box, CT-motif-box, GATA-motif, Sp1, GT1-motif I-box, LAMP-element, ACE, ATC-motif, ATCT-motif, chs-CMA1a,chs-CMA2a,GA-motif,Gap-box,TCCC-motif,Pc-CMA2c,C-box,chs-CMA2b,sbp-CMA1c,AAAC-motif,AT1-motif,GRA,L-box,and Box II,**.** The transcription of light-controlled genes was regulated by the Box4 and G-box motifs**.** In development related cis elements, AAGAA-motif CCGTCC-box, AP-1, AC-I, as-1, CARE, circadian, CAT-box, dOCT, F-box, E2Fb, MSA-like, AC-II, GCN4_motif, HD-Zip 1,HD-Zip 3,O2-site,re2f-1,CCGTCC motif, CT. Pathogenesis-related promoters contain the common as-1 element and an AGAA-motif. The CCGTCC-box, the CARE box, and the CAT-box work as cis elements in the stimulation of meristem-specific genes, the activation of xylem-specific AC-1 and AC-II, the differentiation of palisade mesophyll cells, the formation of blood vessels, and the control of macromolecule processes. The E2Fb and F-box regulate the cell cycle, the RY element aids in seed-specific regulation, the AACA-motif and GCN4-motif are crucial for endosperm expression, the circadian rhythm regulates circadian rhythms, the O2 site regulates zein metabolism, and the MSA-like is a mitosis-specific activator as given in **(Figure 4.10).** Unnamed__2 Unnamed__4 Unnamed__6 are considered the pattern with unidentified functions **(Figure 4.10.D).** Biotic stress related cis elements were box S,W box,WRE3,WUN-motif and their concentration was only 2% of total cis elements **(Figure 4.10.E)**. Abiotic stress sensitive cis elements contained the motifs AT-rich sequence, ARE, CCAAT-box, DRE core, DRE 1, GC-motif, LTR, MBS, MBS 1,

STRE, TC rich repetitions, MYB, MYC, MYB recognition site, MYB binding site, MYB like site, and AT-rich Element (**Figure 4.10.F.).** MYB and MYC are dehydration sensitive motifs that are mainly found in StTrihelix gene promoters, whereas MBS and MBS1 are MYB that act as binding sites that are implicated in drought inducibility and gene regulators of flavonoid biosynthesis. When elicitors are detected, the AT-rich region and AT-rich sequence act as a binding site for AT-rich binding proteins and a promoter of maximum activation. Hormone-related cis regulatory elements identified in StTrihelix include ABRE, ABRE3a, ABRE4, AuxRR-core, CGTCA-motif, ERE, GARE-motif, TATC-box, TCA, TCA-element, TGA-element, 3-AF1, binding site, AuxRE, ABRE2, RY-element-box, and TGA-box. (**Figure 4.10.G.).**

These elements were also reported in *S. lycopersicum*. In response to different stresses, these hormone-related components may interact with signaling pathways directly or indirectly. Parallel investigations identified conserved motifs that may have a significant role in a variety of biological processes. These motifs were discovered in the StTrihelix genes' promotor region, showing that they controlled and regulated transcription to control potato growth and development. Gene ontology annotation was used to examine the molecular functions and functional genomics of each StTrihelix gene. Go annotations results showed that StTrihelix genes are localized in cytosol, membrane and nucleus **(figure 4.7).** Subcellular localizations predications confirm that StTrihelix genes present in other 13 organelle as given in heat map (**Figure 4.8).**

Conclusion:

In conclusion characterizations and identifications of Trihelix genes *Solanum tuberosum* were investigated thoroughly in detailed analysis. Bioinformatic tools were used to thoroughly evaluate phylogenetic analysis, gene structures, conserved motifs, chromosomal localization, gene duplication events, synteny analysis for the detection of orthologs in interspecies, gene ontology, protein modelling, prediction of pocket binding sites, disorder region analysis, and interactive networks with other proteins. Total 27 *StTH* protein having a conserved tryptophan have identified and these are unevenly present on 12 chromosomes.

Based on sequence alignment and phylogenetic analysis, StTH genes were classified into five subgroups (GT-1, GT-2, SH4, GT-Y, SIP1) which aligned to previous Arabidopsis studies. Motif patterns and Gene structures showed that variations among these structures were present in all subgroups. Tandem and segmental duplications aided the expansion of the Trihelix gene family in Potato. StTrihelix were subjected to significant purifying selection during plant evolution, according to evolutionary divergence analyses (Ka/Ks). According to a synteny relationship analysis, 10 and 19 StTH genes are orthologous to Arabidopsis and S. lycopersicum, respectively. In total, 3103 cis-regulatory promoter elements were classified into seven functional classes.

The data acquired revealed a number of motifs that were determined to be involved in a number of activities, including biotic stress responses, hormone induction, light regulation, cell development, and abiotic stress responses (drought and low temperature). The StTrihelix genes were mostly identified in the nucleus, but they were also discovered in 13 other organelles, according to subcellular localization. According to gene ontology (GO) annotation, the bulk of the StTrihelix genes demonstrated DNA binding activities, implying that they function as substantial transcriptional activators. According to functional enrichment, St Trihelix genes have critical roles in cell development, immunological responses, and hormone signaling. Gene expressions results showed that the Trihelix genes (StTH22, StTH25) showed high expression in leaves as comparable to shoot and root. The findings will be used to begin understanding molecular processes and further functional characterization of the StTrihelix gene family, hence providing resources for plant breeding and genetic engineering.

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Abstract

Abstract

The Trihelix transcription factors are referred as a GT element because of their binding capacity with GT element. Trihelix transcription factors influence how plants grow and develop, as well as how they respond to abiotic stresses. Although Trihelix transcriptome factors has described and investigated in numerous plants, there is limited available knowledge regarding their presence in Solanum tuberosum. The current investigation involved the characterization of trihelix genes in *Solanum tuberosum*. Total of 27 StTH genes have been identified in Solanum tuberosum. All of these 27 StTH were divided into five subgroups as previously described in *Arabidopsis thaliana* (GT-2, GT-1, SH4, GT-γ and SIP1). The genomic positioning and occurrences of gene duplication revealed the arbitrary dispersion of StTH genes across 12 chromosomes, with duplication events happening both in tandem and segmental patterns, involving 11 pairs of genes. An analysis of synteny unveiled 19 orthologs of StTH genes within Solanum tuberosum and 16 orthologs within *Arabidopsis*. Through motifmer grammer analysis, diverse arrays of conservation and functional variations were disseminated, with light-responsive and hormone-responsive elements, along with development-linked, abiotic stress-responsive, and promoter-associated elements, prevailing in St Trihelix genes. In various biological and molecular pathways, gene ontology unveiled the involvement of StTrihelix proteins, including defense response, regulation of transcription, nucleotide transmembrane processes, ATP binding, and phosphorylation. Two genes StTH 22 and StTH 25 were checked for drought stress. Both of these gene's show expression in stem, root and leaves. Hence the stem expression was analyzed in leaves. This is the first study to look into how potato trihelix genes respond to drought stress. The insights from our findings will be valuable for forthcoming research on the comparative and molecular mechanisms of Trihelix genes in different plant species. To sum up, our observations indicate the potential significance of StTH22 and StTH25 in contributing to essential roles under drought stress conditions in potatoes.

Keywords: Trihelix, Solanum tuberosum, Gene ontology, MYB/SANT, Gene duplication and Cis-elements.

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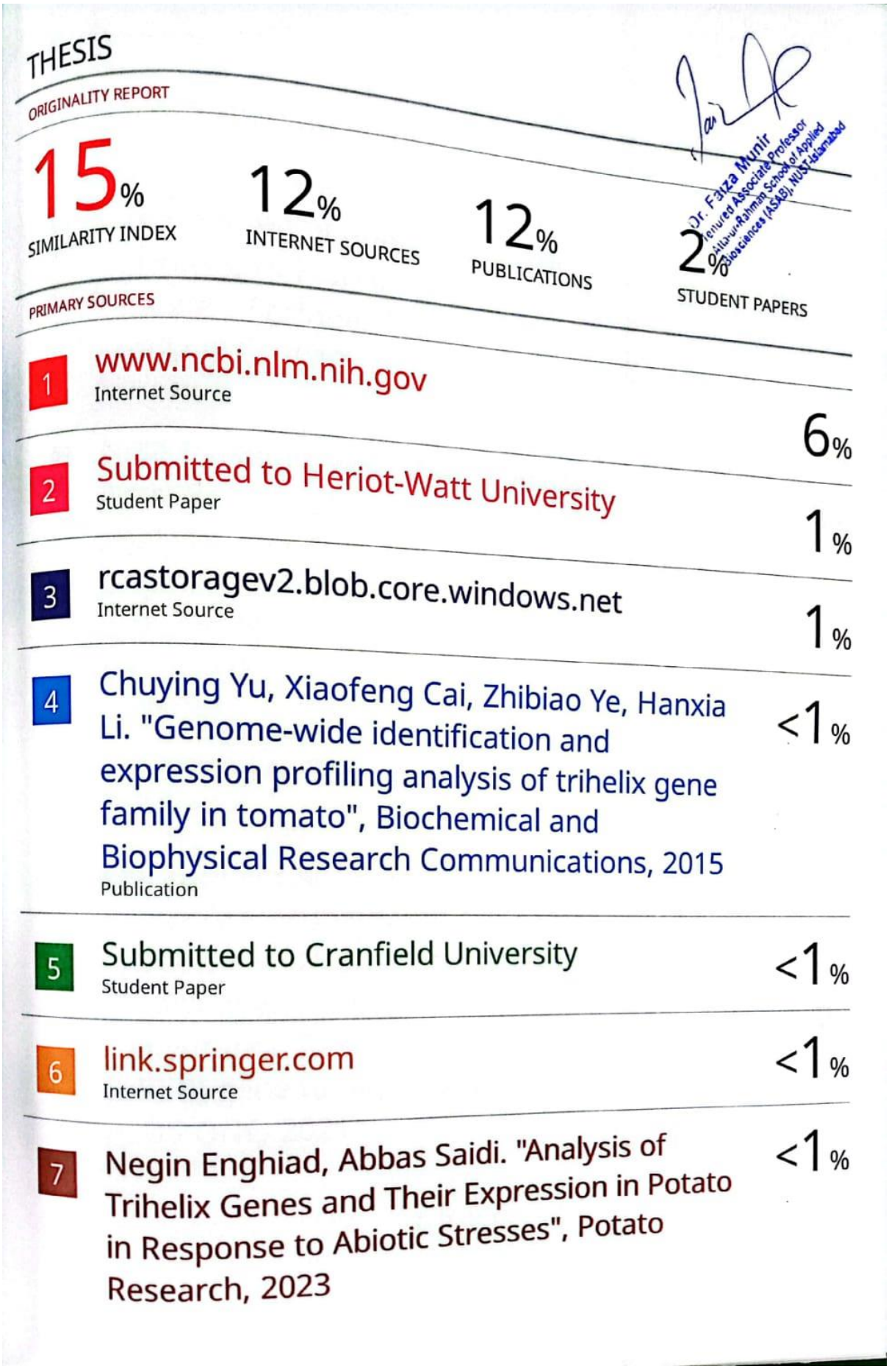

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