Characterization of ACO gene under Salinity Stress in *Triticum aestivum*

By Warda Imtiaz

(Registration no 399944)

Department of Agricultural Sciences and Technology

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

National University of Sciences and Technology (NUST)

Islamabad, Pakistan

2024

Characterization of ACO gene under Salinity Stress in *Triticum aestivum*

By Warda Imtiaz

(Registration no 399944)

Department of Agricultural Sciences and Technology

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

National University of Sciences and Technology (NUST)

Islamabad, Pakistan

20

Thesis acceptance certificate

It is confirmed that the final copy of Warda Imtiaz MS/MPhil thesis (Registration No. 00000399944) from the Atta-Ur-Rahman School of Applied Biosciences, NUST, has been reviewed by the undersigned and found to be complete in accordance with NUST regulations and statutes, free of plagiarism and errors, and acceptable for partial fulfillment toward the awarding of an MS/MPhil degree.

Supervisor: _____

Dr. Alvina Gul
Tenured Associate Professor
Deptt of Plant Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST, Islamabad

Dr. Alvina Gul

Atta-ur-Rahman School of Applied Biosciences

National University of Sciences & Technology

Head of Department: _____

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

Prof. Dr. Muhammad Faraz Bhatti

Atta-ur-Rahman School of Applied Biosciences

National University of Sciences & Technology

Principal: _____

Prof. Dr. Muhammad Asghar
Principal & Dean
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST, Islamabad

Prof. Dr. Muhammad Asghar

Atta-ur-Rahman School of Applied Biosciences

National University of Sciences & Technology

FORM TH-4

National University of Sciences & Technology

MS THESIS WORK

We hereby recommend that the dissertation prepared under our supervision by: Warda Imtiaz

Regn no 399944

Titled: Characterization of ACO gene under Salinity stress in Triticum Aestivum be accepted in partial fulfillment of the requirements for the award of MS Degree in Plant Biotechnology degree with(A grade).

Examination Committee Members

1. Name: Dr. Mustafeez Mujtaba Babar Department: Shifa College of Pharmaceutical Sciences — Signature:

DR. MUSTAFEEZ M. BABAR
Pharm.D, M.Phil, PhD
Associate Professor & Advisor Students Affairs
Shifa College of Pharmaceutical Sciences
Shifa Tameer-e-Millat University
Islamabad

2. Name Dr. Rabia Amir Department: Agricultural Sciences and Technology — Signature:

3. Name: Dr. Faiza Munir Department: Agricultural Sciences and Technology — Signature:

Dr. Alvina Gul
Tenured Associate Professor
Deptt of Plant Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST, Islamabad

Supervisor's name: **Dr. Alvina Gul** Signature:

Date: 5th Sept, 2024

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

5th Sept, 2024
Date

Head of Department

COUNTERSINGED

Date: 5th Sept, 2024

Prof. Dr. Muhammad Asghar
Principal & Dean
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST, Islamabad

Dean/Principal

CERTIFICATE OF APPROVAL

This is to certify that the research work presented in this thesis, entitled "Characterization of ACO gene under Salinity Stress in *Triticum aestivum*" was conducted by Warda Imtiaz under the supervision of Dr. Alvina Gul

No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the Department of Agricultural Sciences and Technology in partial fulfillment of the requirements for the degree of Master of Science in the Field of Plant Biotechnology, Department of Agricultural Sciences and Technology, National University of Sciences and Technology, Islamabad.

Student Name: Warda Imtiaz Signature: Warda

Examination Committee:

a) External Examiner 1: Dr. Mustafeez Mujtaba Babar Signature:

DR. MUSTAFEEZ M. BABAR
Pharm.D, M.Phil, PhD
Associate Professor & Advisor Students Affairs
Shifa College of Pharmaceutical Sciences
Shifa Tameer-e-Millat University
Islamabad

(Associate Professor, Shifa Tameer-e-Millat University)

b) Examiner 2: Dr. Rabia Amir Signature:

(Associate Professor, ASAB, NUST)

c) Examiner 3: Dr. Faiza Munir Signature:

(Associate Professor, ASAB, NUST)

Supervisor Name: Dr. Alvina Gul Signature:

Dr. Alvina Gul
Tenured Associate Professor
Dept. of Plant Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST

Name of HOD: Dr. Muhammad Faraz Bhatti Signature:

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

Declaration

I certify that this research work titled "Characterization of ACO gene under Salinity Stress in *Triticum aestivum*" is my work. The work hasn't been submitted for evaluation elsewhere. All cited material has been appropriately cited and acknowledged.

Warda Imtiaz

Master of Science in Plant Biotechnology Registration # 00000399944

Copyright Statement

Certificate for plagiarism

This is to confirm that the MS thesis entitled "Characterization of ACO gene under Salinity Stress in *Triticum aestivum*" of Warda Imtiaz Reg No. 00000399944 has been examined by me. I undertake that,

1. There is substantial new work and knowledge in the thesis that has not been previously documented. Verbatim copies of any sentence, table, equation, diagram, paragraph, or section from prior works are strictly prohibited and must be enclosed in quotation marks and appropriately referenced.

2. The presented work is authentic and the author's own creation; no instances of plagiarism were detected. There has been no presentation of external ideas, results, or work as the author's own.

3. No attempt is made to falsify data or results to the detriment of the accuracy of the research records.

4. The thesis has undergone a Turnitin check; a copy of the original report is appended. It has been meticulously edited in accordance with HEC plagiarism policy and periodic instructions.

Warda Imtiaz Warda

Date: 5th Sept, 2024

Supervisor: Dr. Alvina Gul

Signature:

Dr. Alvina Gul
Tenured Associate Professor
Deptt of Plant Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST, Islamabad

Dedication

My dissertation is dedicated to my family and instructors. I have an exceptional sense of appreciation for my Father, Mother and my siblings, who are the precious and loving assets of my life.

Acknowledgment

All praise to **Allah Almighty**, the Most Beneficent and the Most Merciful, who has granted me the ability to seek knowledge and eased my journey by guiding me along righteous paths.

I express my sincere appreciation to **Dr. Alvina Gul**, my supervisor, for her invaluable counsel. The constructive criticism she provided had a profound impact on my intellectual development and has fostered a stronger sense of self-control.

I am indebted to my senior colleague **Arzoo Ahad** for her unconditional support. Thanks for always guiding me through every thick and thin of a research project. I am thankful for the unwavering support and companionship of my friends **Tayyaba Tariq** and **Rida Naseer**. They have made my journey at NUST memorable, transforming it into one of the best chapters of my life. A special thanks to Bisma Ahmad, Safia Bibi, Hajira Imran, Sarah Fatima, and Mahnoor Ilyas for helping me in completing the project.

Lastly, but most importantly, I recognize that none of my achievements would have been possible without the prayers and support of my parents. I dedicate every inch of my success to my father and my mother who stands as an ideal and an inspirational figure to me.

Table of Contents

Table of Figure

List of Tables

List of abbreviations

CDD	**Conserved Domain Database**
BLAST	**Basic Local Alignment Search Tool**
NCBI	**National Centre for Biotechnology Information**
GSDS	**Gene Structure Display Server**
ACO	**1-aminocyclopropane-1-carboxylate oxidase**
SAM	**S-Adenosyl methionine**
NaCl	**Sodium chloride**
q-rt	**Quantitative real time**
MEME	**Multiple em for Motif Elicitation**

Abstract

Wheat is among the world's most consumed staple food crops, growing in over 120 countries. Cold, salt, drought, and heavy metals all have a substantial consequence on plant development and agricultural productivity. Salinity has a negative influence on numerous physiological and metabolic processes in plants. Ethylene, which is a stress hormone, can be produced in a variety of conditions. Wheat quality is affected by a variety of factors, including composition (protein, starch, and ash content), safety and sanitation (fungal infections, mycotoxins), physical (kernel moisture content [MC], mass and density, size, colour and hardness) and functional. It is well recognised that biotic and abiotic stressors can diminish crop yield and degrade wheat grain quality. The ethylene production process is divided into two parts. S-adenosyl-L-methionine (SAM) is first transformed to 1-aminocyclopropane-1-carboxylic acid (*ACC*) by *ACC* synthase. The enzyme ACC-oxidase (*ACO*) then converts *ACC* to ethylene. Targeting *ACO* rather than ACS or ethylene signalling components, for example, may reduce the likelihood of interfering with other pathways because it catalyses the terminal step in the ethylene synthesis process. Comparing the ethylene pathways and ACO genes in different wheat varieties and comparable cereal crops could provide valuable insights on evolutionary methods and potential breeding gene targets.

Keywords: salinity stress, ACO gene, wheat, ethylene

Chapter 1: Introduction

Cereals are the only main source of calories to most of the world's population, where near 60% or 80% of these calories are obtained entirely from them in developing countries, respectively (USDA, 2019). FAO's latest forecasts for world cereal production in 2020 point to an increase of 1.2% compared to 2018, achieving 2700 million tons (FAO, 2019). Amongst cereals, wheat is one of the greatest vital foods for 40% of the world's population, with a present yearly production of 761.88 million tons (USDA, 2019), predominantly for people living in Europe, North America, the Western and Northern parts of Asia, and the Americas (FAO, 2018). However, this cereal is substantially sensitive to environmental conditions, i.e. temperature, water reduction, and saline soils (Ibarra-Villarreal et al., 2021).

1.1 Poaceae Family

Monocotyledonous plants have received less attention in terms of ACO gene structure and functional study. Several grass species have ACO genes that have been isolated and characterised. Although looking the Rice Genome Annotation Project for ACO genes, observing that it has 23 relevant loci, there are solely three paralogs of the ACO gene in rice separated and characterized so remotely with discrete annotations in the GenBank database(Ouyang et al., 2007). The first, OS-ACO1, was separated by Mekhedov and Kende. Two others, OS-ACO2 and OS-ACO3, were indicated by Chae et al. (2000). In comparison to ACO genes from other species, most *OS-ACOs* assumed in the rice genome, do not demonstrate the usual four-exon structure (Ouyang et al., 2007).

Although searching for ACO genes in the Rice Genome Annotation Project yielded 23 relevant loci, there are only three paralogs of ACO genes in rice that have been recognized and

considered with different annotations in the GenB2ank database (Ouyang et al., 2007).The total nucleotide sequence homology of *OS-ACO2* and *OS-ACO3* was 72.6%. The coding areas of *OS-ACO2* and *OS-ACO3* share 92 and 72% amino acid sequence identity with *OS-ACO1*, respectively, demonstrating that the *OS-ACO3* gene is a further diverged member of the gene family. In comparison to ACO genes from other species, the majority of *OS-ACOs* expected in the rice genome do not show any activity (Ouyang et al., 2007).

Gallie & Young showed that the maize ACO gene family is, *ZM-ACO15*, *ZM-ACO20*, *ZM-ACO31*, and *ZM-ACO35*, which can be divided into two subfamilies. A few of them is made up of *ZM-ACO35*, which are quite comparable in amino acid sequence (91% amino acid identity). Another subfamily is made up of *ZM-ACO15* and *ZM-ACO31*, which are similarly quite close.

These two subfamilies have diverged significantly, with members of the maize ACO gene family showing more divergence than members of other species such as petunia and tomato. *ZM-ACO15* and *ZM-ACO31* are more like Arabidopsis and rice ACOs than to *ZM-ACO20* and *ZM-ACO35*, implying that gene replication and deviation of the two subfamilies happened before the split of monocotyledonous and dicotyledonous species. *ZM-ACO20* has all twelve conserved amino acid residues among iron- and ascorbate-dependent dioxygenases, whereas *ZM-ACO35*, *ZM-ACO15*, and *ZM-ACO31* have most but not entirely the conserved residues.(Rudus´• et al., 2012).

The ACO genes have also been linked to reactions to environmental stress and hormones (Houben & Van de Poel, 2019a). Salinity stress can negatively alter *TaACO1* in Arabidopsis. Furthermore, flood tolerance occurs in Arabidopsis leading to the ACO gene overexpression. Under salinity stress, nitric oxide was discovered to influence lateral root development in sunflowers via modulating ACO gene activity. Wounding can promote ACO gene expression

in cucumbers and tomatoes. Following *F. eumartii* inoculation, the level of mRNA in *ST-ACO3* in potatoes increases (Zanetti et al., 2002).

ACO genes are shown to give response to ABA in tomato and cauliflower. External mechanical damage can also enhance *OsACO1* expression, and IAA can considerably boost *OsACO2* expression while entirely suppressing *OsACO3* expression. *AsACO* is substantially increased in Agrostis stolonifera in response to ethephon, methyl jasmonate and cold temperature, while in drought and salt stress, is regulated less (Wei et al., 2021a).

1.2. Wheat (*Triticum aestivum)*

Wheat is one of the consumed grain crops in whole of the cereals and is categorized as first in the grain producing crops, particularly for human usage.(Giraldo et al., 2019). Wheat is a staple food for about 36% of humans. Wheat consumption supplies 20% calories proportion and 55% carbohydrates. Salinity imposes a decline in the development and production of wheat (Royo & Abió, 2003).

Wheat is grown globally on a land area of more than 220 million hectares and provides a total of about 20 % of commercial dietary protein necessities. Although wheat is cultivated on a huge area, its production is considerably less than maize and rice and there is a need to enhance production by 60% by 2050 to fulfil 9.6 billion worldwide population under climate modification. The production of wheat is more affected by abiotic stresses rather than biotic. Some of the big abiotic challenges that harm wheat production and quality includes drought, salinity and heat (Abhinandan et al., 2018).

Wheat is grown on nine million hectares, which is huge land area occupied under one crop. It covers 70% rabi crops, 37% of whole cropped area and almost 74.92% of the whole land area occupied by the grain food crops in the region. The land covered by wheat in Pakistan elevated

from 21612 thousand tonnes to 24214 thousand tones. Wheat is of family Poacae, has a significant part in progress of Human in several countries comprising Pakistan. It occupies two third of the area covered by the cereal crops globally. It has a contribution of 14.4% value included in agriculture and 3% in GDP (M. Iqbal et al., 2014).

1.3. Morphology of Wheat Plant

The plant comprises a system of roots and shoots. There are two kinds of roots: seminal roots and nodal roots, which grow from the shoot's lower nodes. The shoot is composed of a sequence of phytomers, each of which may have a node, a leaf, an extended internode, and a bud The vegetative component of each shoot is made up of 6 to 16 or more of these units. An extended internode can be found in four to seven of the most distal units. The internode is still present in the proximal or basal units.

Tillers emerge from the basal leaves axils and have the same fundamental structure as the main stem. Only a subset of the tillers that have formed survive to produce an ear during anthesis. Others perish and may be difficult to locate in a mature plant. The ligule and a pair of tiny, hairy projections, the auricles, are situated at the junction of the sheath and lamina. The base of the culm's leaves thickens to create a stiff knot, or pulvinus. The length of the elongated distal internodes increases from the most distal to basal, the peduncle (Botany of the Wheat Plant - E.J.M. Kirby, n.d.).

1.4. Salinity Stress and Wheat Production

..

Salinity stress negatively affects wheat crop production. The yield of wheat begins to fall off in salinity stress range of 6-8dS m-1. Corresponding to FAO report. 397 million area of hectares

is seriously altered due to salinity stress and that’s becoming a serious challenge for food security. Salinity stress imposes ion toxicity and disturbance of nutritional balance, which destroys the plant's biological processes and ultimately a severe decline in ultimate yield. At first salinity stress significantly reduces seed germination and then it changes evolution and reproducing patterns which results in severe yield decline. Moreover, it also interrupts photosynthesis, the balance of hormones, the uptake of nutrients and water, and causes oxidative stress.

Wheat undergoes adaptations at the organ and cellular level to manage well with salt stress. The salt tolerance resistance mechanisms in wheat are complicated as the plant makes several changes in stomatal conductance, hormonal balance, antioxidant defense mechanism adjustment, and ion exclusion. Wheat undergoes adjustments at the organ and cell stage to cope well with salt stress. It has a complicated mechanism for resistance as it undergoes many changes in the conductance of stomata, balancing of hormones, ion exclusion, and regulation of osmosis. To cope with challenges, breeders do a significant struggle to develop cultivars bearing salt stress in Australia and Pakistan and India. Despite that, the struggle is markedly slow. Within Pakistan, Agriculture University Faisalabad in association with the Saline Agriculture Research Centre made salt bearing lines (LU26S and SARC-1)

Plants based on adaptive evolution can be categorized into two main types: Halophytes (which can tolerate salinity) and glycophytes (that cannot endure salinity and ultimately die). Mostly big crop species fall in the second category. Salinity stress includes modifications in several biological processes, relying upon the time and severity of stress, and eventually stops crop production. In the initial stages salinity effects plant development by inducing osmotic stress and then causing ion toxicity. During the start of salt stress, root systems' capacity to absorb water reduces, and loss of water from leaves is enhanced because of osmotic stress, generated due to huge salt deposits in plants and soil consequently salinity stress is referred to as

hyperosmotic stress. Osmotic stress in the initial stage of salt stress brings many physiological modifications like membrane interruption, unbalancing nutrients, diminishing the strength to decontaminate ROS, and reduced photosynthetic functioning. Salinity stress is known as hyper ionic stress.

Among the damaging consequences of salinity stress, the significant one is the Na+ and Cl- ions accumulation gathered in plant tissues, when given exposure to soils of elevated NaCl concentration. The appearance of Na and Cl inside cells brings acute imbalance of ions and severe concentration may cause physiological disorder. Because of salinity stress, ROS production is increased. Salinity-promoted ROS generation can cause oxidative loss in many plants' cellular parts, thus interrupting normal plant processes. Genetic alterations in bearing salt stress are present and the level of salt tolerance changes with species of plant and varieties within species. Comparing big crops, barley (Hordeum vulgare) tends to have a higher salt tolerance level compared to rice and wheat (Gupta & Huang, 2014).

Wheat is a relatively salt-bearing crop (Qureshi and Barrett-Lennard, 1998), but increasing salinity lowers seed growth and germination (Younis and Hatata, Citation2005). The variability in salinity tolerance among wheat genotypes may be attributable to xylem loading and the ability to absorb and confiscate sodium.

Salinity impacts plant growth by interfering with metabolic processes, low productivity, and changes in biomolecule concentrations. Due to osmotic adjustment, free proline increases in the plants tissue cultivated in saline. Plants respond to salinity stress by altering many biochemical processes and adopting resistance mechanisms as well as anatomical modifications (Hussain et al., 2015).

1.5. Wheat Adaptation to Salinity Stress

A significant factor that can regulate the salt tolerance of species of wheat is their genetic composition. Triticae members possess a significant change in tolerating salt stress and a few of these species are halophytes. It is preferable to utilize wild species for producing wheat species which are salinity tolerant. The diminished Na+ concentration in tissues and an increased Na+/K+ are encouraging traits of the developed improved species.

One of the significantly important side effects that salinity causes on wheat development is the reduced nutrient uptake by plant. When exposed to stressful conditions, the plants Ca, S and Mg level in levels reduces and improved the quantity of P and K in leaves of seedling developed below stress situations (Miransari & Smith, 2019).

Different plants tend to have different salt tolerance as many of cereals, also wheat, are categorized as glycophytes which are salt tolerant to a moderate level. Salinity largely reduces uptake of nutrients (Ashraf et al., 2017). Germination of seed and stages of early seedling establishment are usually most prone to salinity stress. Commercial crop germination may be highly decreased in salinity-prone soil.

In the late stages after stand establishment, the high level of Na+ concentration in leaves promotes premature leaf ageing and a decline in photosynthesis by restraining carbon assimilation. Physiological factors such as chlorophyll contents were considered a useful parameter for wheat tolerance level. If the side effects of salt stress can be lessened at the stage of seedling growth, the chance of growing a crop in salinity soil conditions will be much enhanced (Saddiq et al., 2019).

In current years, the signaling of ethylene has been demonstrated to participate a part in salt acceptance. In the past current years, ethylene biosynthesis has also remained essential not only for growth but for stress management. For instance, the application of ethylene could improve

rice crop drought tolerance. As seen in maize, *ZmACS6* which is an mutant of *ACC* synthase, influencing the ethylene biosynthesis first step, was reported to demonstrate improved drought tolerance and reduced drought-affected leaf senescence. The *AtACS7* expression is controlled by abiotic stresses like heat, salt, light, and ABA treatment. Another essential enzyme in the pathway of ethylene biosynthesis is *ACC* oxidase. It speeds up the decisive ethylene biosynthesis step, in which it converts 1-aminocyclopropane -1 carboxylic acid into ethylene. A divergent multigene family encodes *ACC* oxidase, and environmental factors differentially modulate each member's expression. However, the ACO gene's physiological function in the stress responses of plants is still largely not known (Chen et al., 2014).

1.6 Ethylene Hormone under Salinity Stress

Hormones are the chemicals generated in plants and they control regular plant processes, and growth and encounter different stresses specifically under salinity. The external consumption of various plant hormones moderates the consequence of different abiotic stresses. Auxin enhances germination and dry weight of the shoot, thus balancing the ionic homeostasis under salinity, well recognized as a supporter of growth. It lessens salinity to a level of 15dSm-1 by balancing the concentration of hormones in the plant and boosts production by enhancing the absorption rate in wheat cultivars, which are salt-resistant and salt tolerant. GA3 priming speeds up the development of photosynthetic pigments and plant development under stress situations. ABA-enriched seeds showed elevated salinity patience by increased production of chlorophyll and reduced Na^+ uptake (M. Seleiman, Aslam, et al., n.d.).

Ethylene is regarded as a plant stress-dealing hormone, as its synthesis is stimulated by several abiotic and biotic stresses. Just like several other stresses, salinity triggers the formation of ethylene in several species by altering the enzymatic activity of ethylene biosynthesis pathway enzymes. In a study, endogenic overrun of ethylene or treating with *ACC* precursor of ethylene

can combat the salt-promoted restraint in seed germination of Arabidopsis. Utilizing the soil-based mutant screen system, it was found that, at the vegetative propagation stage, a rise in in vivo production of ethylene enhances the salinity tolerating level of Arabidopsis plants which are planted on saline soil when actively transcribed, despite of other plants brought up in in vitro environment with no transpiration. This led to indicate that ethylene induced salinity tolerance is a potential enhancer of salt tolerance at several developmental phases and in different conditions. A boost to these ideas is given by a recent rice study SALT TOLERANCE1 (SIT1, a lectin receptor-like kinase, positively maintains the salinity tolerance by upregulating the MAPK 3 /6 activity and enhancing the production of ethylene Nevertheless, in some other scenarios, upregulated levels of ethylene can negatively disturb salinity tolerance. In this case, Arabidopsis plants overproducing wheat *ACO1* showed increased ethylene levels but reduced salinity tolerance (Chen et al., 2014b).

In addition, acs7 mutant of Arabidopsis, which showed lower ethylene production, demonstrated higher salt tolerance at the germination stage of the seed (Dong et al., 2011a).A current report also referred to the rice plants, given the ethylene treatment converse salt hypersensitivity. Thus, the ethylene levels of plants can either positively or negatively regulate plant salt sensitivity, proposing that ethylene biosynthesis must be fine-tuned for plants to tolerate salt stress (M. Zhang et al., 2016).

Phytohormones are most significant endogenous substances involved in the tolerance mechanism or plants suspectibility to salinity stress. The phytohormones role under salinity stress is crucial in modulation of physiological responses that will ultimately cause adjustment to an adverse environment. Gibberellins and ethylene are helpful in combating the salinity stress unfavourable effects by initiating defense responses or uplifting growth of plants. However, both these phytohormones influence each other's actions. On the one hand, GA is recognized to enhance ethylene synthesis, and on another hand ethylene impact its signalling,

and therefore, this interaction opens a cross-talk between them. Ethylene has been thought to be a stress hormone and many stresses stimulate it (Ethylene in Plant Biology - Frederick B. Abeles, Page W. Morgan, Mikal E. Saltveit Jr. - Google Books, n.d.). However, its part in salt stress is unclear. (El-Iklil et al., 2000) has described that reduced ethylene production was linked with salt tolerance. On the other side, increased ethylene production has been observed in rice as a salt tolerance indicator. (Khan et al., 1987). A recent study states that ethylene signalling encourages salt acceptance in Arabidopsis.

This investigation posits that the functionality of ethylene receptors contributes to sensitivity to salt, while *ACC* seems to inhibit such sensitivity to salt, suggesting that the involvement of ethylene signaling is essential for salt tolerance in plants The plant's reaction to saline stress could be contingent upon the equilibrium and/or interplay between the ethylene and receptor.

In instances where receptor signaling prevails, the plant displays sensitivity to saline stress, alongside a significant rosette size and delayed flowering. Conversely, in situations where ethylene signaling dominates, the plant exhibits tolerance to saline stress, coupled with a diminutive rosette size and early flowering.

The plant must acclimate to these contrasting circumstances. By finely regulating various aspects, plants can achieve an active state of homeostasis, thereby enhancing survival under stressful conditions and promoting relatively normal growth.(N. Iqbal et al., 2022).

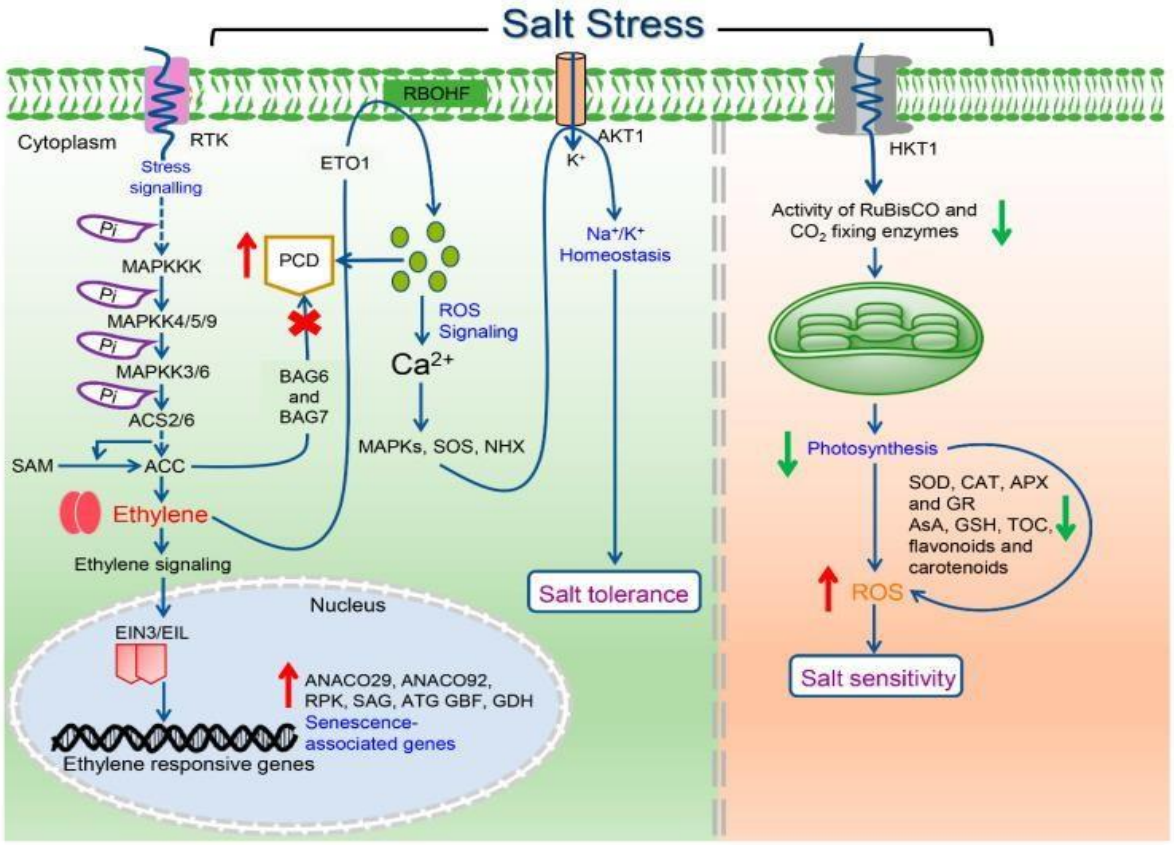

FIGURE 1:ACTIVATION OF ETHYLENE BIOSYNTHESIS AND SIGNALING PATHWAY UNDER SALT STRESS (Riyazuddin et al., 2020a)

1.7 ACC oxidase: Ethylene Forming Enzyme

ACC oxidase is an enzyme that helps in the transition of ACC to ethylene (Yang and Hoffman 1984). It has been proposed that ACC increases ethylene synthesis by considerably increasing *TaACO1* expression. In vitro, *TaACO1* protein consistently exhibits ACC oxidase activity. *TaACO1* overexpression plants have reduced size of seed under normal conditions when *TaACO1* in Arabidopsis is constitutively expressed. This finding shows that the transgenic plants may possibly create more ethylene as a result of their ACC oxidase activity, as in plant development ethylene is considered to be a negative controller. Although the ethylene level in acs7 was lesser, the germination of acs7 seeds was quicker than the wild type, and the ultimate germination rate indicated no variation.(Dong et al., 2011). The ferrous-dependent nonheme

oxygenase ACC oxidase uses 2-oxoglutarate as a co-substrate (Mirica and Klinman 2008). There are two different domains in its sequence: an N-terminal extremely conservative non-heme dioxygenase DIOX_N region and a C-terminal 2OG-FeII_Oxy region (Punta et al., 2012). The Fe2OG dioxygenases catalyse two-electron oxidations such as hydroxylation's, desaturations, and oxidative ring closures. Although there is little sequence commonality among these enzymes, all of them have a single ferrous ion bound in a tridentate ligand arrangement known as a "2-His-1-carboxylate facial triad" (Mirica and Klinman 2008). Yoo et al. (2006) discovered that the positively charged surface in the *ACO's* C-terminal helix, which spans the motif (Lys296-Glu301), is exclusively conserved and that between the C-terminus and the active site, positive charge network is crucial for *ACO* activity (Rudus´• et al., 2012).

On the other hand, the *TaACO1*-transgenic plants slower germination could be due to ethylene-induced lignification, which strengthens the seed cell wall. *TaACO1* develops to be a functioning ACC oxidase in plants; nevertheless, its precise function in ethylene production is unknown(Chen et al., 2014). The amino acid methionine is the typical pioneer of the ethylene production route. SAM synthetase uses ATP to convert methionine into S-adenosyl-L-methionine (SAM) in the initial but general reaction (Adams et al., 1977).The following chemical stages are specific to the ethylene production pathway. ACC-synthase (ACS) first converts SAM to 1-aminocyclopropane-1-carboxylic acid (ACC) and 5′-methylthioadenosine (MTA). ACS fits to the family of aminotransferases that require pyridoxal-5′-phosphate (PLP) as a cofactor (Boller et al., 1979). To avoid methionine depletion through high rates of ethylene synthesis, the Yang cycle recycles the byproduct MTA back to methionine.

In a further stage, ACC-oxidase (ACO) (Hamilton et al., 1990a)releases ethylene from ACC, a process that needs molecular oxygen (Burg and Burg, 1965). ACC can also be transformed into malonyl-ACC, -glutamyl-ACC (GACC; Martin and Saftner, 1995), and jasmonyl-ACC (JA-ACC; Staswick and Tiryaki, 2004).

1.8 ACO Biotechnology and Applications

Since ethylene is vital in several plant processes, incorporating critical fruit ripening and aging, too much ethylene can cause plant-based food to deteriorate. As a result, ethylene production and signalling genes have frequently been aimed in biotechnological and transgenic efforts to extend the qualiy life of foods that are plant based. Because *ACO* catalyses the final step in the ethylene biosynthesis route, it is a good choice to target (rather than, say, ACS or ethylene signalling components), as there is less chance of interfering with further pathways (e.g., ACC metabolism) (Houben & Van de Poel, 2019b).

1.9 ACO gene structure

ACO is determined via a short multigene family with three to four members (Pech et al., 2010.The majority of *ACO* gene family, with seven homologs in the genome, has been found in Populus trichocarpa. In many cases, just one *ACO* gene is recognized for a species; nevertheless, additional research should eventually lead to the discovery of some more homologs.

Chapter 2: Literature Review

Wheat (*T. aestivum*) is the main staple cereal after rice. The growing population's need for food is pushing up demand for wheat in both developed and developing countries, and demand is increasing daily. Wheat output in developing countries may increase by 60% by 2050 to meet rising demand caused by the world's burgeoning population, and drought reduces wheat production by 20-30%, particularly in poor countries. As a result, new solutions for sustainable wheat production are required to reduce the negative effects on wheat productivity while still ensuring the food and nutritional security of the rising population (Hossain et al., 2021). Wheat is a vital food crop ranked highest in grain production globally. It is used in food industry, providing nutrition to 36% of the world's population, and it allocates 20% of the calories and 55% of the carbohydrates worldwide.

Wheat (*T. aestivum*) has a significant position among cereals of its area of development (8.6 million acres), production (21 million tons), and utilization in Pakistan. Punjab is the major agricultural hub of Pakistan, with 72% cultivation of wheat and in the total national economy has a significant part of 75%. The uplift in wheat production in the previous two decades in Pakistan is striking (1643 to 2627 kg/ha− 1), but significant less than North America (6425 kg/ha− 1) and numerous countries of the world such as India (4400 kg/ha− 1), and it is unlucky that although Pakistan is the 7th major wheat producing country of the world and it stands 59th

in prospect of production per hectare, and still trade in wheat from further countries to complete the requirements of growing population in the country (Wahid, 2003).

2.1 Abiotic Stress

Abiotic stress illustrates the negative influence of non-living materials on living things in a specific ecosystem. Among the stresses are drought, salt, temperature extremes, and other environmental factors. Drought and hyper salinity are the common abiotic stresses that affect crop loss worldwide. Heat, salt, and drought, on their own, have been the subject of extensive research. However, crops and other plants in the field are routinely subjected to several unique abiotic stresses. Many crops in drought-affected areas, for example, endure a combination of drought and other stressors such as heat or salt (Mittler, 2006).

2.2 Salinity stress

Excessive salt concentrations in the soil, known as "salt stress," gradually hinder crops from growing and, eventually, cause crop fatality. When it comes to rice crop growth, salt is the most dangerous element on the earth (Rattan et al., 2022).

Soil salinity is a worldwide issue that impacts approx. 20 % of irrigated land and decreases crop yield greatly. The physical response of a plant to salinity are mostly complicated and multi-faceted, because of which experiments are complex to explain and design. Now plant physiology has progressed, given the development of so-called ‘omics-driven’ research. Biological measurements have been advanced by modern technologies, such as high-data phenotyping, bioinformatics, and new systematic methods that have make fields like metabolomics to rise.

At a core level, the plants response to salinity can be explained in two major phases: the shoot ion-independent response happens promptly, within minutes to days, and is supposed to be

linked to Na+ signalling. In this initial phase, the effect of salinity on water relations can be crucial, affecting closure of stomata and the stopping of leaf expansion. The second phase, the ion-dependent response to salinity, progresses within long time (days to weeks) and includes the buid up of ions in the shoot to lethal concentrations, chiefly in old leaves, causing premature aging of leaves and ultimately decreased production and even plant deterioration (Negrão et al., 2017).

2.3 Wheat and Salinity Stress

Wheat is the best globally cultivated and consumed grain crop on the planet. Salinity stress, on the other hand, is a severe danger to world wheat productivity, food security, and dietary security. Salt stress harms seed propagation, plant development, photosynthesis, nutrient uptake, and yield due to a variety of factors. Salt causes oxidative stress, as well as ionic and hormonal abnormalities. Under salinity stress, the wheat crop exhibits a varied spectrum of structural, functional, and molecular responses. The biological and molecular mechanisms are critical because they can assist wheat breeders in developing salt tolerance. Wheat has well-understood systems for dealing with salt stress. However, further research is needed in several disciplines, particularly the physical source of integrate partitioning from plant sources to sinks.

Germination is important in the plant life cycle because it supports progress, development, and production characteristics. Salinity stress prevents seed germination, leading to significant drop in wheat crop output. Salt stress lowers osmotic potential, interferes with the simple work of enzymes required for metabolic activity, and reduces final stand establishment and production. Salt stress decreased characteristics such as spikelet quantity, productive tillers and biomass output. Plant seedlings are extremely susceptible to pressure conditions, and salt stress causes seedling death (Saddiq et al., 2021).Salinity stress has negative role on root and shoot growth (M. Seleiman, Alhammad, et al., n.d.).

Salinity stress has a lethal effect on root and shoot parameters as well. It was discovered that salt stress declined wheat growth relative to normal conditions (Guo et al., 2015).Similarly, there was a deterioration in root and shoot lengths and dry weight below salt stress (100 mM NaCl) (Zou et al., 2016). Almost all crops' yields are significantly reduced by salinity stress. However, the proportion of yield decline may differ between salt-tolerant and sensitive types.

2.4 Mechanisms of Salinity Stress in Wheat

Wheat develops modifications at the cellular and organ levels to operate well underneath salt stress. Wheat has complex salt resistance mechanisms because it changes many components of its stomatal conductance, hormonal stability, antioxidant protection system, osmotic adjustment, and ion transport. (M. Seleiman, Alhamdi, et al., n.d.).

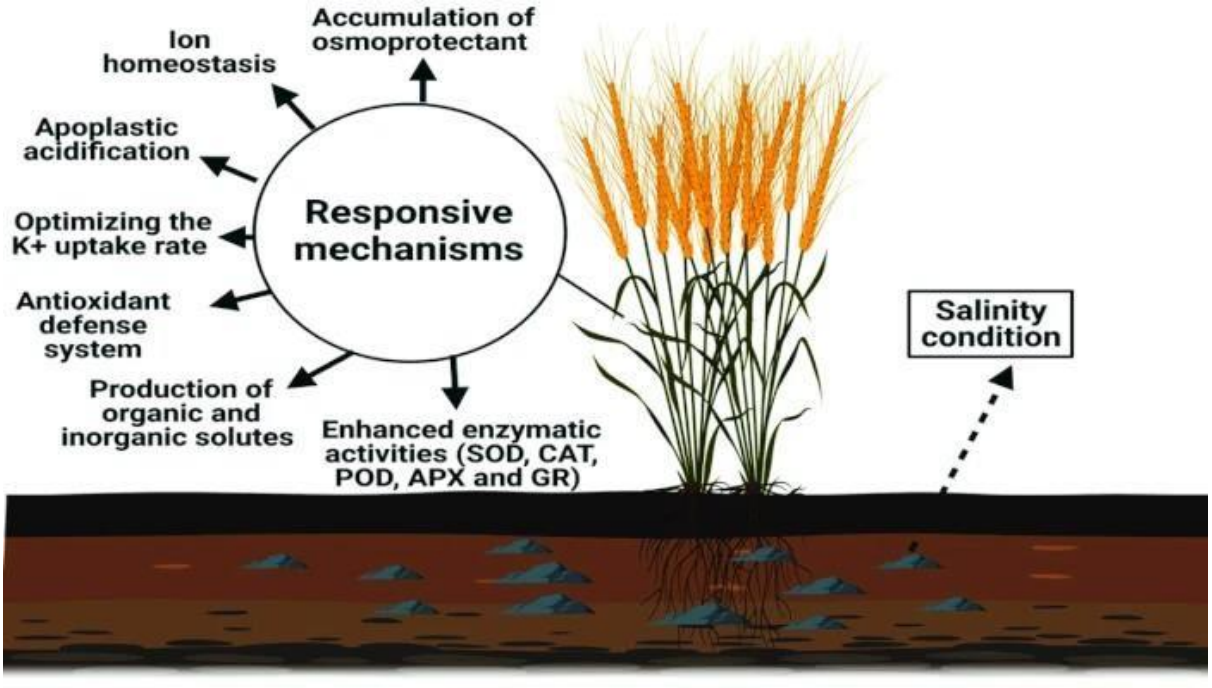

FIGURE 2:RESPONSIVE MECHANISM OF WHEAT CROP TO SALINITY STRESS

(M. F. Seleiman et al., 2022)

2.5 Adverse Effects of Salinity Stress on Wheat

Very salinity-varying agricultural fields are found in dry or semi-arid locations(Liu et al., 2020. Salinity is the most detrimental factor to wheat production and quality because it changes the biological and physiological processes in plants. Under salt stress, the generation of ROS because of Na+ toxicity is a constant occurrence, causing cellular damage and altering redox balance. However, because of the conditions, it is difficult to recover soils damaged by salt.

Ions such as Na+ and Cl are exclusively active in soils. Additionally, it is usually a pricey band-aid solution to a continuing problem. Third, the nature of salinity in the soil is dynamic, with variations in salinity caused by the interaction of various edaphic effects (soil pH, bulk density) terrestrial factors, agronomic practices (tillage, crop rotation, and fertilization), and climatic effects (temperature, humidity, precipitation, wind, and To achieve salinity resistance, it is necessary to employ cohesive agronomical, physiological, and soil management strategies that target many qualities at once. As a result, it is necessary to replace Na^+ with Ca^{2+} before removing or leaching salt for sustainable crop production.

Salinity reduces plant growth and crop productivity by delaying germination, lowering seedling development and dispersal of germination events, and modifying seedling metabolism One critical method is to learn how plants respond to salt stress. Plants respond to salinity in two stages. Salt set off osmotic stress in the primary phase due to a decline in soil water potential. Another phase takes a few days or weeks to mature, depending on the degree of salinity. Na+ ions accumulate in numerous plant tissues currently, reducing production and potentially killing the plant (Munns et al., 2008).

Furthermore, salinity reduces biomass output and phenological growth of wheat, such as leaf number. The saline environment disrupts plant water relations such as relative water content, leaf water potential and water usage efficiency(Nishida et al., 2009).

Salinity has a detrimental effect on crop plant growth and yields because it limits soil moisture availability and because high concentrations of salt and chloride ions are toxic to plants (Munns et al., 2008).Salinity stress reduces the number of fertile tillers, accelerates all wheat phenological phases, and reduces the number of spikes. Yield losses in salt-stressed wheat, for example, have been reported to reach 45%, and discovered that salt stress (15 dSm1) significantly reduces grains per spike, 1,000-grain weight, and seed output in both tolerant and sensitive wheat cultivars. Figure 1 depicts the effects of salinity stress on essential plant processes, yield, morphological characteristics, germination, root activity, and yield attributes in wheat (EL Sabagh et al., 2021).

2.6 Structure of ACO Genes

The ethylene production system in plants is strongly reliant on ACO genes, which regulate a variety of physiological processes (Houben & Van de Poel, 2019c).Gene expression is regulated by the promoter region, which is positioned upstream of the gene. It has numerous regulatory components that regulate the timing and intensity of ethylene production in answer to developmental cues and ecological stimuli (Dugardeyn & Van Der Straeten, 2008).Exons, which are coding portions of the gene, include the sequences that code for ACO enzyme synthesis. Several enzymes are required for ACC to be transformed into ethylene.

After being transcribed, they are normally spliced off during the development of mature mRNA. The function of introns in ACO genes is still being studied (Rudus´• et al., 2012b).

The precise structure of ACO genes varies between plant species, and different triggers can induce different members of the ACO gene family to express themselves in diverse ways. Understanding the impact of ACO genes on ethylene synthesis, which impacts plant growth, development, and environmental adaptability, necessitates knowledge of ACO gene structure.

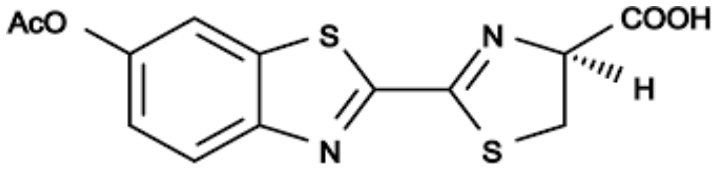

FIGURE 3: STRUCTURE OF ACO **GENE**

2.7 Discovery of ACO and its reaction mechanism

ACO (formerly known as the Ethylene Forming Enzyme, or EFE) was thought to be a membrane-bound protein that lost activity after homogenization, making it impossible to purify and assess its in vitro activity for a long time. Membranes from pea (Guy and Kende, 1984) bean (Guy and Kende, 1984; Mayne and Kende, 1986), Sprenger's asparagus, and kiwi fruit all retained some remaining or incomplete in vitro *ACO* activity, but solitary a fraction (5-0.5%) of the total in vivo ethylene yielding capacity. The revelation that the tomato clone pTOM13 encodes a possible ACO gene was a big step forward (Hamilton et al., 1990b).

Iron is a compulsory metal cofactor for the enzyme activity of *ACO* (Bouzayen et al., 1991).Iron contributes in the attachment of the *ACC* amino group to H177 and the *ACC* carboxylate group to D179, two important *ACO* deposits in the reaction centre. These bonds are coordinated by iron. The ascorbate coenzyme is employed as a reducing agent to open the ACC-ring (Z. Zhang et al., 2004).

Furthermore, employing bicarbonate and molecular oxygen as activators, the *ACO* reaction mechanism catalyses the transformation of *ACC* into ethylene. Throughout this reaction, an uneven intermediate cyanoformate ion [(NCCO2)-] is formed, which swiftly degrades into CO2 and CN-. The responsive cyanide ion (CN-) is cleaned to produce -cyan alanine (Peiser et al., 1984).

ACC-oxidase belongs to the 2-oxoglutarate-dependent dioxygenase (2OGD) class of non-heme iron-containing proteins. The majority of 2OGD superfamily members are involved in oxygenation and hydroxylation activities. However, 2OGD enzymes can participate in a broader range of activities, including plant DE methylenation, halogenation, ring closure and desaturation (Farrow & Facchini, 2014).

The double-stranded -helix core structure, which is also seen in *ACO*, is shared by all 2OGDs and contains the 2-His-1-carboxylate motif essential for iron compelling. This motif, which comprises of two His residues and a carboxylate group from either an Asp or a Glu residue, is required for ACC binding because it induces Fe (II) to bind in the enzyme's catalytic domain (Martinez & Hausinger, 2015).

Although 2OGD enzymes are commonly detected in the cytosol, the specific subcellular location of *ACO* is still being debated. According to several studies, ACO is found at the plasma membrane, as first postulated by Kend. However, recent studies have shown that ACO is cytosolically localised, which is consistent with the general position of 2OGD enzymes (Chung et al., 2002).

Several experiments have been conducted to assess *ACO* activity in membrane/apoplast and intracellular preparations. All these studies utilized immunolocalization in conjunction with subcellular fractionations, which may not have offered adequate accuracy to pinpoint ACO location. In latest study, a safflower *ACO* was tagged with GFP and ectopically localised in onion epidermis cells.(Bouzayen et al., 1990).

CtACO1 was discovered in the cytoplasm (perhaps connected with membranes) and the nucleus, however their pictures do not have organelle markers. The overall results of these studies are inconclusive regarding the specific *ACO* localization (Tu et al., 2019).

2.8 ROLE OF ACO GENE

ACO genes show a substantial part in the alteration of plant growth, advancement, and response to environmental incentives. These genes are primarily implied in the production of ethylene, a gaseous plant hormone that appears as a signalling molecule.

2.8.1 Ethylene Biosynthesis

The enzyme 1-aminocyclopropane-1-carboxylate oxidase, which is encoded by ACO genes, converts (ACC) to ethylene. Ethylene is implicated in many physiological processes. Beginning with S-adenosylmethionine (SAM), ethylene synthesis comprises two critical processes, both of which have been studied by Yang and Hoffman (1984). The cyclization of SAM to 1-aminocyclopropane-1-carboxylic acid (ACC) is catalysed first by 1-aminocyclopropane-1-carboxylate synthase (ACS) [EC 4.4.1.14], and this reaction is usually regarded as the rate-limiting step in the route. *ACS* also generates 5′-methylthioadenosine, which is reused to make methionine. The decisive step, the oxygen-dependent conversion of ACC to ethylene, is catalysed by *ACO* (ACC oxidase) [EC 1.14.17.4]. ACC's carbons C-2 and C-3 are modified to produce ethylene, while C-1 is transformed to cyanide and the carboxyl group is transformed to carbon dioxide (Rudus´• et al., 2012c).

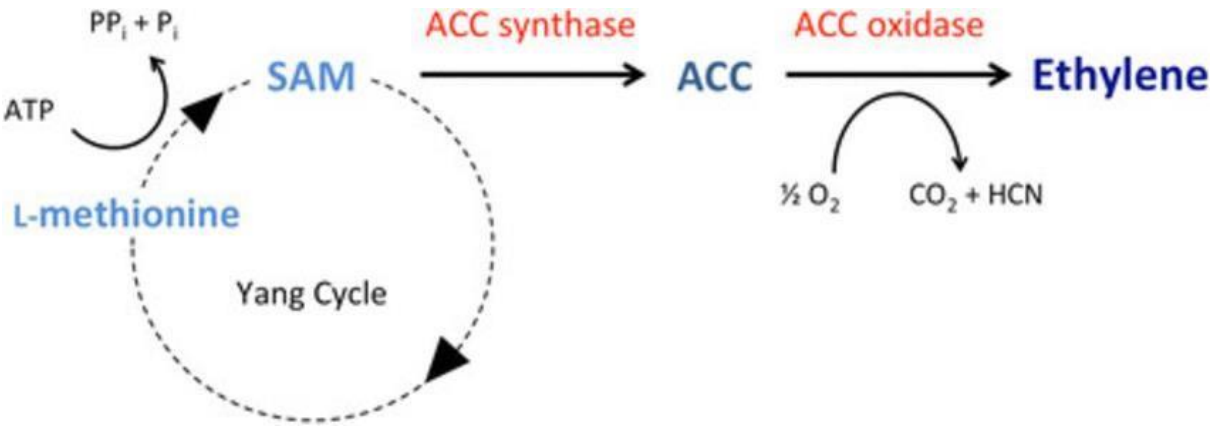

FIGURE 4:ETHYLENE BIOSYNTHESIS PATHWAY

(Wang et al., 2002)

2.8.2 Fruit Ripening

ACO1 and *ACO2* have the most pronounced ripening-regulated expression patterns whereas *ACO4* expression increases steadily but slightly during ripening. Six ACO genes were found in the tomato genome during a genome-wide search The current work backs up previous findings that identified *ACO1* and *ACO4* as the key ACO genes involved in ripening-linked ethylene generation. (Nakatsuka et al., 1998).The transcript levels of *ACO3, ACO5*, and *ACO6* are still relatively low, indicating that they play a minor role in the climacteric ethylene synthesis process.

Two ethylene biosynthetic processes in climacteric fruits have been suggested (Mcmurchie et al., 1972).System 1 produces basal ethylene levels in all tissues, including non-climacteric fruit tissues. System 2 is autocatalytic and active throughout critical ripening, whereas System 1 is ethylene autoinhibitory and active throughout fruit growth. System 2 is triggered by ethylene-induced upregulation of *ACS2* and *ACS4*, whereas System 1 is relied on ACS1A and ACS6, each of them are adversely synchronized by ethylene.

Both *ACO1* and *ACO4* have low expression in immature green fruits, where system 1 is active; however, the switch to system 2 is induced by transcript accumulation with the climacteric

increase in ethylene synthesis. Furthermore, *ACO4* expression is maintained during fruit ripening. It has been shown that developmental controllers, like RIN and LeHB-1, which wholly bind to the promoters of *ACS2* and *ACO1* to regulate their expression, govern the expression of ethylene-producing genes. Thus, it is possible that processes other than system 2 ethylene synthesis are involved in the autocatalytic regulation of climacteric ethylene (Van de Poel et al., 2014).

2.8.3 Senescence

The ethylene-sensitive floral senescence is caused by the two primary enzymes implied in ethylene production, ACC synthase (*ACS*) and ACC oxidase (*ACO*). Several investigations have shown that the *ACO* action instantly transforms the *ACC* into ethylene(Sornchai et al., 2020). It has been determined that the expression of the ACS and ACO genes rises during petal senescence in many flowers. Downregulation of these two genes was found to be linked with a decline in ethylene production in petunia, campanula, carnation, and rose. The suppression of PhEOL1 by VIGS accelerated floral aging and elevated ethylene production in the corolla. In *Arabidopsis thaliana*, EOL1 (ETHYLENE-OVERPRODUCER1-like) protein inhibits ethylene production.

Senescing blooms of Hibiscus rosa-sinensis have been demonstrated to activate *ACS* and *ACO* via an increase in ethylene in a variety of floral organs. Hibiscus rosa-sinensis global transcriptome study demonstrates that transcriptional control of the ethylene production pathway amplifies the signals that would normally occur with ageing, promoting senescence. Furthermore, transcripts associated with ethylene response factors (ERFs) and biosynthetic genes (*ACS* and *ACO*) were differentially regulated in each floral tissue during senescence (Dar et al., 2021).

2.8.4 Stress responses

Salinity increases ethylene biosynthesis during the germination period of many lettuce cultivars, as well as pepper, broccoli, and beetroot. Melon, spinach, and tomato are all declining at the same time. Indeed, salt stress increased ethylene biosynthesis; nevertheless, it was the stress of ethylene that needed to be decreased to an prime level that supported plant growth. *ACC* was found to impair the growth of tomato seedlings under salt stress. The ethylene production in response to salt stress has also been shown to be connected to plant sensitivity; for instance, pepper shoot is the highest susceptible to saline treatment, with the largest fresh weight inhibition and total *ACC* concentration increase (Siddikee et al., 2012a).

In contrast, beetroot responds to saline treatment with little effect on total *ACC* content and is less sensitive to salt. As a result, as salinity resistance increases, so does a plant's reaction to salinity, including stress ethylene production. Salinity treatment raises the complete *ACC* intensity in the roots and shoots of utmost of the plant species studied, including Cucumis melo, Lactuca sativa, Capsicum annum, and Lycopersicon esculentum (Zapata et al., 2007).

When exposed to salinity, *ACO* activity increases, causing Cicer arietinum root ethylene levels to rise. It was revealed that salt and other abiotic stresses produce a decrease in wheat ACO1 transcripts, which reduces ethylene synthesis. Wheat *ACO1* constitutive expression in Arabidopsis inhibited the stress-responsive and salt-sensitive AtRAB18, AtCBF1, and AtCBF3 genes while increasing the expression of AtMYB15 (Fatimaet al., 2022a).

2.8.5 Hormone Crosstalk

Probably, ethylene role in development and growth is not isolated. Through interactions with other phytohormones, it establishes a network of signalling pathways and influences the control of various activities. Understanding how different phytohormones, such as ethylene, interact

to influence growth and senescence could provide a valuable technique for changing the molecular makeup of these hormones to produce specific plant responses.

Ethylene is important in the alteration of plants from the vegetative to reproductive stages, as well as in senescence, and it interacts with further plant hormones in this process. This networking influences tissue sensitivity as well as ethylene concentration. Few studies have investigated the molecular adjustments that occur in plant tissues because of ethylene and other combination treatments.

Furthermore, the balance between ethylene biosynthesis and insight influences crop performance and tolerance to diverse stress conditions. Other plant hormones have been shown to influence this equilibrium, either positively or negatively. It is also required to conduct post-translational analyses of the effect of ethylene and plant hormones on performance of plant (N. Iqbal et al., 2017).

2.9 ACO gene under Salinity Stress

It was found that both brief and lasting salt treatments improved the regulation of numerous *ACO*s in cotton. According to these findings, plants exposed to salinity and other biotic and abiotic pressures make more ethylene, mostly through the augmentation of *ACS*s and *ACO*s. Wheat *ACO1* transcipts were lowered by salinity and more abiotic stresses (Chen et al., 2014d).

ACSs and ACOs, two key enzymes for ethylene synthesis, are normally up regulated in salinity, albeit they may have negative consequences on plant salt response. The lack of function of *ACS7* in Arabidopsis boosted salt tolerance, encouraged vegetative development, and reduced ethylene emission. In Arabidopsis, salinity sensitivity was achieved through constitutive expression of wheat *ACO1*, probably by upregulating the AtMYB15 expression and downregulating certain stress-reactive genes such AtRAB18, AtCBF1, and AtCBF3. While the damage in function of mutant mkk9 was more resistant to salinity, MKK9 expression in

transgenic plants stimulated the endogenous MPK3 and MPK6 kinases, promoting the production of ethylene and camalexin, and eventually conferred enhanced salinity sensitivity (Xu et al., 2008).

TaACO1 expression was greatly elevated by *ACC*, which was considered to raise ethylene production. *TaACO1* protein constantly demonstrates activity of *ACC* oxidase in vitro. *TaACO1* overexpression plants exhibit smaller seeds when expressed constitutively in Arabidopsis. Because ethylene has long been thought to be a adverse controller of plant development, this observation propose that transgenic plants may generate additional ethylene because of higher ACC oxidase activity (Ecker 1995). Previously, it was discovered that acs7 seeds developed quicker than wild type seeds despite having a reduced ethylene level.

Similarly, the *TaACO1*-transgenic plants in our study accumulated considerable amounts of *TaACO1* transcripts; yet the ultimate seed propagation percentage did not differ between the *TaACO1* OE plants and the wild type. Within 48 hours, the seeds of the *TaACO1*-transgenic plants evolved somewhat gradual than those of the wild type and VC plants. Because imbibition can effectively drive *TaACO1* expression, the phenotype of *Ta*ACO1-transgenic plants may be the result of less successful imbibitions of smaller seeds. Alternatively, it was proposed that *Ta*ACO1-transgenic plants delayed germination could be due to ethylene-induced lignification, which reinforced seed cell walls Its precise role in the ethylene production process is uncertain (Chen et al., 2014b).

2.9.1 Cotton

ACO is an enzyme in the ethylene synthesis process and shows a crucial function in the adaptation of plant development and growth. Nevertheless, little consideration has been given to the role of ACO genes in cotton. G. hirsutum was discovered to have 332 *GhACOs* respectively. According to a gene duplication study, whole-genome duplication was the

primary processes compelling the synthesis of cotton ACO genes. There were cis-acting elements in *GhACO* promoters that reacted to stress, phytohormones and circadian cues, demonstrating that *GhACOs* may be involved in these techniques. According to expression and co-expression analyses, the majority of *GhACOs* were discovered to be extensively expressed in a variety of tissues.

Overexpression of GhACO106_At in Arabidopsis improved salt tolerance and promoted flowering. These findings require a complete outline of cotton's ACO genes and pave the way for future practical studies on these genes (Wei et al., 2021b).

2.9.2 Petunia

Recently, drought provoked expression of *ACO1* and ethylene synthesis in petunias were described. The fact that the WT was more stress-tolerant than the mutations in this investigation revealed that ethylene was needed for the petunia's stress retort and adaption. Editing the *PhACO1* or *PhACO3* genes also appeared to decrease ethylene production below the tolerance level mandatory for stress adaption, making the variations more vulnerable to stress than the WT. This discovery was relevant with previous study that found ethylene to be beneficial in other plant species' ability to survive abiotic stress (Naing et al., 2022).

2.9.3 Red Pepper (*Capsicum annuum L.*)

Because *ACO* substrate is *ACC,* decreases in the substrate level would result in lower *ACO* activities, lowering the quantity of ethylene produced by oxidation of *ACC*. Halotolerant bacteria used the enzyme *ACC* deaminase to hydrolyse *ACC* into ammonia and -ketobutyrate. Diminished ACC levels, *ACO* substrate, led in lower ethylene levels in stressed plants. Higher ACC concentrations in petals (Bufler and others 1980), higher ACC synthase and ACC oxidase

expression of both ACS (Park and others 1992) and ACO genes in senescing petals were related with increased ethylene production (Siddikee et al., 2012b).

2.10 Regulation of ACO gene in wheat

Understanding how the wheat ACO (1-aminocyclopropane-1-carboxylate oxidase) gene is controlled is critical for understanding how the gene influences the plant's response to environmental challenges such as salt stress. ACO genes produce enzymes involved in the synthesis of ethylene, a plant hormone vital for plant development and stress responses.

Numerous internal and external elements influence the tightly regulated process of ACO gene regulation in wheat. Understanding the regulatory systems that govern the expression of ACO genes is critical for establishing the function of ethylene in wheat's response to stress, namely salt stress.

2.10.1 Transcriptional Regulation

Transcription is the principal regulator of the ACO gene's expression. Certain transcription factors (TFs) are required for either activating or decreasing the expression of the ACO gene in salinity-stressed wheat plants. These transcription factors (TFs) identify and attach to certain promoter regions in the ACO gene, causing transcription to begin or halt (Rudus´• et al., 2012d).

2.10.2 Ethylene-Responsive Elements (EREs)

EREs (ethylene-responsive elements) are recognized in the promoter regions of ACO genes in wheat. EREs are specific DNA sequences recognised by TFs such as ERFs (ethylene response factors). In answer to salt stress or extra environmental inducements, ERFs bind to EREs, causing ACO gene transcription (Zhu et al., 2014)).

2.10.3 Epigenetic Regulation

Histone acetylation and DNA methylation are two epigenetic modifications that can influence the accessibility of ACO gene promoters to transcription factors. These modifications may either raise or reduce the expression of the ACO gene in response to stress (Prescott, 2000).

2.10.4 Post-Transcriptional Regulation

Following transcription, post-transcriptional regulatory mechanisms, like as microRNAs, can precisely regulate ACO gene expression by lowering or inhibiting *ACO* mRNA translation. This adds another level of control to ensure precise regulation.

ACO gene regulation in wheat is also altered by environmental factors like as temperature, light, and nutrition availability. These elements can change the transcriptional machinery and thus the ACO gene expression.

Numerous *ACO* transcriptional regulators from other animals have been discovered. *ACO* transcription is regulated similarly to *ACS* transcription by hormonal interactions including auxins and abscisic acid. There is evidence that *ACO* is both transcriptionally and post transcriptionally controlled at the mRNA transcript level (Pattyn et al., 2021).

2.10.5 Posttranslational regulation of ACO

The *ACO* protein family can be divided into three categories made on the amino acid sequence resemblance of the RXS motif, which is required for catalytic activity. Although the *ACS* post translational regulation is well characterised, there is a paucity of evidence on the *ACO* post

translational regulation. Although this was not tested, possible places for glycosylation and phosphorylation inside the *ACO* protein sequences was discovered. More facts of redox-specific posttranslational changes of certain *ACO* cysteine residues have been discovered. For instance, Arabidopsis *ACO1* has been demonstrated to be S-glutathionylated, however it is unclear how this modification impacts *ACO1* activity.

While S-sulfhydration of *SlACO1* and *SlACO2* was discovered to impede *ACO* enzyme action in tomatoes, Arabidopsis also showed S-Sul hydration of *ACO4*. S-nitrosylation, another cysteine modification of *ACO*, has been demonstrated in Arabidopsis and tomato). Two recent investigations using site-directed mutagenesis assays endorsed the consequence of redox-specific cysteine alterations in regulating *ACO* activity and fundamental strength. Taken together, the evidence for *ACO* being redox controlled in plants is growing stronger. It is still unknown what impact these cysteine alterations have in *ACO* stability or action. Protein-protein interactions allow *ACO* to function (Pattyn et al., 2021).

2.11 ACO gene and salinity stress in Wheat

Salinity stress, or high salt concentrations in the soil, is a severe threat to the world's wheat supply. Wheat is a vital cereal crop that is particularly prone to the detrimental effects of salt stress. Significant study has been conducted to determine how ACO genes function in the response of wheat to this stress. ACO genes are particularly noteworthy because they are involved in the production of ethylene, a plant hormone that is required for plant stress responses. This detailed note delves into the intricate relationship between wheat salinity stress and ACO genes.

2.11.1 ACO Gene Family in Wheat

Wheat contains ACO genes, which encode the ACO enzymes that convert ACC into the plant hormone ethylene. These ACO genes exhibit distinct temporal and spatial expression patterns in various wheat plant tissues and developmental stages (Kesawat et al., 2022).

2.11.2 Ethylene's Role in Salinity Stress

Ethylene, a versatile signalling chemical, regulates how plants respond to environmental stimuli such as salt stress. Wheat plants frequently demonstrate increased ethylene production as a stress response mechanism in high saline situations. Numerous studies have shown that ethylene production increases significantly in response to environmental stresses, leaf withering, and fruit ripening.

Ethylene interacts with nutrients and/or phytohormones, influencing several processes. It regulates photosynthesis, proline, sulphur, and nitrogen metabolism, as well as the synthesis of glycine betaine (GB) and the antioxidant defence mechanism that protects plants from environmental stresses. Ethylene participates in the creation of secondary metabolites that promote stress tolerance in stressful setting. Ethylene regulates plant metabolism to allow for stress tolerance (Fatma et al., 2022b).

2.11.3 ACO Genes as Ethylene Regulators

ACO genes are the key regulators of ethylene synthesis in plants. They catalyse the conversion of *ACC* to ethylene, and a variety of factors, including salt stress, can influence the amount of these genes produced.

Elevated ethylene production is frequently the result of increased ACO gene expression in response to salt stress. *ACS* was first assumed to be the pathway's rate-limiting enzyme, prompting extensive studies into the control of ACS protein stability and activity. Nevertheless, a growing body of evidence suggests that *ACO* is the rate-restraining step in the assembly of ethylene throughout specific focused operations. This shows that the *ACO* protein family is also subject to severe control.

Because all vegetative tissues express ACC-oxidase to varying degrees, it was once thought that *ACO* proteins were constantly extant and set to generate ethylene. Moreover, applying *ACC* to plant tissue usually causes a quick ethylene synthesis. Thus, it has been suggested that the rate-limiting enzyme in the production of ethylene is not *ACO* but rather *ACS* (Adams and Yang, 1979). The community has accepted this theory with ease, which has resulted in a profusion of research endeavours aimed at elucidating the regulation and operation of ACS concerning its primary function in the generation of ethylene). Nonetheless, an expanding body of research indicates that *ACO* rather than *ACS* is crucial for managing ethylene production in plants.

Strong overexpression of the related ACO genes has also been linked to ethylene-induced cotton fibre cell elongation. *ACO* has recently been discovered to have a critical role in determining the sex of cucumber blooms. According to these studies, *ACO* can be rate-limiting, which means it regulates ethylene production. This shows that ACO expression, stability, and/or activity are governed by a stringent regulatory system.

2.11.4 Physiological Effects of Ethylene in Salinity Stress

Wheat's response to salt stress is greatly influenced by ethylene, which affects a variety of physiological processes:

Stomatal closure: In salty settings, ethylene can cause stomatal closure to reduce water loss and conserve water resources. It influences the structure and growth of the roots, which may improve the plant's ability to absorb nutrients and water.

Antioxidant defence: Ethylene can reduce oxidative damage caused by salinity stress by activating antioxidant systems.

Phytohormones that interact with ethylene and are important for adaptation to salt stress include auxin (IAA), cytokinin (CK), and abscisic acid (ABA). In response to environmental changes, calcium-dependent protein kinases and MAPK signalling cascades are activated in tandem, and their partial overlap contributes to the expression of stimulus-specific response. Ethylene is responsible for promoting communication between these two pathways and inducing the desired response (Ludwig et al., 2005). Furthermore, gibberellins and ethylene have an antagonistic connection that influences salt tolerance by either activating or deactivating the defence system.

Until date, research on ethylene and salinity stress has established that ethylene levels in plants can impact how they respond to salt stress in either a good or negative way. This shows that plants' ethylene action may need to be fine-tuned to tolerate salinity stress. Future study could focus on dissecting ethylene-induced signalling during salt stress utilising a multi-omics approach. Furthermore, future study should concentrate on understanding how posttranslational changes affect how ethylene signalling is regulated in settings including salt stress. Plant biologists must be motivated by compelling experimental findings to continue their research on the biological function of ethylene under salinity stress (Riyazuddin et al., 2020).

2.11.5 Implications for Salinity Tolerance

The ethylene pathway and the ACO genes are substantially responsible for wheat's tolerance to salinity stress.

Understanding the control of ACO genes and their interaction with the ethylene pathway is critical for developing strategies for increasing salinity tolerance in wheat through genetic engineering and breeding efforts (Tao et al., 2015).

2.12 Challenges and Future Directions

Understanding how ACO genes affect wheat's response to salt stress is a developing subject of research.

Subsequent research efforts are expected to focus on optimising *ACO* gene expression to improve salt tolerance while conserving other essential plant functions (Nongpiur et al., 2016).

2.12.1 Prospects for ACO Gene Research in Wheat

It's probable that different *ACO* homologs play diverse roles in the body's response to salt stress. Subsequent research will attempt to unravel the different activities of each ACO gene and their contributions to salt tolerance.

Wheat's ACO (1-aminocyclopropane-1-carboxylate oxidase) genes have the potential to improve crop output and stress tolerance, particularly when evaluated in the context of salinity stress. As this field of study grows, many potential future directions and issues of interest become apparent. It’s probable that different *ACO* homologs play diverse roles in the body's response to salt stress. Subsequent research will attempt to unravel the different activities of each ACO gene and their contributions to salt tolerance.

Integrating many omics approaches, such as transcriptomics, proteomics, metabolomics, and genomics, will provide a comprehensive understanding of ACO gene control and operation in the context of salinity stress (Ullah et al., 2022). This all-encompassing technique will reveal intricate gene networks and pathways. Examining the epigenetic control of ACO genes, which includes histone modifications and DNA methylation, will reveal how environmental factors, such as salt stress, influence gene expression. Understanding these pathways allows for the refinement of ACO gene responses.

The development of precise gene editing tools such as CRISPR-Cas will allow for targeted modifications of ACO genes to improve their performance under salt stress.(Erdoğan et al., 2023).Future research may investigate multigene approaches that target many genes involved in ethylene synthesis, signalling, and downstream responses to improve salt tolerance.

A comparison of the ethylene pathways and ACO genes in different wheat varieties and comparable cereal crops can provide valuable information about evolutionary adaptations and potential breeding gene targets. If the research findings are successful in the real world, they could lead to the development of salt-tolerant wheat cultivars that can grow in saline soils, boosting global food security in salt-affected areas. ACO gene research will contribute to climate-adaptable agriculture. Wheat farmers with improved salt tolerance can withstand changing environmental conditions such as growing salinity caused by climate change.

Prospects call for increasing collaboration among wheat ACO gene researchers, groups, and institutes. This area of research will move faster. Wheat ACO gene research has a promising future, with the potential to make significant advances to sustainable agriculture and global food security. Finally, salt-tolerant wheat varieties and novel solutions will emerge from a detailed investigation of ACO genes and how they are regulated under salinity stress, benefiting both farmers and consumers. Utilizing research findings in wheat breeding and crop

management will be critical in ensuring wheat production's tolerance to environmental challenges as the field evolves.(Li et al., 2021).

Objectives of Research

- **1.** To identify the ACO gene family in *Triticum aestivum* (wheat) to understand its molecular diversity
- **2.**To analyse the expression patterns of ACO genes in response to salinity stress

Chapter 3: Materials and Methodology

3.1 In silico approach

3.1.1 Sequence retrieval

To perform the comparative investigation, *Oryza sativa* was used as model plant and its sequences were recovered from Rice Genome Annotation Project. The amino acid sequences of OsACO gene were used as queries to retrieve against *T. aestivum* via the Ensemble Plants Database (https://plants.ensembl.org/index.html) (Garcia et al., 2021).

3.1.2 Conserved Domain confirmation

The second stage of our inquiry was conserved domain confirmation. The retrieved amino acid sequences were further verified for the presence of functional *ACO* domains using NCB-CDD search. Later, the domains visualization was done using Tbtools. (Conserved Domains Database (CDD) and Resources, n.d.).

3.1.3 Primer designing

Primer designing was done using online available tool, primer 3. The parameters to design primers were set to 18 to 22 nucleotides length, 40-60% GC content, and 55-60 degree melting temperatures (Tm). To avoid the creation of primer-dimers and self-complementarity the primers were further checked by primer stat (Untergasser et al., 2007).

3.1.4 Phylogenetic Analysis of *ACO* gene

We employed the MUSCLE approach with the default settings to align the full amino acid sequences of the *ACO* and Oryza sativa proteins to study their evolutionary relationships. Following alignment, we used the NG Phylogeny platform (https://ngphylogeny.fr/) to create phylogenetic trees. To ensure statistical robustness, we used a maximum likelihood technique based on the Poisson replacement model, which comprised 1000 bootstrap samples. (Dunn et al., 2013)

3.1.5 Motif and Gene structure Analysis

The genomes and entire coding sequences (CDS) of each ACO gene were retrieved from Ensemble Plant to study the exon-intron architecture. The CDS sequences of the ACO genes can be determined using the Gene Structure Display Server by comparing them to the matching genomic sequences. Gene structure display server was used to provide a full graphical interpretation of the exon-intron layout. (GSDS 2.0) (http://gsds.gao-lab.org/) (Kesawat et al., 2022b).

3.2 Wet lab

Three varieties of wheat seeds (Bakhar star, Markaz -19, Faisalabad-08) were obtained from National Agricultural Research centre (NARC), oil, seed and research department in Islamabad.

3.2.1 Sterilization and Germination of *T. aestivum* seeds

Following the selection of healthy seeds, they were surface sterilized for 30 minutes with 1% sodium hypochlorite They were then thoroughly rinsed with autoclaved distilled water for five minutes. Using forceps and moist autoclaved filter paper, the seeds were inoculated in 125 mm autoclaved glass Petri plates (Whatman No 40). Petri plates were kept at 25°C in the dark for 48 hours. After that, the plates were placed in a 25°C growth room with a diurnal cycle of 16 hours of light and 8 hours of darkness. Using autoclaved water, the filter paper was kept moist during the experiment. On the fourth day, seedlings of the same size were chosen for transplantation

3.2.2 Set-up of Hydroponic System

The hydroponic system was comprised of three black boxes measuring six inches in length, twelve inches in width, and six inches in depth. Each box held 3.5 L of fluid and included 32 one-inch-diameter lid holes. Each hydroponic box was supplied with pump having a power range of 220-240 volts.The air pressure was controlled by a knob, and the tubes were connected at a distance of ten inches apart.

A standard Lombnaes medium was designed for cultivating *T. aestivum*. All glassware was dried after being rinsed with de-ionized water. The stock solution for each nutrient was prepared and added to the medium in accordance with the final concentration. The pH of the solution was 5.7. Details of all the chemicals mixed in the solution are mentioned in the table.

Table I Composition of stock solutions for Lombnaes media

Stock solutions (500ml each)	**Final concentration in media**	**Volume per liter in media**
MES	1 mM	195.2 mg
10 mM MnCl2.4H2O	0.6 µM	60 µl
1 M Ca (NO3)2.4H20	2 mM	1 ml
1 M KNO3	1mM	1 ml
80 mM KH2PO4	80 µM	1 ml
0.5 M MgSO4.7H2O	0.5mM	1 ml
0.9 M NaOH	0.9mM	1 ml
75 mM Fe (NO3)3	75 µM	1 ml

3.2.3 Glass house Growth Conditions

Every plant was cultivated with 8 hours of light each day at 21°C and 16°C at night. The humidity in a controlled atmosphere is 55% during the day and 65% at night, and the light level is 220 mol m-2 s-1 in the glasshouse.

3.2.4 Plantation of Wheat in Hydroponic media

Following the selection of healthy seeds, they were surface sterilized for 30 minutes with 1% sodium hypochlorite They were then thoroughly rinsed with autoclaved distilled water for five minutes. Using forceps and moist autoclaved filter paper, the seeds were inoculated in 125 mm

autoclaved glass Petri plates (Whatman No 40). Petri plates were kept at 25°C in the dark for 48 hours. After that, the plates were placed in a 25°C growth room with a diurnal cycle of 16 hours of light and 8 hours of darkness. Using autoclaved water, the filter paper was kept moist during the experiment. On the fourth day, seedlings of the same size were chosen for transplantation.

3.2.5 Growth under Salinity stress

The plants were harvested after 28 days of growth on normal Lombnase medium and divided into four groups: control (0 mM NaCl), low-stress treatment (100 mM NaCl), medium-stress treatment (150 mM NaCl), and high-stress treatment (200mM NaCl). The plants were cultivated on that medium for a total of 28 days.

3.2.6 Sampling and Collection of Data

28^{th} day after the transplantation, morphological data of three replicates were obtained. The overall length, root and shoot lengths in centimetres, total fresh weight, shoot weight, and root weight in grams were all measured. For biochemical analysis and RT expression, the shoot and root samples were immediately placed in liquid nitrogen, then stored at -80 degrees Celsius for further analysis.

3.2.7 RNA Extraction

For this work, total genomic RNA was isolated from roots and shoots using the TRIzol reagent. Using liquid nitrogen and an autoclaved pestle and mortar, 200 mg of fresh material (leaf and root separately) were crushed to a fine powder.

Each sample tube was loaded with 1 ml of Trizol and placed in a 1.5 ml micro-centrifuge tube. The tubes were shaken for 15 seconds before being placed in a 4°C incubator for 10 minutes. In an Eppendorf centrifuge (5810R), sample tubes were centrifuged for 10 minutes at 14000rpm and 4°C.

After that, the supernatant was moved to a brand-new 1.5 ml micro-centrifuge tube. For the chloroform phase separation, 200 l of chloroform (Sigma ALDRICH®, Germany) was applied. After being manually agitated for 15 seconds, the supernatant was kept at 4°C for two to three minutes. The tubes were then centrifuged for 15 minutes at 4°C on a 14000-rpm machine. The upper aqueous phase was transferred to a new 1.5 ml micro-centrifuge tube (Rio et al., 2010).

After adding an identical volume of 100% isopropanol (Sigma ALDRICH®, Germany), the mixture was incubated at 4°C for 10 minutes to precipitate the RNA. To extract the RNA, the sample was spun at 14000 rpm for 15 minutes at 10°C. The RNA pellet was cleaned in 75% ethanol for five minutes at 4°C and 7500 rpm. Following that, the pallet was immersed in 30 microliters of RNase-free water.

3.2.8 cDNA synthesis

To avoid genomic contamination, the isolated total genomic RNA from the roots and shoots was treated with DNase enzyme (Thermo Scientific, Lithuania) according to 34. agreement with the manufacturer's specifications. To make first-strand complementary DNA (cDNA), an RNA sample treated with the DNase enzyme was used. A 200 L PCR tube was filled with one microgram of isolated total RNA. The riboLock RNase Inhibitor Enzyme (20 u/L), dNTPs Mix (10 mM), reversed reverse transcriptase Enzyme (200 u/L), and 4 L of 5x reaction buffer were then added.

The final 20 liters were made with RNase-free water. The tubes were quickly spun in a mini-centrifuge and then incubated for 60 minutes at 42 °C, five minutes at 70 °C, and thirty minutes at 4 °C in a fast TM Max Pro. Thermal Cycler by ESCO® Micro Pte. Ltd. After the cycle was completed, the cDNA tubes were kept at -80 °C until the expression analysis. The reagents and tubes used in the cDNA synthesis procedure were always kept on ice.

3.2.9 q-RTPCR

The expression of ACO genes in hydroponically grown wheat tissue samples was studied using qRT-PCR with EvaGreen qPCR Mix Plus (Solis BioDyne, Estonia).

Table 2: Ingredients of qrtPCR

Reagents	**Volume**
Template DNA	1 µl
Forward primer	0.2 µl
Reverse primer	0.2 µl
5x HOT FIREPOL EvaGreen qPCR Mix Plus	2.5 µl
H2O PCR grade	1.1µl
Total	5µl

3.3 Qualitative analysis of Phytochemicals

Detection of phytochemicals in leaf extracts of *Triticum aestivum* was carried out according to methods described by (*(PDF) Determination of Antioxidant Properties and the Bioactive Compounds in Wheat (Triticum Aestivum L.)*, n.d.).

3.3.1 Detection of alkaloids

The presence of Alkaloids was checked by adding Mayers reagent. In 2ml of methanolic extract 2ml Mayers reagent was added, and sample was checked for white precipitate formation.

3.3.2 Detection of Phenols and Tannins

Tannin's presence was tested by using Braymer's test**.** An equal proportion of plant extract and 10% ferric chloride was fused and examined for the appearance of red, green or blue colour showing presence of phenol. The appearance of dark blue coloration shows hydrolysable tannins while green colour formation shows condensed tannins.

3.3.3 Detection of flavonoids

Using quercetin equal volume of plant extract, as a positive control, 20% NaOH were added in it and analyzed for the appearance of yellow to orange colour. By adding HCL in it makes it transparent (Ali Redha et al., 2018).

3.3.4 Detection of Deoxy sugars

A few drops of FeCl3, 2 ml of acetic acid, 1 ml of H2SO4, and 2 ml of methanolic extract were combined. A dark ring forms in the extract, indicating the presence of deoxy sugars.

3.3.5 Detection of Carbohydrates

Fehling's test was used to detect the presence of carbohydrates. 1 millilitre of Fehling solutions A and B were heated and mixed with 2 millilitres of methanolic extract. Red precipitates indicate the presence of carbohydrates.

3.3.6 Detection of saponins

The Emulsion test was done to check whether saponin was present. A few drops of olive oil were combined with 5 mL of methanolic extract. The production of an emulsion was noticed.

3.4 Phytochemical Quantification

The three extracts of plant taken either from hydroponics or from soil were analyzed for checking total phenol, flavonoid, tannins, and carbohydrates estimation in accordance with methods described below (Oshadie et al., 2017).

3.4.1 Total Flavonoid Estimation

100µl of 10% Aluminum chloride in methanol was mixed with 500µl of 4mg/ml plant extract and to make certain of acidic environment 100µl of sodium acetate was added. The absorbance was measured at 415nm after placing it in dark for 40min of incubation. Quercetin was taken as standard under the same conditions. The blank was extracts without adding AlCl3.

3.4.2 Total Phenol Estimation

Taken 500µl of plant extracts was dissolved with 150µl of Folin Ciocalteu reagent and was allowed to stand for 5min. 500µl of 1M Na2 CO3 was assorted in samples and vortexed to mix well. The absorbance at 660nm was checked after placing sample in darkness for 1hour. Gallic acid was used as standard under the same condition.

3.4.3 Total Tannin estimation

Tannic acid was taken as standard to estimate total tannin estimation. 500µl of 4mg/ml extracts was dissolved with 200µl of FeCl3 and then with 200 µl of potassium ferrocyanide. The samples absorbance was taken within 10 minutes at 395nm.

3.4.4 Total Carbohydrates estimation

Using modified Anthron method, total carbohydrates content was quantified. D- glucose was taken as standard. 2ml of anthron reagent was added in 500 µl of extracts (4mg/ml). The solutions were placed in boiling water bath for 10min and then rapidly cooled under tap water to measure absorbance at 630nm.

Chapter 4: Results

4.1 Insilico Results

4.1.1 Identification and sequence retrieval of ACO gene in *T. aestivum*

To observe the *ACO* genes in wheat, the amino acid sequences of ACO genes in Arabidopsis thaliana were utilised as query sequences against the ACO genes of *T. aestivum* in a BLASTp homology search. After removing more redundant hits with minimal e-values
6 ACO genes—known as *ACO 2* through *ACO 7*—were identified in *T. aestivum*. Using InterProScan, each *ACO* gene was discovered to include a2OG-FeII_Oxy domain as well as DIOX_N domain, which is typical of this gene family.

4.1.2 Phylogenetic Analysis of ACO gene

To explore the evolutionary relationship and sequence homology of *Oryza sativa* and *T. aestivum* Trihelix protein sequences, a maximum likelihood phylogenetic tree with 1000 bootstrap replicates was created. The phylogenetic tree revealed that *TaACO* genes were classified into six groups. The ACO gene family's proteins range in length from 477 to 1007 amino acids. The tree was constructed using Ng phylogeny (**Fig 9**) (https://ngphylogeny.fr)

4.1.3 Gene structure (Introns exons) analysis

The wheat ACO gene structure is made up of exons, introns, and untranslated regions. Gene structure is envisioned using gene structure display server (Fig 6). Gene structure evaluation of *TaACOs* has displayed a unique evolutionary path. The TaACO gene family is extended in the wheat and comprised other ACO genes contrasted to the prior depicted ACOs in *A. thaliana*.

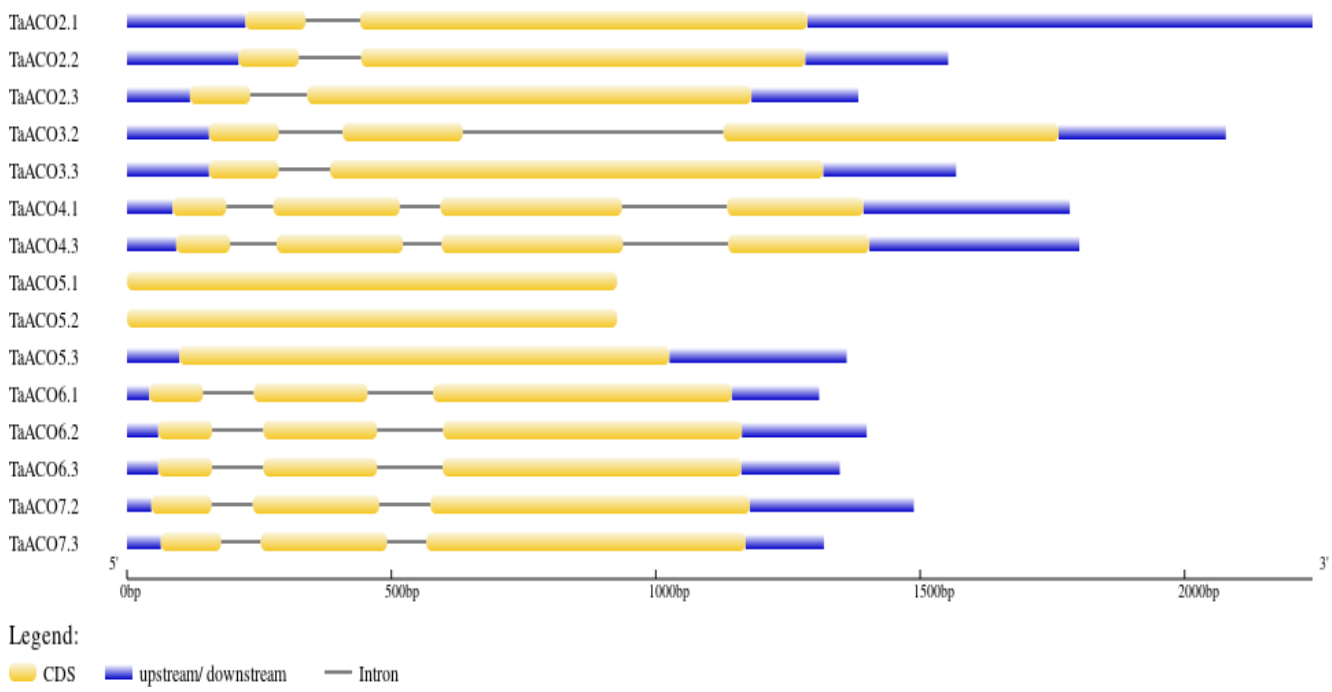

FIGURE 5:GENE STRUCTURE ANALYSIS OF ACO GENE

All the sequences contain a minimum of 1 intron and maximum of 3 introns. TaACO 5.1 and TaACO 5.2 do not contain any intron. (GSDS, http://gsds.cbi.pku.edu.cn)

4.1.4 Motif and Domain Analysis

A total of 10 motifs were discovered in ACO gene (**Fig 7**). Motif 1,2,3 ,4 ,5,6,7,8 and 9 were present in *TaACO 2, TaACO 3, TaACO 4, TaACO 5, TaACO 6* and *TaACO 7* while motif 10 was present in only *TaACO 2*. Motif 1,2,3,4,5,6,7,8 and 9 are highly conserved. The motif analysis was done using MEME suite software. (https://meme-suite.org/meme/)

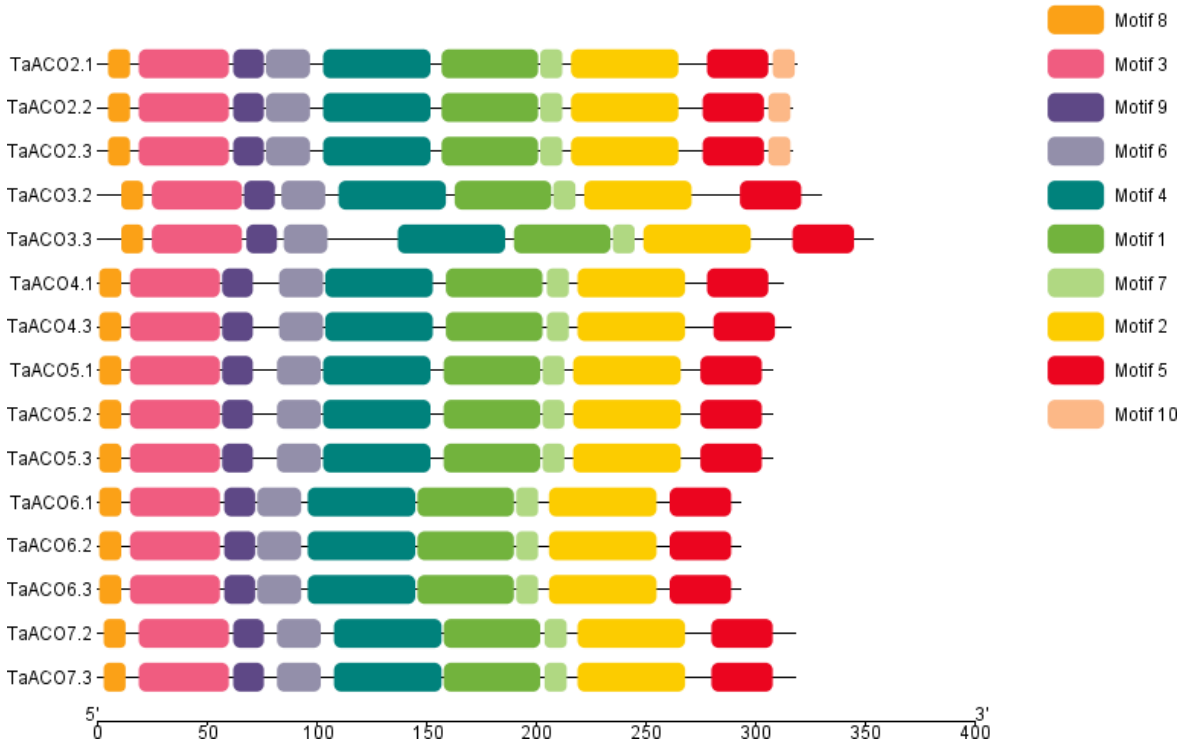

FIGURE 6:MOTIFS ANALYSIS OF ACO GENE

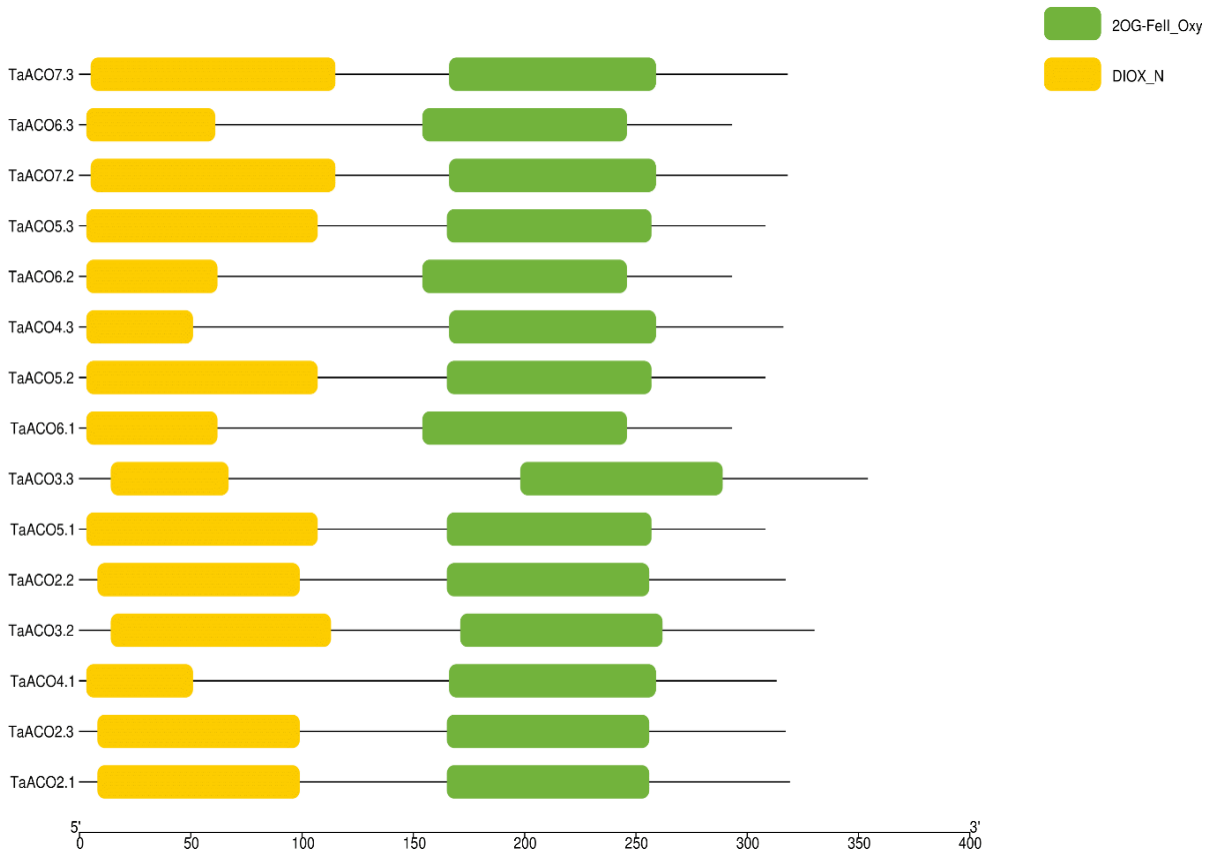

FIGURE 7: DOMAIN ANALYSIS OF ACO GENE

The domains 2OG-Fell_Oxy and DIOX_N are present in all sequences of *TaACO*2,3,4,5,6 and 7. They are highly conserved domains. The domains were analysed by using NCBI cdd software. (**Fig 8**) (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml)

FIGURE 8:PHYLOGENETIC TREE AND MOTIF ANALYSIS

4.1.5 Subcellular localization of protein

4.1.5.1 Heat map

The subcellular localization of proteins was established using a heat map approach (**Fig 10**) According to the study's findings, *ACO 4.1,4.3 and 5.1* are largely located in the cytoplasm. *ACO* 3.3 was discovered to be subcellularly localized in the chloroplast. *TaACO* 6.3 in the cytoskeleton.

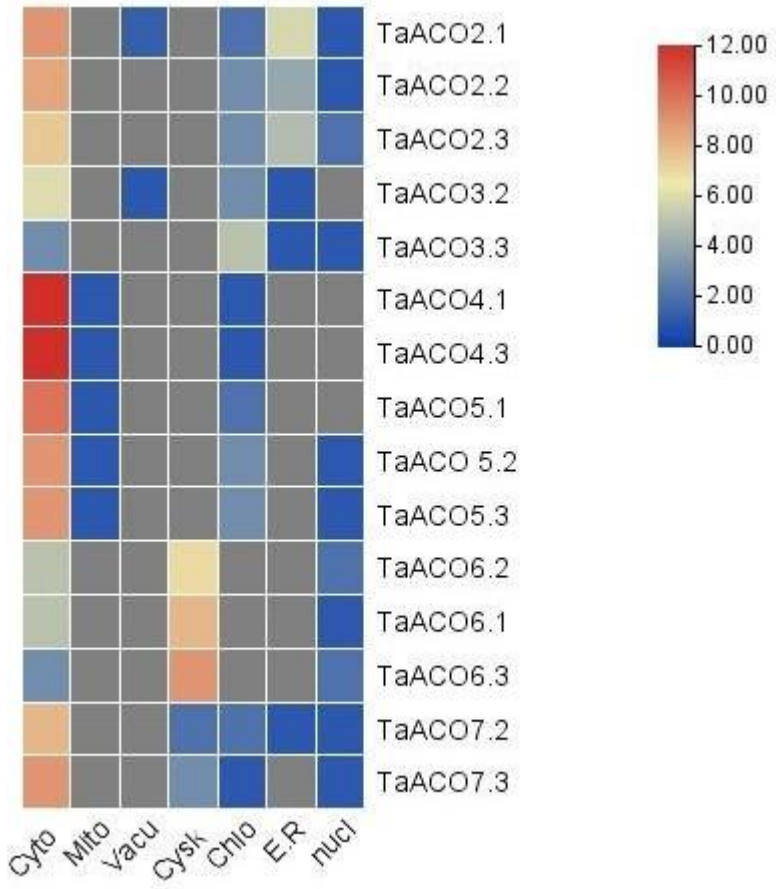

FIGURE 9:SUBCELLULAR LOCALIZATION OF ACO PROTEIN

4.1.6 Protein Similarity

TaACO 2.1,2.2 and 2.3 show protein similarity, *TaACO* 3.2 and *TaACO*3.3 are similar to each other. *TaACO* 4.1 and *TaACO* 4.3 show resemblance meanwhile *TaACO* 5.1,5.2 and 5.3 are similar. *TaACO* 6.1,6.2 and 6.3 are linked and *TaACO* 7.2 and *TaACO*7.3 have protein similarity. *TaACO* 4.1 ,4.3 ,5.1 .5.2,5.3,6.1,6.2,6.3 does not show that resemblance with *TaACO* 2.1,2.2,2.3,3.2,3.3. TaACO 6.1,6.2,6.3,7.2 and 7.3 show least similarity with others.

	TaACO2.1	TaACO2.2	TaACO2.3	TaACO3.2	TaACO3.3	TaACO4.1	TaACO4.3	TaACO5.1	TaACO5.2	TaACO5.3	TaACO6.2	TaACO6.1	TaACO6.3	TaACO7.2	TaACO7.3
TaACO2.1	100	98.12	97.18	80.65	74.93	63.88	62.99	63.94	63.64	64.85	62.39	62.08	61.77	60.54	58.21
TaACO2.2	98.12	100	97.79	81.55	75.21	64.37	64.18	65.24	64.94	65.85	64.8	64.49	64.17	61.21	58.04
TaACO2.3	97.18	97.79	100	81.25	75.21	64.16	63.96	65.05	64.95	65.65	62.7	63.55	63.24	60.91	60.12
TaACO3.2	80.65	81.55	81.25	100	85.24	60.52	60.69	63.53	62.94	63.53	59.23	59.52	58.63	56.57	56.29
TaACO3.3	74.93	75.21	75.21	85.24	100	54.3	54.32	58.4	57.69	58.4	54.32	54.6	53.76	55.08	53.74
TaACO4.1	63.88	64.37	64.16	60.52	54.3	100	97.47	76.51	76.73	77.46	54.6	54.6	54.29	53.57	53.45
TaACO4.3	62.99	64.18	63.96	60.69	54.32	97.47	100	75.63	75.79	76.1	53.96	53.96	53.05	53.61	52.69
TaACO5.1	63.94	65.24	65.05	63.53	58.4	76.51	75.63	100	98.38	98.38	57.55	57.55	56.92	57.14	57.14
TaACO5.2	63.64	64.94	64.95	62.94	57.69	76.73	75.79	98.38	100	99.03	58.41	58.41	57.78	57.45	58.05
TaACO5.3	64.85	65.85	65.65	63.53	58.4	77.46	76.1	98.38	99.03	100	57.86	57.86	57.23	57.75	58.05
TaACO6.2	62.39	64.8	62.7	59.23	54.32	54.6	53.96	57.55	58.41	57.86	100	100	98.98	64.78	64.47
TaACO6.1	62.08	64.49	63.55	59.52	54.6	54.6	53.96	57.55	58.41	57.86	100	100	98.98	65.09	64.78
TaACO6.3	61.77	64.17	63.24	58.63	53.76	54.29	53.05	56.92	57.78	57.23	98.98	98.98	100	64.78	64.47
TaACO7.2	60.54	61.21	60.91	56.57	55.08	53.57	53.61	57.14	57.45	57.75	64.78	65.09	64.78	100	96.23
TaACO7.3	58.2	58.56	60.12	56.29	53.74	53.45	52.69	57.14	58.05	58.05	64.47	64.78	64.47	96.23	100

FIGURE 10:: PROTEIN SIMILARITY OF ACO GENE

4.2 Morphological Analysis

4.2.1 Stress application and Sampling of plants

The plants were harvested after 28 days of growth on Lombnase medium and divided into four groups: control (0 mM NaCl), low-stress treatment (100 mM NaCl), moderate treatment (150 mM NaCl), and toxic treatment (200mM NaCl). After 28th days of growth morphological data of three replicates were obtained. The overall length, root and shoot lengths in centimeters, total fresh weight, shoot weight, and root weight in milligrams were measured. For biochemical analysis and RT expression, the shoot and root samples were harvested and instantly placed in liquid nitrogen, then stored at -80 degrees Celsius for further analysis.

4.2.2 SPAD

The graph shows SPAD (Soil Plant Analysis Development) of different wheat varieties under varying different level of doses (mM of salinity doses). Bakhar star shows the highest level of

Spad values. With a relative increase in salinity concentration, the spad value declines in comparison with control in three varieties. Bakhar star has a higher spad value than FSD-08. Likewsie FSD-08 has high spad value than Markaz-19.

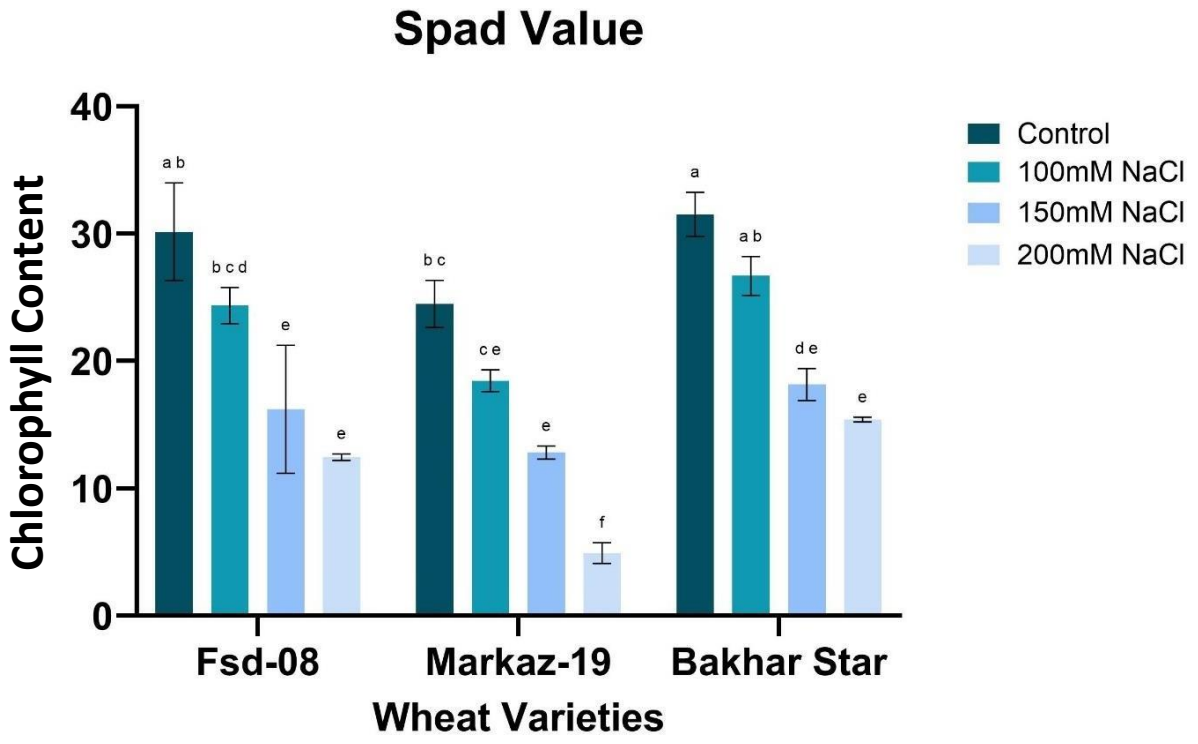

FIGURE 11:SPAD VALUE OF WHEAT VARIETIES

4.2.3 Shoot length

The graph depicts shoot length of three wheat varieties under different treatment of salinity doses. In all three wheat varieties, higher salinity doses(200mM) frequently result in shorter shoot lengths; the control variety(0mM) has the longest shoot length. Bakhar star has the highest shoot length in comparison with FSD-08 and Markaz-19. FSD-08 shows lowest shoot length.

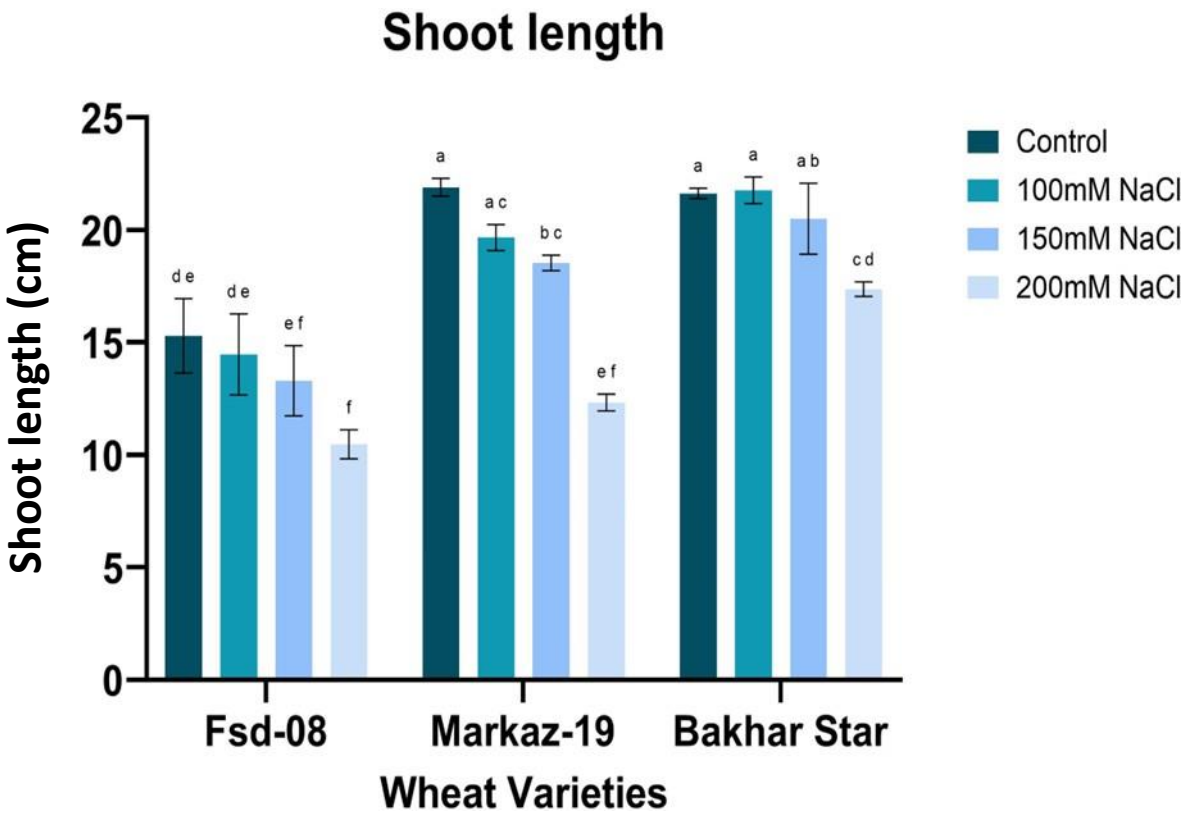

FIGURE 12:SHOOT LENGTH OF WHEAT VARIETIES

4.2.4 Root length

The graph depicts the root length of wheat varieties under different stress treatments. Higher doses typically reduce the root length in all three wheat varieties, with the longest roots being the control type. This suggests that the maximum dose (200mM) significantly limits root growth, although lower doses(150mM,200mM) may have insignificant side effects. The statistical significance letters support the idea that shorter root lengths correspond to higher dosages. Bakhar star showing longest root length in comparison with FSD-08 and Markaz-19. FSD-08 and Markaz-19 almost follow the same trend of root length variation.

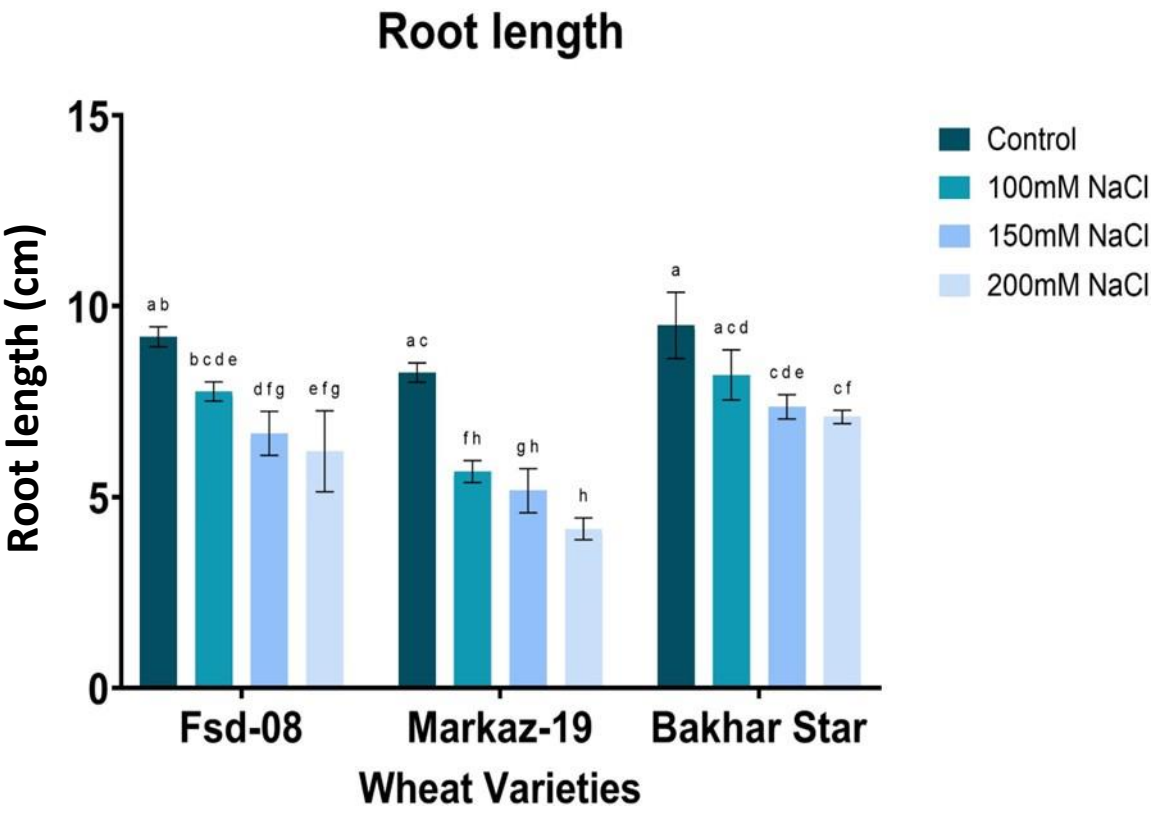

FIGURE 13:ROOT LENGTH OF WHEAT VARIETIES

4.2.5 Shoot fresh weight

The control condition of Bakhar star yields the highest shoot fresh weight, significantly exceeding any other dosage. Weight decreases as dosages increase(150mM,200mM); Dose 3(200mM) has the lowest weight. Bakhar star, being tolerant shows highest fresh weight than FSD-08 and Markaz-19. As the salinity treatment dose increases, the shoot fresh weight of all three wheat varieties declines. When using the Control condition, all varieties consistently produce the higher shoot length.

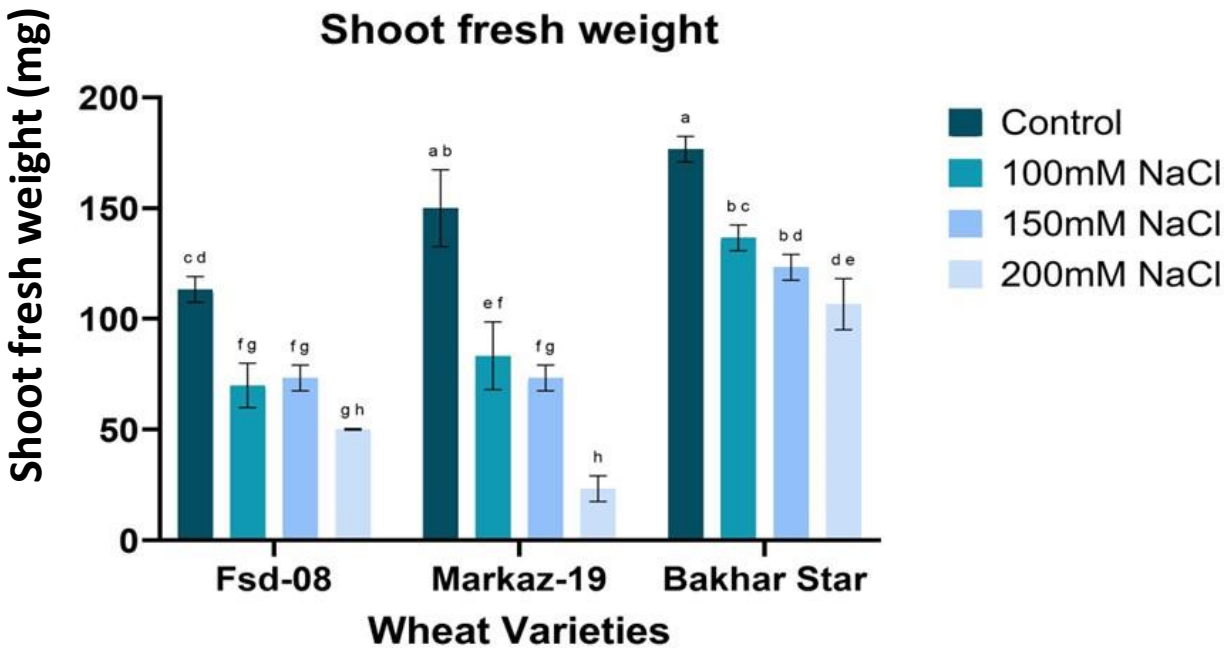

FIGURE 14:SHOOT FRESH WEIGHT OF WHEAT VARIETIES

4.2.6 Root fresh weight

Bakhar Star, in all doses, has a relatively high root fresh weight, with the highest in the control condition. Fresh root weight of FSD-08 decreases with increasing salinity dose, with the highest weight under Control and the lowest weight under Dose 3. Markaz-19, Like Fsd-08, it shows a decreasing trend as dosages are increased, with the Control condition having the highest weight. In the Control condition, all three varieties usually have the highest root fresh weight. In Fsd-08 and Markaz-19, there is a noticeable drop in root fresh weight as salinity treatment dosages are increased. Bakhar Star maintains a more consistent weight than the other two varieties.

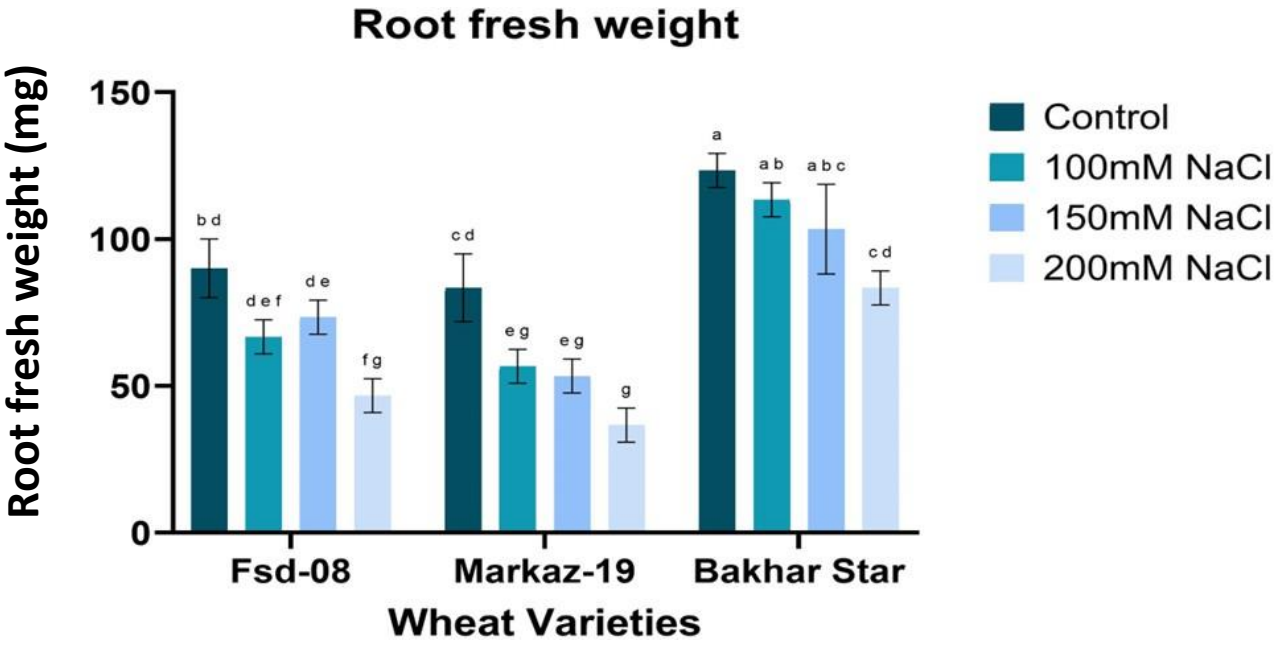

FIGURE 15:ROOT DRY WEIGHT OF WHEAT VARIETIES

4.2.7 Shoot dry weight

Bakhar Star shows a decrease in shoot dry weight from Control to salinity Dose 1(100mM), followed by a slight increase at Dose 2(150mM), and finally a significant decrease at Dose 3(200mM). FSD-08 indicates a slight increase at Dose 1, then further declines at Dose 2 before decreasing at Dose 3. Markaz-19, From Control to Dose 3, the shoot dry weight remains relatively consistent decreasing. At the maximum salinity treatment dose (200mM), all three types show a decrease in shoot dry weight. Bakhar shows the highest trend than FSD-08 and Markaz-19.

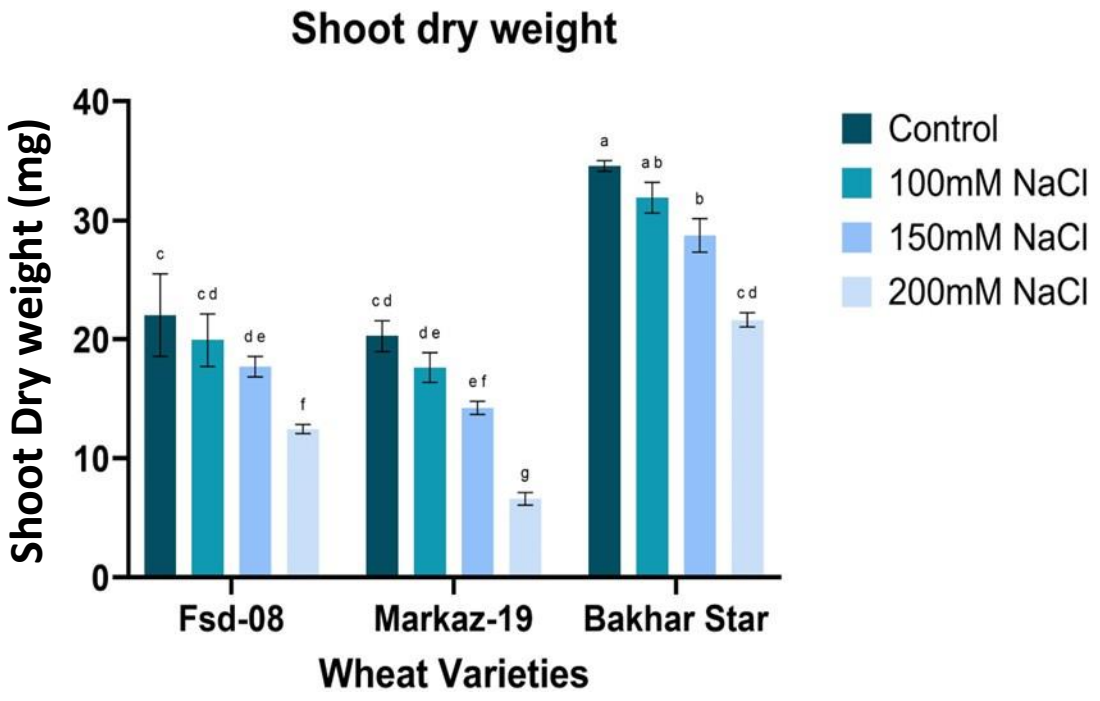

FIGURE 16:SHOOT DRY WEIGHT OF WHEAT VARIETIES

4.2.8 Root dry weight

Bakhar star Shows a decrease in root dry weight between salinity Dose 3(200mM) and Control. The weight is highest in the Control condition, and lowest in Dose 3. FSD-08 root dry weight decreases from Control to Dose 3, just like Bakhar Star. With a significant decrease in Dose 3, the Control condition has the highest weight. Markaz-19, there is a considerable decline in root dry weight between salinity Dose 3 and Control. Compared to all other doses, the weight under control is significantly higher, with Dose 3 being the lowest. There is a general trend in all varieties of decreasing root dry weight as salinity dose level increases, indicating that higher doses may be detrimental to these wheat varieties' ability to generate roots.Bakhar star shows highest root dry weight than FD-08 and Markaz-19 . Markaz-19 has the lowest root dry weight compared to others.

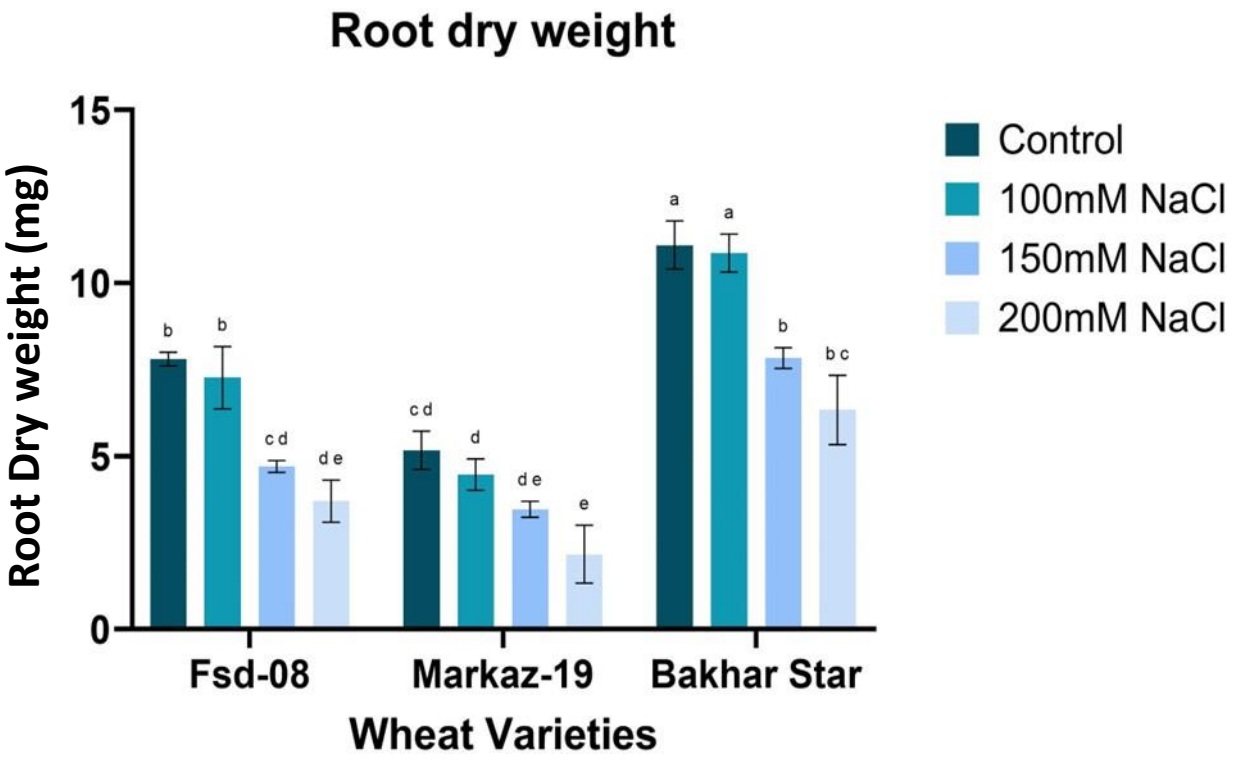

FIGURE 17:ROOT DRY WEIGHT OF WHEAT VARIETIES

4.3 Phytochemical analysis

Codes: FSD-C: Faisalabad control

FSD-D1: Faisalabad Dose1

FSD-D2: Faisalabad Dose2

FSD-D3: Faisalabad Dose3

M-C: Markaz Control

M-D1: Markaz Dose1

M-D2: Markaz Dose2

M-D3: Markaz Dose3

B.s-C: Bakhar Star Control

B.s-D1: Bakhar star Dose1

B.s-D2: Bakhar star Dose2

B.S-D3: Bakhar star Dose3

Table 3: Phytochemical Analysis

Compounds	FSD-C	FSD-D1	FSD-D2	FSD-D3	M-C	M-D1	M-D2	M-D3	B.S-C	B.S-D1	B.S-D2	B.S-D3
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+
Phenol	+	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-
Flavanoids	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+	+	+

4.3.1 Carbohydrate estimation

Graph displays the carbohydrate quantity present in three varieties of wheat. Bakhar Star control has the highest amount of carbohydrate estimation while Fsd-08, all salinity doses, has the lowest amount of carbohydrate quantity. Markaz 19 has the moderate amount of

carbohydrate present, with exception of Markaz-19 control (0mM), showing high amount of carbohydrate present.

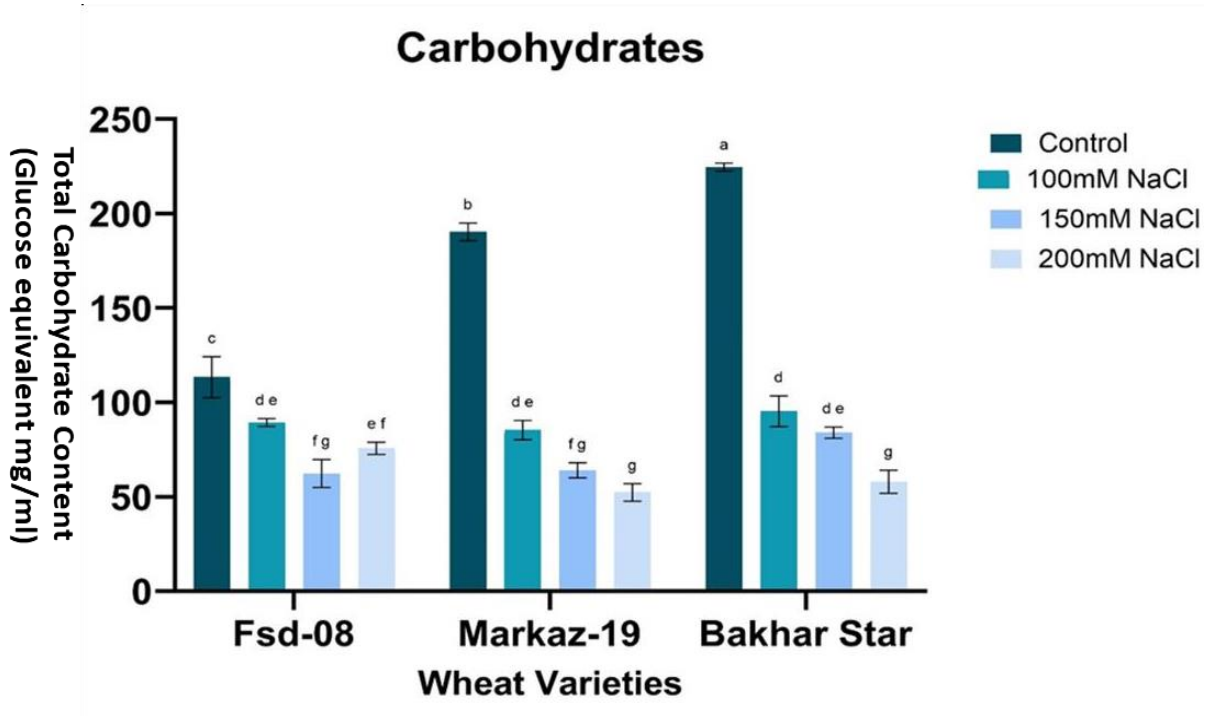

FIGURE 18:CARBOHYDRATE DETERMINATION OF WHEAT VARIETIES

4.3.2 Flavonoid Determination

Graph displays Flavonoid determination in three wheat varieties. Bakhar star salinity dose 2(150mM) has highest flavonoid quantity while doses of Markaz and FSD-08 has moderate amount of Flavonoid estimation. FSD- 08 control has less flavonoid content than salinity doses(100mM,150mM,200mM). Markaz-19 variety shows a consistent trend in doses and control.

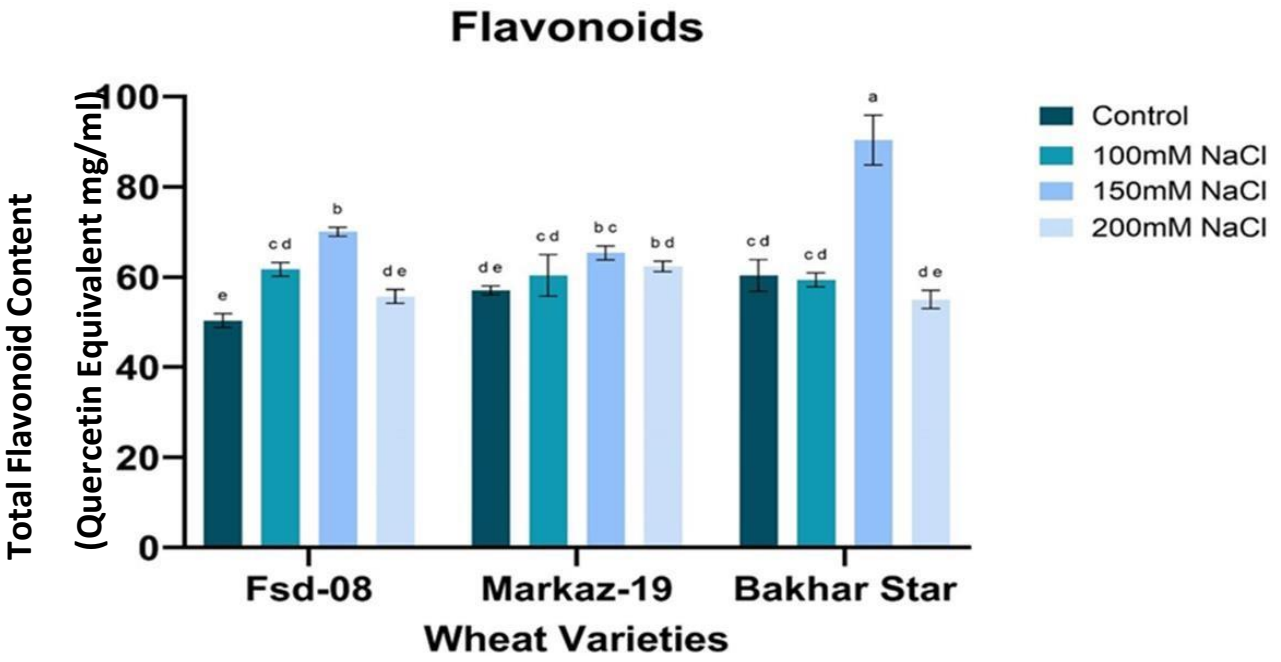

FIGURE 19: FLAVONOID ESTIMATION OF WHEAT VARIETIES

4.3.3 Phenol Estimation

The Graph displays phenol estimation in varying wheat varieties. Bakhar star dose3 has maximum phenolic content in comparison with fsd-08 and Markaz-19. With the exception of Bakhar star dose3, similar trends can be seen in terms of phenolic content estimation in wheat varieties (Markaz-19, FSD-08, Bakhar Star).

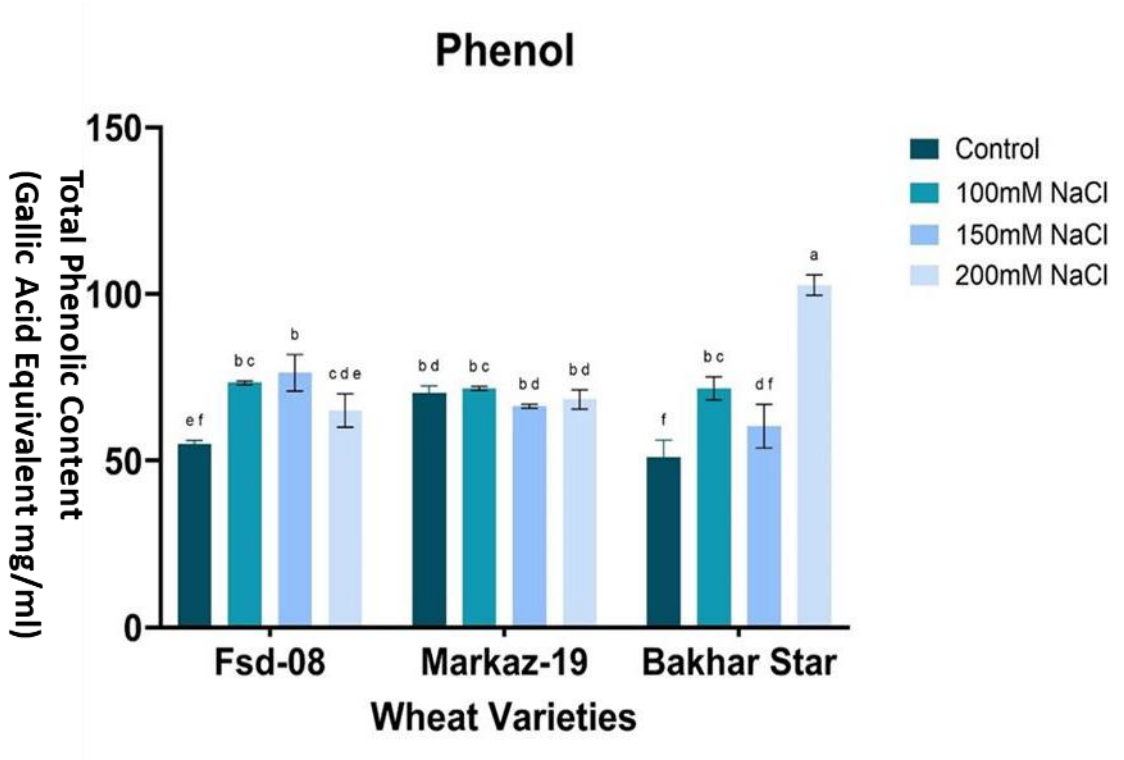

FIGURE 20:PHENOL ESTIMATION OF WHEAT VARIETIES

4.3.4 Tannins determination

Graph displays Tannins estimation in wheat varieties. A high amount of Tannin concentration is observed in three varieties (FSD-08, Markaz-19, Bakhar Star).FSD-08 control(0mM)and dose3 (200mM) has low tannin content .Markaz-19 shows high tannin concentration than other two varieties(Bakhar star,FSD-08).

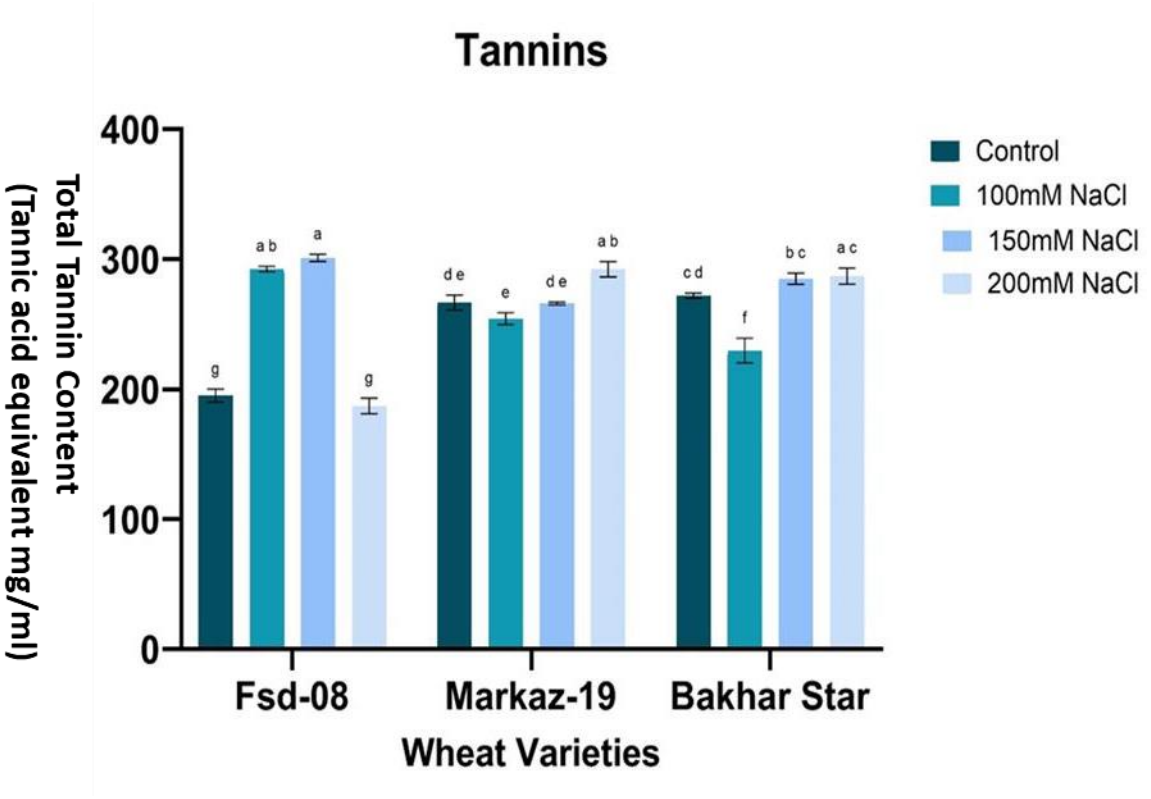

FIGURE 21:TANNIN ESTIMATION OF WHEAT VARIETIES

5. Gene Expression Results

The gene expression of ACO genes were checked to determine whether they are upregulated or downregulated under Salinity stress in Triticum aestivum. The expression of *TaACO2.1*, *TaACO2.3*, *TaACO 3.2*, *TaACO5.1*, *TaACO6.3* and *TaACO7.3* genes were checked in root and shoot under Salinity stress. The results are as follows:

5.1 Gene Expression of *TaACO2.1*:

Shoot

Bakhar star showed upregulation while Markaz-19 and Bakhar star showed down regulation in shoot. Two doses are compared, one of control(0mM) and Dose 3 (200mMNaCl). Markaz-19 and Bakhar star showed similar trend of down regulation in shoot.

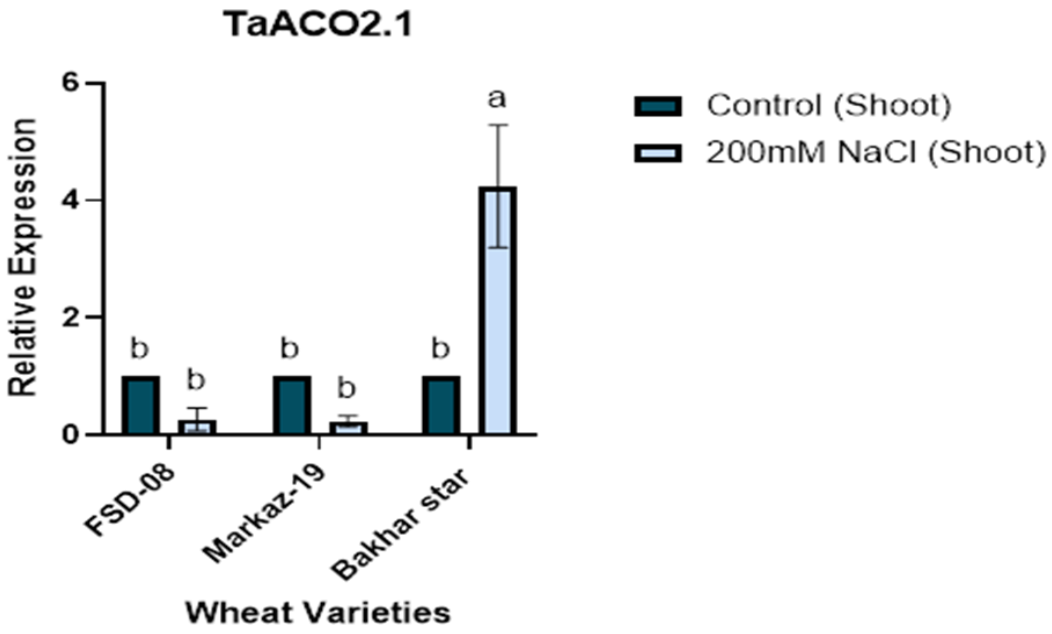

FIGURE 22:GENE EXPRESSION OF *TAACO2.1* SHOOT

Root:

A different trend in root of *TaACO2.1* is observed compared to shoot. FSD-08, being moderately salt tolerant variety showed significant rise in gene expression compared to control. Markaz-19 showed down regulation while Bakhar star showed a slight decline in expression level. The trend was discontinuous**.**

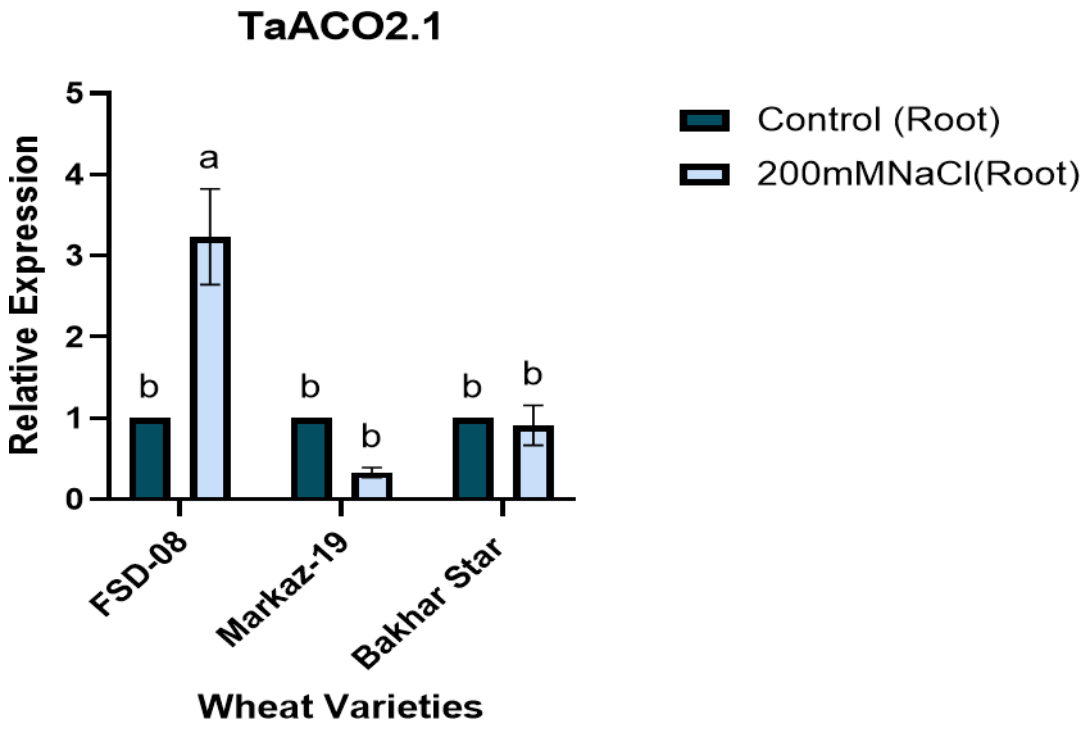

FIGURE 23: GENE EXPRESSION OF *TAACO2.1* IN ROOT

5.2 Gene Expression of *TaACO 2.3*

Shoot

Gene expression analysis of *TaACO 2.3* shows that FSD-08 is upregulated and Bakhar star and Markaz-19 are also upregulated in shoot. Bakhar star and Markz-19 show a similar trend in the upregulation of Gene.

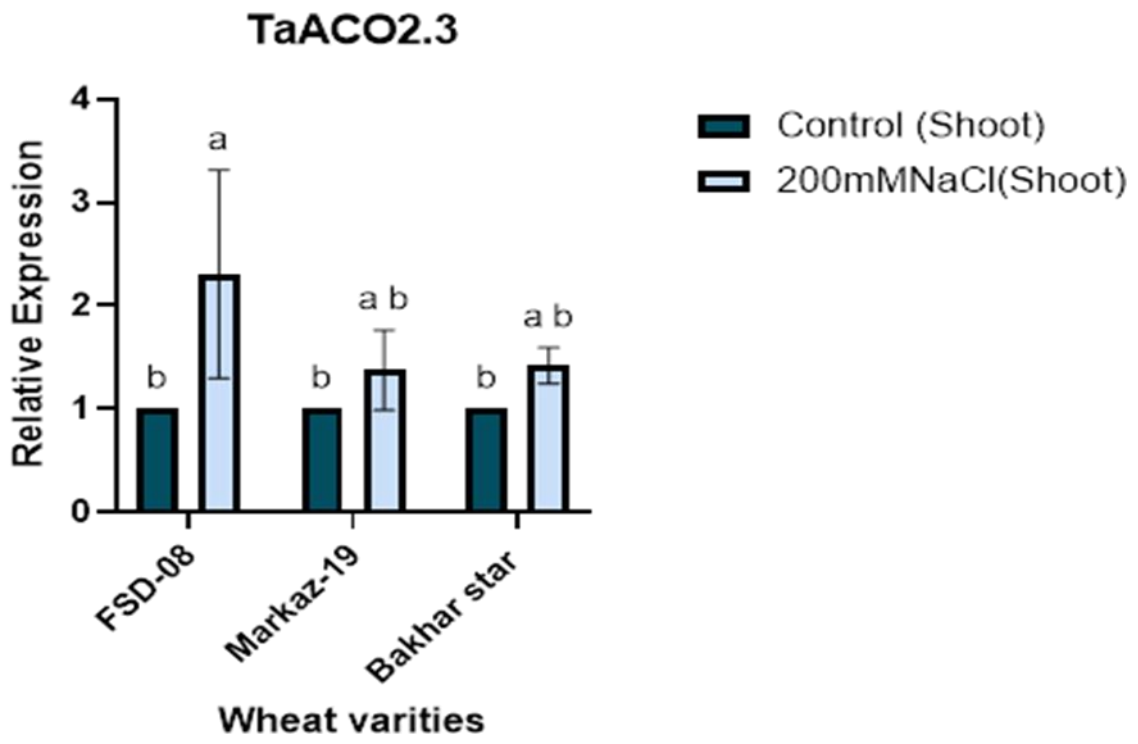

FIGURE 24:GENE EXPRESSION ANALYSIS OF *TAACO2.3* IN SHOOT

Root

FSD-08 is showing consistent trend in upregulation in roots and shoots. FSD-08 is significantly upregulated in roots while Bakhar star and Markaz-19 showed a similar trend of upregulation in roots. All three varieties of wheat were upregulated in response to stress.

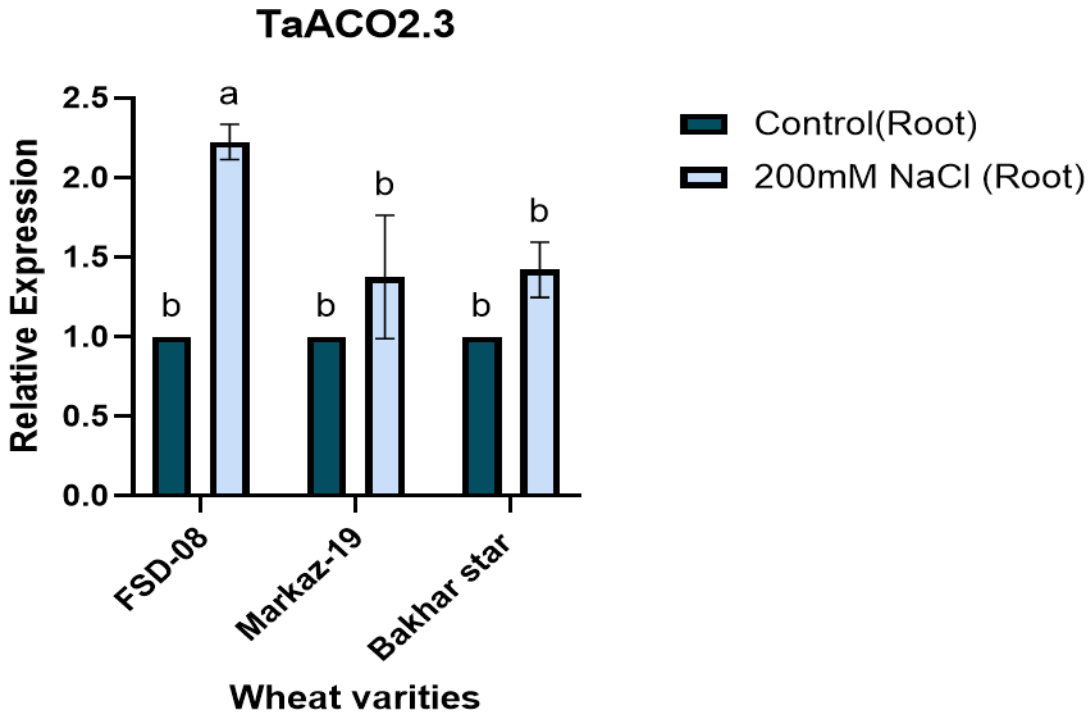

FIGURE 25:GENE EXPRESSION ANALYSIS OF *TAACO2.3*

5.3 Gene Expression Analysis of *TaACO 3.2*

Root

The TaACO3.2 expression in Markaz-19 is significantly higher than Bakhar star and FSD-08. Fsd-08 showed a slightly higher expression**.**

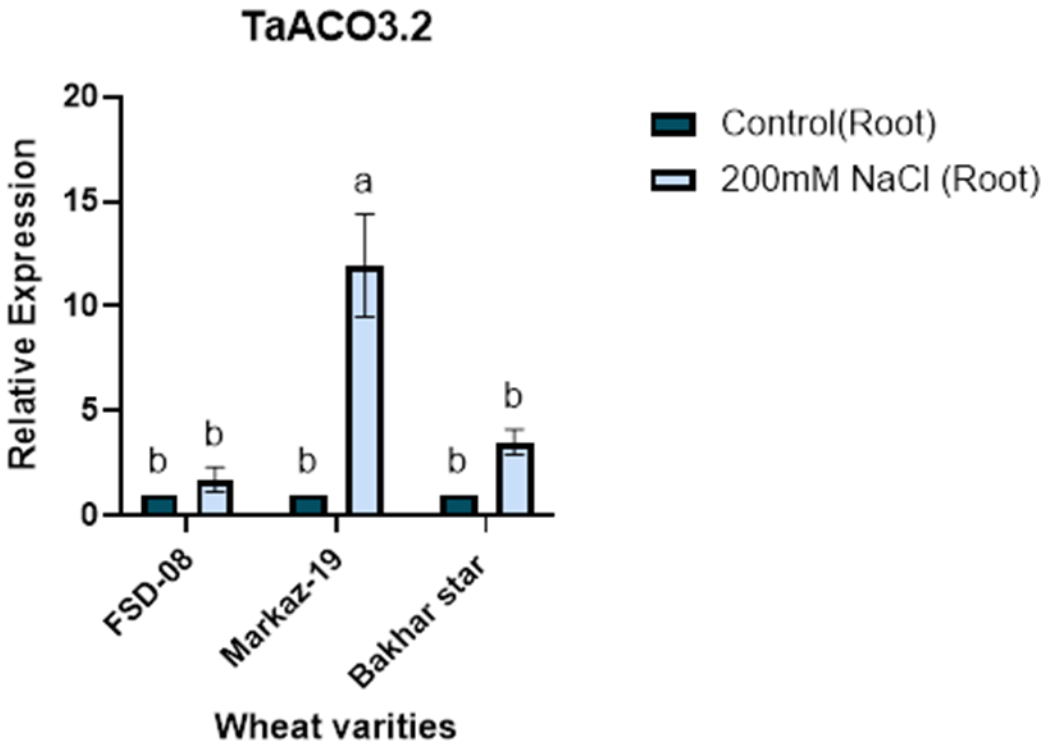

Figure 26: Gene expression analysis of *TaACO3.2* in Root

SHOOT

Markaz-19 showed a similar trend in root and shoot. Bakhar star is slightly upregulated and FSD-08 showed a consistent response in comparison with control.

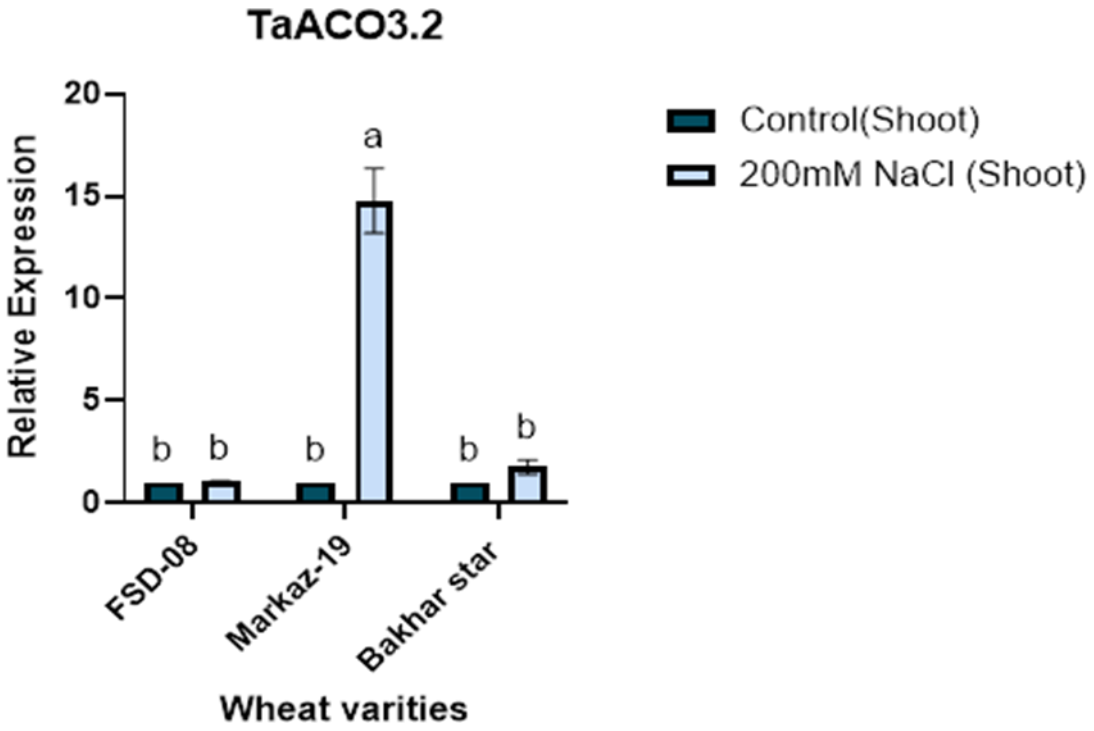

FIGURE 27: GENE EXPRESSION ANALYSIS OF *TaACO3.2* IN SHOOT

5.4 Gene Expression analysis of *TaACO 5.1*

Shoot

Bakhar star showed an upregulation of this gene higher than markaz-19 and FSD-08. Markaz-19 and FSD-08 showed same upregulation response as indicated by lettering.

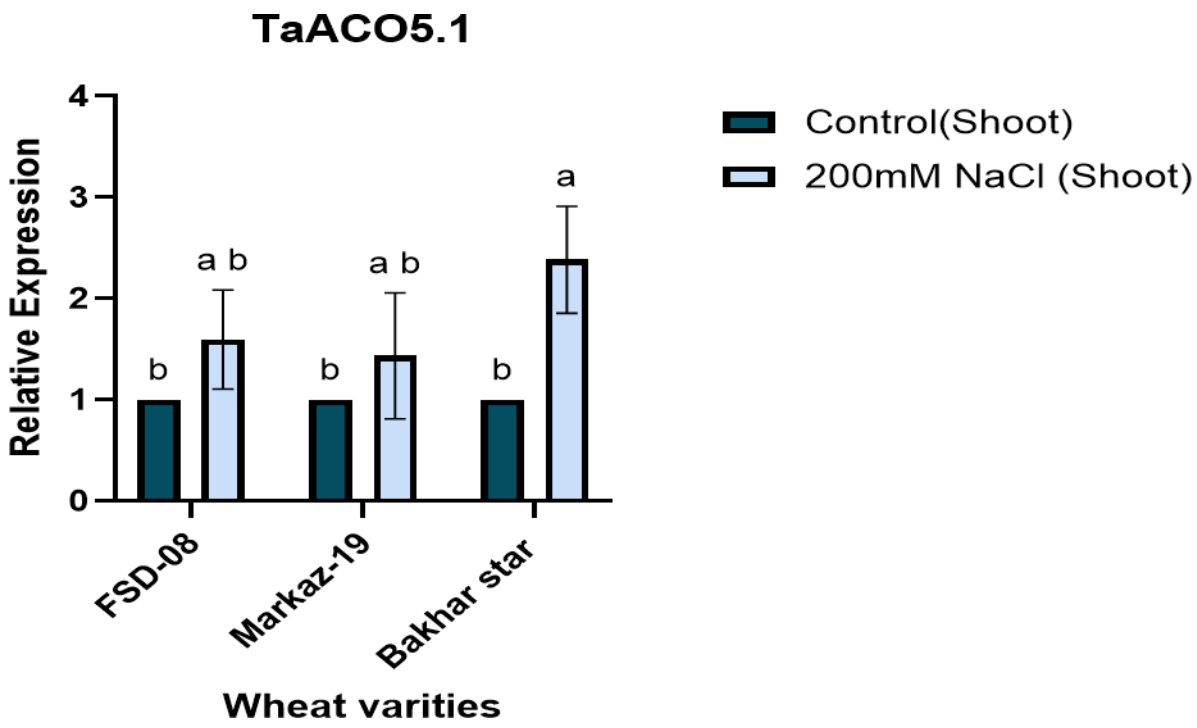

FIG 28: GENE EXPRESSION ANALYSIS OF *TaACO 5.1* IN SHOOT

Root

Bakhar star showed upregulation in root also Markaz-19 was having moderate response and FSD-08 was having lower upregulation than both other varieties.

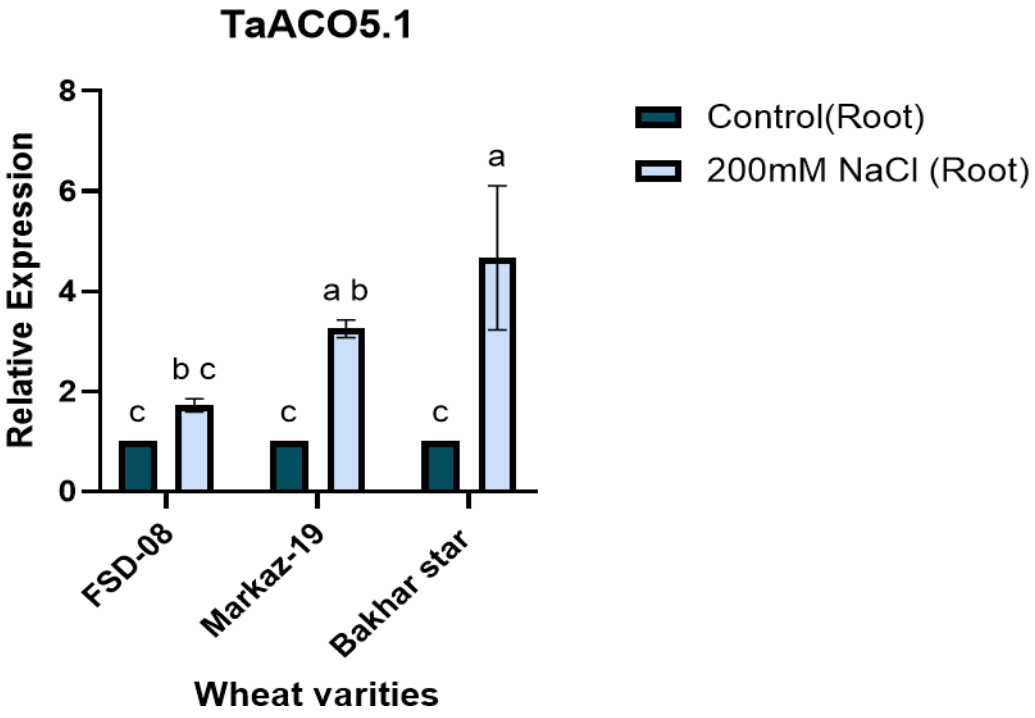

FIG 29: GENE EXPRESSION ANALYSIS OF *TaACO 5.1* IN ROOT

5.5 Gene Expression Analysis of *TaACO 6.3*

Shoot

Bakhar star showed a significant upregulation in shoot of *TaACO6.3*. FSD-08 was also upregulated while Markaz-19 was showing downregulation.

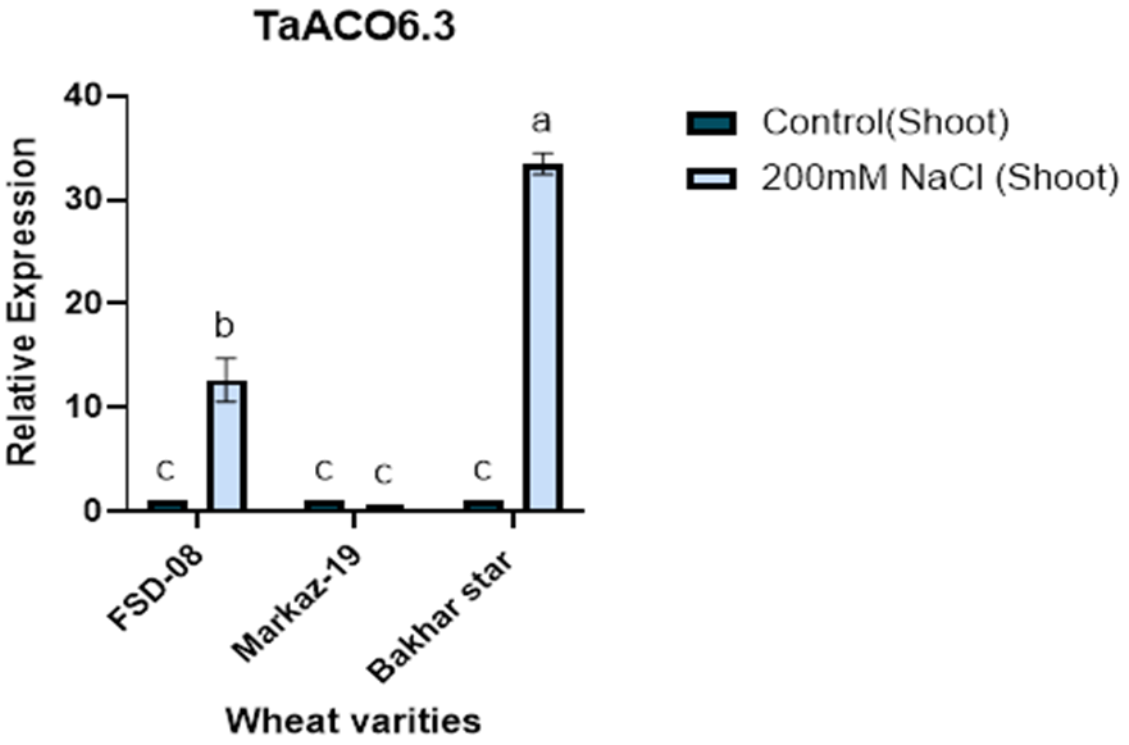

FIG 30: GENE EXPRESSION ANALYSIS OF *TaACO6.3* IN SHOOT

Root

Bakhar star is showing significant upregulation in root of *TaACO 6.3* while Markaz-19 and FSD-08 showed down regulation.

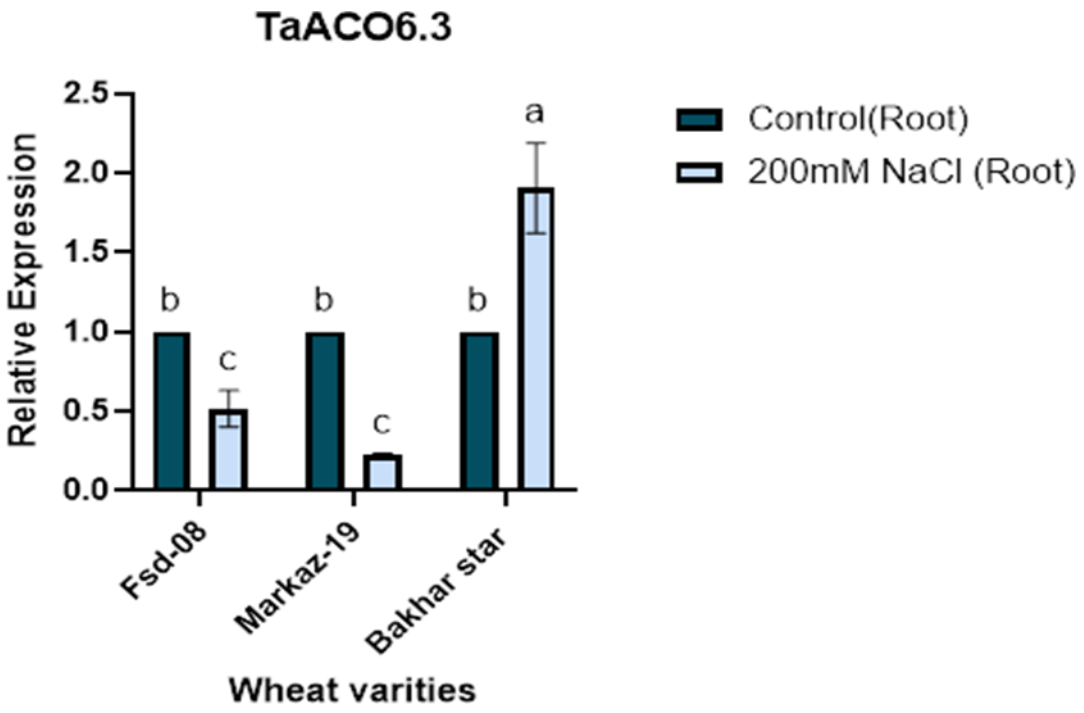

FIG 31: GENE EXPRESSION ANALYSIS OF *TaACO 6.3* IN ROOT

5.6 Gene Expression Analysis of *TaACO 7.3*

In the root at 200mM NaCl, *TaACO7.3 wa*s upregulated in Bakhar star and FSD-08, while downregulation in Markaz-19.

Shoot

At 200mM NaCl concentration, *TaACO7.3* was significantly upregulated in Markaz-19 followed by Bakhar star while downregulation was noticed in Fsd-08 shoot samples.

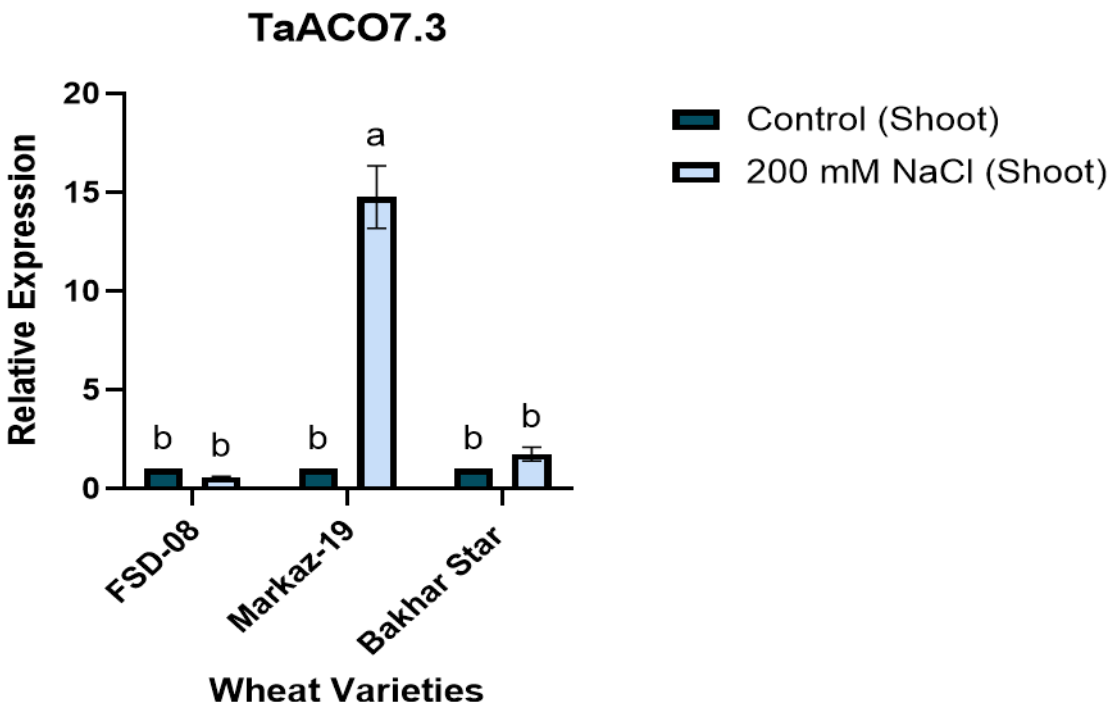

FIG 32: GENE EXPRESSION ANALYSIS OF *TaACO 7.3* IN SHOOT

Chapter 5: Discussion

Wheat one of the world's most influential crops, is a robust cereal that can grow in several temperate zones. Crop quality enhancement is the most challenging aspect of wheat production due to naturally occurring environmental changes and changing weather conditions. Wheat is cultivated in over 120 countries and is one of the world's most important staple food crops. Given the crop's global significance, the market seeks high-quality wheat products. Wheat quality is known to be influenced by a variety of factors, including composition (protein, starch, and ash content), safety and sanitation, physical and functional (viability and milling yield). It is widely established that biotic and abiotic stressors can degrade wheat grain quality and impair crop yield (Filip et al., 2023).

Drought, cold, salt, and heavy metals all have a significant effect on plant growth and crop yield. Abiotic stress impacts plants at the molecular level starting at the morphological levels and can appear at any point in the growth cycle when drought is prevalent. Pre-anthesis, terminal phase, and vegetative growth are the three key plant phases that are impacted by the drought Plants respond physiologically to stress in a variety of ways, including wilting, abscission, reduction in leaf area, and slower water loss through transpiration. The issue of excessive water use in agriculture during drought circumstances is significantly mitigated by crop development. These abiotic stresses, which account for more than half of the average returns for major crops, are the root cause of the global crop disaster. Thus, increased cultivation is critical to closing the widening gap between food production and population growth via promoting stress tolerance. Plants can respond to and acclimatize to abiotic stress

by undergoing certain molecular, cellular, and physiological changes. Improving stress tolerance will be easier with a better understanding of how plants react to abiotic stress in both traditional and modern breeding applications. Studies on stress-tolerant wild plant species have also substantially advanced our understanding of the subject.

Salinity stress is caused by an increase in soil salt concentration. It usually happens in semi-arid and dry locations, when plants experience higher rates of transpiration and evaporation than precipitation volume throughout the year. Primary soil salinity arises from naturally occurring salt deposition in the subsoil, whereas secondary soil salinity is caused by human activities such as environmental contamination. Changes in soil composition, increasing fertilizer use, and the use of saline irrigation water can all lead to secondary soil salinity. Soil salinity is a global issue that threatens the agriculture sector by reducing plant production. Wheat is the principal crop, which is grown mostly during the Rabi season. It is commonly grown under a variety of agro-ecological situations (Fahad et al., 2017).

Because of mutually natural and agricultural phenomena, such as irrigation systems, the quantity of agricultural land altered by high salinity is rising worldwide. Osmotic stress and ionic stress are the two most common salinity-related concerns in plant growth. It also shows symptoms of oxidative stress.

Oxidative stress, stomatal closure, and photosynthetic suppression all contribute to reduced photosynthesis. Osmotic stress can inhibit cell development directly or indirectly by interfering with abscisic acid metabolism and translocation. Because there are too many sodium ions close to the root zone, potassium does not reach the plant's root surface. Because potassium and sodium ions function similarly biochemically, sodium effectively inhibits potassium absorption by roots. Potassium is the most abundant cell cation and supports cell turgor, enzyme activity, and membrane potential, hence a potassium deficit would surely impede growth. Many

enzymes lose their capacity to function when sodium reaches the cytoplasm. This inhibition is also dependent on the amount of potassium available; a high sodium/potassium ratio is more harmful. (Rowe et al., 2016).

Nutritional changes caused by salinity have an impact on nutrient accessibility and partitioning, inhibiting plant development. High salt concentrations can lead to nutrient imbalances or shortages, since Na+ and Cl− compete with K+, Ca 2+, and NO3−. Specific ion toxicity of Na+ and Cl-, as well as ionic imbalances, have an impact on plant growth's biophysical components and/or metabolic in saline settings. To combat salinity stress, most crops accumulate low molecular weight organic solutes such as cyclic polyols (inositol and various mono- and dimethylated inositol derivatives), amino acids (proline or glutamate), and betaine (betaine glycine or betaine alanine).(Yadav et al., 2020).

Ethylene is hypothesized to affect plant physiological and developmental processes, such as seed germination and senescence. Furthermore, plants regulate ethylene to stimulate signaling pathways that defend them against the negative influences of abiotic stress. As a result, it is a key signalling molecule in the abiotic stress process. Nevertheless, it is now unable to say with certainty if ethylene contributes positively to plants' responses to abiotic stress. Several studies have proven the favourable effects of ethylene, or its precursor (ACC), on the ability of several plant species, including maize, tomato, grapevines, Arabidopsis, and others, to survive stress. (Naing et al., 2022b).

The ethylene production mechanism consists of two distinct steps. ACC-synthase (ACS) first transforms (SAM) into (ACC). In the afterwards step, ACC-oxidase (ACO) converts ACC to ethylene. *ACS* was first assumed to be the pathway's rate-limiting enzyme, prompting extensive research into the adjustment of *ACS* protein stability and activity. Over time, however, a significant body of evidence has emerged indicating that *ACO* is the rate-limiting step in the

production of ethylene during devoted operations. This shows that the *ACO* protein family is also subject to strict control(Dubois et al., 2018)

ACO genes influences responses to hormones and environmental stress. In Arabidopsis, salinity stress can reduce *TaACO1* levels. Furthermore, when the ACO gene is overexpressed in Arabidopsis, flood tolerance develops. Nitric oxide was discovered to adjust the formation of lateral roots in sunflowers under salt stress by altering the activity of the ACO gene Wounding can enhance ACO gene expression in tomato and cucumber]. After *F. eumartii* inoculation, the mRNA level of ST-ACO3 in potatoes enhances ACO genes have been observed to counter to mechanical damage and ABA in tomato and cauliflower.(Wei et al., 2021c).

The genes length and complexity vary widely, with some having longer upstream/downstream sections or more introns. The number of introns and exon lengths differ between TaACO genes, which may affect gene expression and protein function.(Su et al., 2023)

TaACO2.1, TaACO2.2, and TaACO2.3 genes have several introns and long upstream and downstream regions, implying that their regulation is complicated and that there may be alternative splicing alternatives.*TaACO3.1, TaACO3.2,* and *TaACO3.3* have different coding sequence and intron lengths, indicating that the proteins they encode have separate functional domains.TaACO4.1 and TaACO4.3 genes have fewer introns and are shorter, which could indicate a simpler regulatory structure and faster transcription and translation. *TaACO5.1,* 5.2, and 5.3 have a mix of short and long coding sequences with different intron patterns, indicating a variety of roles and regulatory mechanisms.

TaACO6.1, TaACO6.2, and TaACO6.3 genes, similar to *TaACO2*, these genes have difficult regulation due to their long upstream/downstream regions and several introns. *TaACO7.2*,

TaACO7.3 have variations in the length of their coding sequences and upstream/downstream regions may affect the regulatory mechanisms that regulate these genes(Kesawat et al., 2022b).

Some motifs (e.g., Motif 2 and Motif 5) appear in most gene variants, indicating that these parts are highly conserved and most likely required for the *ACO* protein's action. Motif variations such as Motif 9 and Motif 10 appear in fewer gene variants, implying possible specialization or functional variation among the TaACO genes.

Variants in TaACO gene expression, regulation, or protein function may be linked to the unique motif combinations and arrangements seen in each TaACO gene variant. The three TaACO2 mutations contain a consistent pattern and a similar motif structure, indicating that they serve a common purpose. This could indicate a function in a certain physiological process that requires precise patterns. *TaACO3* variants Displayed functional diversity using a motif structure that is similar to yet slightly different from *TaACO2* variations. The diverse themes or variations of *TaACO4*, *TaACO5*, *TaACO6*, and *TaACO7*, revealed by each of these categories demonstrate the range of roles that these gene variants may perform in different tissues.

Yellow DIOX_N can be found in the N-terminal region of *TaACO* proteins. Most gene variants have the same length and position of this domain, implying a conserved role related to the enzyme's early activity or substrate recognition.

2OG-Oxy Domain—Fe (II) (Green) domain is critical for the *ACO* enzyme's catalytic activity because it binds the Fe (II) ions and 2-oxoglutarate required for the oxidation reaction. All gene variants contain the 2OG-Fe (II)_Oxy domain in the protein's C-terminal region, indicating that it is essential for enzyme function. The fact that all variants contain the 2OG-Fe (II)_Oxy domain emphasizes its role in the catalytic conversion of ACC to ethylene. The conservation

of this domain indicates that the catalytic mechanism is conserved among *TaACO* proteins. The conservation of the DIOX_N domain suggests that it may function in the early phases of substrate binding and recognition, as well as in preserving the enzyme's structural stability(Martinez & Hausinger, 2015b).

The majority of *TaACO* variants contain both domains, indicating a conserved functional role in ethylene synthesis. The length of these domains varies somewhat between gene variants, which could be related to minor differences in enzyme activity, regulation, or interactions with other molecules.

The subcellular localization in heat map shows that *TaACO4.1* and *TaACO4.3* are highly confident in their cytoplasmic localization, implying that these variants are critical for cytoplasmic functions.*TaACO4.1* and *TaACO4.3* may have a function in energy metabolism and stress response in the mitochondria.*TaACO2.3* and *TaACO4* variants appear to be moderately confident in their involvement in vacuolar functions, which could be related to stress responses or storage. *TaACO6.1's* chloroplast localization is moderately certain, indicating that it participates in photosynthesis or stress responses. *TaACO2.1* and *TaACO5.1's* ER localization suggests that they are involved in signaling or protein processing.(Kesawat et al., 2022c).In the case of tobacco, ethylene treatment can correct the functional defect of female sterility in transgenic plants generated by slowing the demonstration of an ACO-like gene. The formation of the minute hilum seed coat phenotype in soybeans is preceded by the overproduction of an ACO gene.

In Arabidopsis, salinity stress can reduce *ACO1* levels. Furthermore, when the ACO gene is overexpressed in Arabidopsis, flood tolerance develops. Nitric oxide was discovered to adjust the formation of lateral roots in sunflowers under salt stress by altering the activity of the ACO gene. Wounding can enhance ACO gene expression in tomato and cucumber.

With increasing NaCl concentrations, all three wheat varieties show a decrease in SPAD values, indicating that salt stress has a negative impact on chlorophyll content. However, the degree of sensitivity varies between types.

Bakhar Star obtained the highest SPAD score under control conditions, indicating that it may have more chlorophyll under normal circumstances. However, it appears to be more sensitive to salt stress, as demonstrated by a significant drop in SPAD values at higher NaCl concentrations. Markaz-19 maintains higher SPAD values with rising salt concentrations than the other varieties.

Markaz-19 shows the smallest decline in shoot length with increasing NaCl concentrations, indicating that it is the most resistant to salt stress. Fsd-08 is the most sensitive to salt stress, as seen by a substantial decrease in shoot length, particularly at higher NaCl concentrations. Bakhar Star, compared to Fsd-08, has a more progressive fall in shoot length, although it is still more effected than Markaz-19, especially at the highest salt concentration. Fsd-08, as NaCl content increases, this variety's root length decreases significantly. At 200 mM NaCl, the root length is significantly shorter than in the control, indicating a high vulnerability to salt stress. Markaz-19, Like Fsd-08, root length decreases as NaCl concentration increases, although this variety performs slightly better at 150 and 200 mM.. Bakhar Star, this variety operates best with 100 mM NaCl and under controlled conditions. At higher salt concentrations, root length losses are quite minor. This suggests that Bakhar Star, when compared to the other two types, may be more resistant to salt stress.

Fsd-08 Shows the maximum shot fresh weight under regulated conditions. It sees a sharp decline in fresh weight when NaCl concentrations rise. Although this type appears to be more susceptible to salt stress, under the right circumstances, it could produce more. Markaz-19 has an intermediate fresh weight when kept under controlled conditions.

shows a little decline with increasing NaCl concentrations. This variety shows a moderate salt tolerance as it outperforms Fsd-08 but not Bakhar Star under extreme salt stress. Bakhar Star has the lowest shoot fresh weight when conditions are optimal, shows the least amount of initial weight loss as the concentration of NaCl increases. Even at 150 and 200 millimolar NaCl, Bakhar Star maintains a higher root fresh weight, showing enhanced salt tolerance. Root fresh weight losses are more obvious in Fsd-08 and Markaz-19 when NaCl concentrations increase. Bakhar Star shows a greater decline in shoot dry weight at lower concentrations (100 mM NaCl) than Fsd-08 and Markaz-19, indicating that it may be more sensitive to lower salt levels. (Bhutto et al., 2019) At higher concentrations (200 mM NaCl), all varieties grow less, but Bakhar Star maintains the lowest shoot dry weight, indicating that this variety is more susceptible to salinity. All wheat cultivars have a consistent tendency in which root dry weight falls as NaCl content increases. This suggests that higher salt levels have a negative impact on wheat root biomass. The Phytochemical qualitative results showed that bioactive compounds carbohydrate, flavonoid, tannins and phenol are present in all three wheat varieties. Bakhar star, being tolerant variety significantly produced Carbohydrate. In the Markaz-19 and FSD-08, Carbohydrate are produced highest in control, but with increasing doses, as stress is applied, the plant ability to produce naturally carbohydrate declines.

The phenolic compounds tannins, phenol and flavonoid are produced as a defense mechanism against stress conditions. In the control, their concentration is less, but with increasing concentration their content started to rise as protective mechanism against stress conditions in all wheat varities (Pratyusha & Pratyusha, 2022).

Gene Expression analysis of TaACO genes revealed that they are upregulated and downregulated under salinity stress conditions. The expression analysis of *TaACO2.1*,

TaACO2.3, *TaACO 3.1*, *TaACO5.2*, *TaACO6.3* and *TaACO7.3* was checked. The gene expression analysis of *TaACO 2.1* in roots and shoots showed differential expressions. Bakhar star in shoots showed significant upregulation as ACO gene is ACO gene expression is increased to tolerate plant against stress situations while Markaz-19 and FSD-08 are down regulated as their mechanism of ACO expression is not activated. The *TaACO 2.1* roots showed signific upregulation in FSD-08 and down regulation in Markaz-19. FSD-08 showed moderate upregulation in ACO gene as protective mechanism against stress. Markaz-19 showed down regulation as it may perceive stress as mild and activate some other pathways to combat stress rather than *ACO* activation.

The expression pattern of *TaACO 2.3* in roots and shoots revealed that FSD-08 was showing highest upregulation as moderately sat tolerant variety enhanced its mechanism to protect against cellular damage while Markaz-19 and Bakhar star was showing relative trend as they have same threshold response to stress in this gene expression. In The expression of *TaACO 3.2* roots and shoots Markz-19 being susceptible variety showed significant upregulation in comparison with Bakhar star and FSD-08. As Markaz-19 may have strong stress transduction pathways in response to this gene compared to others. Bakhar star and Markaz-19 stress response pathway is not that much activated in response to stress.

In the gene expression of *TaACO 5.1*, Bakhar star showed upregulation as being tolerant variety. The ACO expression is increased to defend against salt stress. FSD-08 and Markaz-19 are also upregulated. The varieties showed a very significant response as highest expression in salt tolerant variety, moderate in moderately tolerant variety and lowest in susceptible varieties.

The expression pattern of *TaACO 6.3* shows that Bakhar star is upregulated due to effective ethylene signalling, effective genetic modifications and effective stress management. The downregulation of Markaz-19 and FSD-08 reveals that disrupted ethylene response and

negative feedback mechanism. The expression pattern of *TaACO 7.3* displays that the pattern was discontinuous. The down regulation of markaz-19 in root and FSD-08 in shoot shows their inefficient stress tolerance mechanism.(Ye et al., 2017)

Chapter 6: Conclusion

This research highlighted the genetic basis of variability of salt tolerant, moderately salt tolerant and salt susceptible wheat varieties. The expression profiling of ACO gene showed that most genes are upregulated and few are downregulated. *ACO* showed differential expression pattern in root and shoot. The presence of Bioactive compounds in all wheat varities were observed. Bakhar star, being tolerant variety performed well in stress tolerance mechanism. FSD-08 showed moderate stress response and Markaz-19 didn't withstand stress tolerance mechanism well. Different ACO gene variants had distinctive roles.The ACO gene showed conserved domain and motif analysis. The research aims for supporting sustainable agricultural practices and understanding salinity stress adverse effects on wheat growth and production. The prospects of research involve either suppressing or overexpress ACO gene for breeding salt tolerant varieties. Using salinity tolerance markers linked to the ACO gene in breeding programs. Introducing ACO gene variants from other salt-tolerant species into wheat.

Appendices

Table:

Ingredients of qrtPCR

Reagents	**Volume**
Template DNA	1 µl
Forward primer	0.2 µl
Reverse primer	0.2 µl
5x HOT FIREPOL EvaGreen qPCR Mix Plus	2.5 µl

Primers used

TaACO25AF	**TaACO36BR**
TaACO25AR	**TaACO51AF**
TaACO25DF	**TaACO51AR**
TaACO25DR	**TaACO67DF**
TaACO36BF	**TaACO67DR**
TaACO77DF	**TaACO77DR**

Reagents	Volume
5XABscript RT mix	2 µl
20XgDNA remover	0.5µl
Total RNA	1 µg
Nuclease free water	10µl

Table: cDNA synthesis

References

Abhinandan, K., Skori, L., Stanic, M., Hickerson, N. M. N., Jamshed, M., & Samuel, M. A. (2018). Abiotic Stress Signaling in Wheat - An Inclusive Overview of Hormonal Interactions During Abiotic Stress Responses in Wheat. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/FPLS.2018.00734

Adams, D. 0, Shang, A., & Yang, F. A. (1977). Methionine Metabolism in Apple Tissue: Implication of S-Adenosylmethionine as an Intermediate in the Conversion of Methionine to Ethylene. *Plant Physiology*, *60*(6), 892–896. https://doi.org/10.1104/PP.60.6.892

Ali Redha, A., Ali Redha, A. A., Hasan, A. M., Mandeel, Q., & Redha, A. A. (2018). *Phytochemical Determinations of Pomegranate (Punica granatum) Rind and Aril Extracts and their Antioxidant, Antidiabetic and Antibacterial Activity*. https://doi.org/10.4172/2329-6836.1000332

Ashraf, M., Shahzad, S. M., Imtiaz, M., Rizwan, M. S., & Iqbal, M. M. (2017). Ameliorative effects of potassium nutrition on yield and fiber quality characteristics of cotton (Gossypium hirsutum L.) under NaCl stress. *Soil Environ*, *36*(1), 51–58. https://doi.org/10.25252/SE/17/31054

Bhutto, T. A., Qadir Gola, A., Bhutto, H., Wahocho, S. A., Buriro, M., Wahocho, N. A., Tofique, C., Bhutto, A., Gola, A. Q., & Wahocho, A. (2019). Evaluation of wheat varieties to salt stress (NaCl) for seed germination and early seedling growth under laboratory conditions. *Pure and Applied Biology*, *8*(1), 609–618. https://doi.org/10.19045/bspab.2018.700223

Boller, T., Herner, R. C., & Kende, H. (1979). Assay for and enzymatic formation of an ethylene precursor, 1-aminocyclopropane-1-carboxylic acid. *Planta*, *145*(3), 293–303. https://doi.org/10.1007/BF00454455/METRICS

Bouzayen, M., Felix, G., Latché, A., Pech, J. C., & Boller, T. (1991). Iron: an essential cofactor for the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene. *Planta*, *184*(2), 244–247. https://doi.org/10.1007/BF00197953/METRICS

Bouzayen, M., Latché, A., & Pech, J. C. (1990). Subcellular localization of the sites of conversion of 1-aminocyclopropane-1-carboxylic acid into ethylene in plant cells. *Planta*, *180*(2), 175–180. https://doi.org/10.1007/BF00193992/METRICS

Chen, D., Ma, X., Li, C., Zhang, W., Xia, G., & Wang, M. (2014a). A wheat aminocyclopropane-1-carboxylate oxidase gene, TaACO1, negatively regulates salinity stress in Arabidopsis thaliana. *Plant Cell Reports*, *33*(11), 1815–1827. https://doi.org/10.1007/S00299-014-1659-7/FIGURES/6

Chen, D., Ma, X., Li, C., Zhang, W., Xia, G., & Wang, M. (2014b). A wheat aminocyclopropane-1-carboxylate oxidase gene, TaACO1, negatively regulates salinity stress in Arabidopsis thaliana. *Plant Cell Reports*, *33*(11), 1815–1827. https://doi.org/10.1007/S00299-014-1659-7/FIGURES/6

Chen, D., Ma, X., Li, C., Zhang, W., Xia, G., & Wang, M. (2014c). A wheat aminocyclopropane-1-carboxylate oxidase gene, TaACO1, negatively regulates salinity stress in Arabidopsis thaliana. *Plant Cell Reports*, *33*(11), 1815–1827. https://doi.org/10.1007/S00299-014-1659-7/FIGURES/6

Chen, D., Ma, X., Li, C., Zhang, W., Xia, G., & Wang, M. (2014d). A wheat aminocyclopropane-1-carboxylate oxidase gene, TaACO1, negatively regulates salinity stress in Arabidopsis thaliana. *Plant Cell Reports*, *33*(11), 1815–1827. https://doi.org/10.1007/S00299-014-1659-7/FIGURES/6

Chen, D., Ma, X., Li, C., Zhang, W., Xia, G., & Wang, M. (2014e). A wheat aminocyclopropane-1-carboxylate oxidase gene, TaACO1, negatively regulates salinity stress in Arabidopsis thaliana. *Plant Cell Reports*, *33*(11), 1815–1827. https://doi.org/10.1007/S00299-014-1659-7/FIGURES/6

Chung, M. C., Chou, S. J., Kuang, L. Y., Charng, Y. Y., & Yang, S. F. (2002). Subcellular Localization of 1-Aminocyclopropane-1-Carboxylic Acid Oxidase in Apple Fruit. *Plant and Cell Physiology*, *43*(5), 549–554. https://doi.org/10.1093/PCP/PCF067

Conserved Domains Database (CDD) and Resources. (n.d.). Retrieved August 30, 2024, from https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml

Dar, R. A., Nisar, S., & Tahir, I. (2021). Ethylene: A key player in ethylene sensitive flower senescence: A review. *Scientia Horticulturae*, *290*, 110491. https://doi.org/10.1016/J.SCIENTA.2021.110491

Dong, H., Zhen, Z., Peng, J., Chang, L., Gong, Q., & Wang, N. N. (2011a). Loss of ACS7 confers abiotic stress tolerance by modulating ABA sensitivity and accumulation in Arabidopsis. *Journal of Experimental Botany*, *62*(14), 4875–4887. https://doi.org/10.1093/JXB/ERR143

Dong, H., Zhen, Z., Peng, J., Chang, L., Gong, Q., & Wang, N. N. (2011b). Loss of ACS7 confers abiotic stress tolerance by modulating ABA sensitivity and accumulation in Arabidopsis. *Journal of Experimental Botany*, *62*(14), 4875–4887. https://doi.org/10.1093/JXB/ERR143

Dubois, M., Van den Broeck, L., & Inzé, D. (2018). The Pivotal Role of Ethylene in Plant Growth. *Trends in Plant Science*, *23*(4), 311–323. https://doi.org/10.1016/J.TPLANTS.2018.01.003

Dugardeyn, J., & Van Der Straeten, D. (2008). Ethylene: Fine-tuning plant growth and development by stimulation and inhibition of elongation. *Plant Science*, *175*(1–2), 59–70. https://doi.org/10.1016/J.PLANTSCI.2008.02.003

Dunn, C. W., Luo, X., & Wu, Z. (2013). Phylogenetic Analysis of Gene Expression. *Integrative and Comparative Biology*, *53*(5), 847–856. https://doi.org/10.1093/ICB/ICT068

EL Sabagh, A., Islam, M. S., Skalicky, M., Ali Raza, M., Singh, K., Anwar Hossain, M., Hossain, A., Mahboob, W., Iqbal, M. A., Ratnasekera, D., Singhal, R. K., Ahmed, S., Kumari, A., Wasaya, A., Sytar, O., Brestic, M., ÇIG, F., Erman, M., Habib Ur Rahman, M., … Arshad, A. (2021). Salinity Stress in Wheat (Triticum aestivum L.) in the Changing Climate: Adaptation and Management Strategies. *Frontiers in Agronomy*, *3*, 661932. https://doi.org/10.3389/FAGRO.2021.661932/BIBTEX

Erdoğan, İ., Cevher-Keskin, B., Bilir, Ö., Hong, Y., & Tör, M. (2023). Recent Developments in CRISPR/Cas9 Genome-Editing Technology Related to Plant Disease Resistance and Abiotic Stress Tolerance. *Biology*, *12*(7), 1037. https://doi.org/10.3390/BIOLOGY12071037

Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S., Ihsan, M. Z., Alharby, H., Wu, C., Wang, D., & Huang, J. (2017). Crop production under drought and heat stress: Plant responses and management options. *Frontiers in Plant Science*, *8*, 265598. https://doi.org/10.3389/FPLS.2017.01147/BIBTEX

Farrow, S. C., & Facchini, P. J. (2014). Functional diversity of 2-oxoglutarate/Fe(II)-dependent dioxygenases in plant metabolism. *Frontiers in Plant Science*, *5*(OCT), 115843. https://doi.org/10.3389/FPLS.2014.00524/BIBTEX

Fatma, M., Asgher, M., Iqbal, N., Rasheed, F., Sehar, Z., Sofo, A., & Khan, N. A. (2022a). Ethylene Signaling under Stressful Environments: Analyzing Collaborative Knowledge. *Plants 2022, Vol. 11, Page 2211*, *11*(17), 2211. https://doi.org/10.3390/PLANTS11172211

Fatma, M., Asgher, M., Iqbal, N., Rasheed, F., Sehar, Z., Sofo, A., & Khan, N. A. (2022b). Ethylene Signaling under Stressful Environments: Analyzing Collaborative Knowledge. *Plants 2022, Vol. 11, Page 2211*, *11*(17), 2211. https://doi.org/10.3390/PLANTS11172211

Filip, E., Woronko, K., Stępień, E., & Czarniecka, N. (2023). An Overview of Factors Affecting the Functional Quality of Common Wheat (Triticum aestivum L.). *International Journal of Molecular Sciences 2023, Vol. 24, Page 7524*, *24*(8), 7524. https://doi.org/10.3390/IJMS24087524

Garcia, D. F., Wang, Z., Guan, J., Yin, L., Geng, S., Li, A., & Mao, L. (2021). WheatGene: A genomics database for common wheat and its related species. *The Crop Journal*, *9*(6), 1486–1491. https://doi.org/10.1016/J.CJ.2021.04.011

Giraldo, P., Benavente, E., Manzano-Agugliaro, F., & Gimenez, E. (2019). Worldwide Research Trends on Wheat and Barley: A Bibliometric Comparative Analysis. *Agronomy 2019, Vol. 9, Page 352*, *9*(7), 352. https://doi.org/10.3390/AGRONOMY9070352

Guo, R., Yang, Z., Li, F., Yan, C., Zhong, X., Liu, Q., Xia, X., Li, H., & Zhao, L. (2015). Comparative metabolic responses and adaptive strategies of wheat (Triticum aestivum) to salt and alkali stress. *BMC Plant Biology*, *15*(1), 1–13. https://doi.org/10.1186/S12870-015-0546-X/TABLES/2

Hamilton, A. J., Lycett, G. W., & Grierson, D. (1990a). Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. *Nature 1990 346:6281*, *346*(6281), 284–287. https://doi.org/10.1038/346284a0

Hamilton, A. J., Lycett, G. W., & Grierson, D. (1990b). Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. *Nature 1990 346:6281*, *346*(6281), 284–287. https://doi.org/10.1038/346284a0

Hossain, A., Skalicky, M., Brestic, M., Maitra, S., Alam, M. A., Syed, M. A., Hossain, J., Sarkar, S., Saha, S., Bhadra, P., Shankar, T., Bhatt, R., Chaki, A. K., Sabagh, A. E. L., & Islam, T. (2021). Consequences and Mitigation Strategies of Abiotic Stresses in Wheat (Triticum aestivum L.) under the Changing Climate. *Agronomy 2021, Vol. 11, Page 241*, *11*(2), 241. https://doi.org/10.3390/AGRONOMY11020241

Houben, M., & Van de Poel, B. (2019a). 1-aminocyclopropane-1-carboxylic acid oxidase (ACO): The enzyme that makes the plant hormone ethylene. *Frontiers in Plant Science*, *10*, 464815. https://doi.org/10.3389/FPLS.2019.00695/BIBTEX

Houben, M., & Van de Poel, B. (2019b). 1-aminocyclopropane-1-carboxylic acid oxidase (ACO): The enzyme that makes the plant hormone ethylene. *Frontiers in Plant Science*, *10*, 464815. https://doi.org/10.3389/FPLS.2019.00695/BIBTEX

Houben, M., & Van de Poel, B. (2019c). 1-aminocyclopropane-1-carboxylic acid oxidase (ACO): The enzyme that makes the plant hormone ethylene. *Frontiers in Plant Science*, *10*, 464815. https://doi.org/10.3389/FPLS.2019.00695/BIBTEX

Iqbal, M., Fahim, M., Zaman, Q., Usman, M., & Rahman, A. U. (2014). *EFFECT OF VARIOUS FACTORS ON WHEAT PRODUCTION*. *30*(1).

Iqbal, N., Khan, N. A., Ferrante, A., Trivellini, A., Francini, A., & Khan, M. I. R. (2017). Ethylene role in plant growth, development and senescence: interaction with other phytohormones. *Frontiers in Plant Science*, *8*, 235913. https://doi.org/10.3389/FPLS.2017.00475/BIBTEX

Iqbal, N., Saffeullah, P., & Umar, S. (2022). Ethylene and Nitric Oxide Under Salt Stress. *Ethylene in Plant Biology*, 312–344. https://doi.org/10.1002/9781119744719.CH14

Kesawat, M. S., Kherawat, B. S., Ram, C., Singh, A., Dey, P., Gora, J. S., Misra, N., Chung, S. M., & Kumar, M. (2022a). Genome-Wide Identification and Expression Profiling of Aconitase Gene Family Members Reveals Their Roles in Plant Development and Adaptation to Diverse Stress in Triticum aestivum L. *Plants*, *11*(24), 3475. https://doi.org/10.3390/PLANTS11243475/S1

Kesawat, M. S., Kherawat, B. S., Ram, C., Singh, A., Dey, P., Gora, J. S., Misra, N., Chung, S. M., & Kumar, M. (2022b). Genome-Wide Identification and Expression Profiling of Aconitase Gene Family Members Reveals Their Roles in Plant Development and Adaptation to Diverse Stress in Triticum aestivum L. *Plants*, *11*(24). https://doi.org/10.3390/PLANTS11243475/S1

Kesawat, M. S., Kherawat, B. S., Ram, C., Singh, A., Dey, P., Gora, J. S., Misra, N., Chung, S. M., & Kumar, M. (2022c). Genome-Wide Identification and Expression Profiling of Aconitase Gene Family Members Reveals Their Roles in Plant Development and Adaptation to Diverse Stress in Triticum aestivum L. *Plants*, *11*(24). https://doi.org/10.3390/PLANTS11243475/S1

Li, S., Zhang, C., Li, J., Yan, L., Wang, N., & Xia, L. (2021). Present and future prospects for wheat improvement through genome editing and advanced technologies. *Plant Communications*, *2*(4). https://doi.org/10.1016/J.XPLC.2021.100211

Liu, X., Chen, D., Yang, T., Huang, F., Fu, S., & Li, L. (2020). Changes in soil labile and recalcitrant carbon pools after land-use change in a semi-arid agro-pastoral ecotone in Central Asia. *Ecological Indicators*, *110*, 105925. https://doi.org/10.1016/J.ECOLIND.2019.105925

Ludwig, A. A., Saitoh, H., Felix, G., Freymark, G., Miersch, O., Wasternack, C., Boller, T., Jones, J. D. G., & Romeis, T. (2005). Ethylene-mediated cross-talk between calcium-dependent protein kinase and MAPK signaling controls stress responses in plants. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(30), 10736–10741. https://doi.org/10.1073/PNAS.0502954102/SUPPL_FILE/02954FIG6.JPG

Martinez, S., & Hausinger, R. P. (2015a). Catalytic mechanisms of Fe(II)- and 2-Oxoglutarate-dependent oxygenases. *Journal of Biological Chemistry*, *290*(34), 20702–20711. https://doi.org/10.1074/jbc.R115.648691

Martinez, S., & Hausinger, R. P. (2015b). Catalytic Mechanisms of Fe(II)- and 2-Oxoglutarate-dependent Oxygenases. *The Journal of Biological Chemistry*, *290*(34), 20702. https://doi.org/10.1074/JBC.R115.648691

Mcmurchie, E. J., Mcglasson, W. B., & Eaks, I. L. (1972). Treatment of Fruit with Propylene gives Information about the Biogenesis of Ethylene. *Nature 1972 237:5352*, *237*(5352), 235–236. https://doi.org/10.1038/237235a0

Miransari, M., & Smith, D. (2019). Sustainable wheat (Triticum aestivum L.) production in saline fields: a review. *Critical Reviews in Biotechnology*, *39*(8), 999–1014. https://doi.org/10.1080/07388551.2019.1654973

Mittler, R. (2006). Abiotic stress, the field environment and stress combination. *Trends in Plant Science*, *11*(1), 15–19. https://doi.org/10.1016/j.tplants.2005.11.002

Munns, R., Biol., M. T.-Annu. Rev. P., & 2008, undefined. (2008). Mechanisms of salinity tolerance. *Annualreviews.OrgR Munns, M TesterAnnu. Rev. Plant Biol., 2008•annualreviews.Org*, *59*, 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Naing, A. H., Campol, J. R., Kang, H., Xu, J., Chung, M. Y., & Kim, C. K. (2022a). Role of Ethylene Biosynthesis Genes in the Regulation of Salt Stress and Drought Stress Tolerance in Petunia. *Frontiers in Plant Science*, *13*, 844449. https://doi.org/10.3389/FPLS.2022.844449/BIBTEX

Naing, A. H., Campol, J. R., Kang, H., Xu, J., Chung, M. Y., & Kim, C. K. (2022b). Role of Ethylene Biosynthesis Genes in the Regulation of Salt Stress and Drought Stress Tolerance in Petunia. *Frontiers in Plant Science*, *13*, 844449. https://doi.org/10.3389/FPLS.2022.844449/FULL

Nakatsuka, A., Murachi, S., Okunishi, H., Shiomi, S., Nakano, R., Kubo, Y., & Inaba, A. (1998). Differential Expression and Internal Feedback Regulation of 1-Aminocyclopropane-1-Carboxylate Synthase, 1-Aminocyclopropane-1-Carboxylate Oxidase, and Ethylene Receptor Genes in Tomato Fruit during Development and Ripening. *Plant Physiology*, *118*(4), 1295–1305. https://doi.org/10.1104/PP.118.4.1295

Negrão, S., Schmöckel, S. M., & Tester, M. (2017). Evaluating physiological responses of plants to salinity stress. *Annals of Botany*, *119*(1), 1–11. https://doi.org/10.1093/AOB/MCW191

Nishida, K., Khan, N. M., & Shiozawa, S. (2009). Effects of salt accumulation on the leaf water potential and transpiration rate of pot-grown wheat with a controlled saline groundwater table. *Soil Science and Plant Nutrition*, *55*(3), 375–384. https://doi.org/10.1111/J.1747-0765.2009.00368.X

Nongpiur, R. C., Singla-Pareek, S. L., & Pareek, A. (2016). Genomics Approaches For Improving Salinity Stress Tolerance in Crop Plants. *Current Genomics*, *17*(4), 343. https://doi.org/10.2174/1389202917666160331202517

Oshadie, G., Silva, D., Abeysundara, A. T., Minoli, M., & Aponso, W. (2017). Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. *~ 29 ~ American Journal of Essential Oils and Natural Products*, *5*(2), 29–32.

Ouyang, S., Zhu, W., Hamilton, J., Lin, H., Campbell, M., Childs, K., Thibaud-Nissen, F., Malek, R. L., Lee, Y., Zheng, L., Orvis, J., Haas, B., Wortman, J., & Buell, R. C. (2007). The TIGR Rice Genome Annotation Resource: improvements and new features. *Nucleic Acids Research*, *35*(suppl_1), D883–D887. https://doi.org/10.1093/NAR/GKL976

Pattyn, J., Vaughan-Hirsch, J., & Van de Poel, B. (2021). The regulation of ethylene biosynthesis: a complex multilevel control circuitry. *New Phytologist*, *229*(2), 770–782. https://doi.org/10.1111/NPH.16873

(PDF) Determination of Antioxidant Properties and the Bioactive Compounds in Wheat (Triticum aestivum L.). (n.d.). Retrieved August 29, 2024, from https://www.researchgate.net/publication/326631826_Determination_of_Antioxidant_Properties_and_the_Bioactive_Compounds_in_Wheat_Triticum_aestivum_L

Pech, J. C., Latché, A., & Bouzayen, M. (2010). Ethylene Biosynthesis. *Plant Hormones: Biosynthesis, Signal Transduction, Action!*, 115–136. https://doi.org/10.1007/978-1-4020-2686-7_6/COVER

Peiser, G. D., Wang, T., Hoffman, N. E., Yang, S. F., Liut, H., WALSHt, C. T., & Stumpf, P. K. (1984). Formation of cyanide from carbon 1 of 1-aminocyclopropane-1-carboxylic acid during its conversion to ethylene. *Proceedings of the National Academy of Sciences*, *81*(10), 3059–3063. https://doi.org/10.1073/PNAS.81.10.3059

Pratyusha, S., & Pratyusha, S. (2022). *Phenolic Compounds in the Plant Development and Defense: An Overview*. https://doi.org/10.5772/INTECHOPEN.102873

Prescott, A. G. (2000). Chapter Eight Two-oxoacid-dependent dioxygenases: Inefficient enzymes or evolutionary driving force? *Recent Advances in Phytochemistry*, *34*(C), 249–284. https://doi.org/10.1016/S0079-9920(00)80009-X

Rattan, A., Kapoor, D., Ashish, Kapoor, N., & Sharma, A. (2022). Involvement of brassinosteroids in plant response to salt stress. *Brassinosteroids in Plant Developmental Biology and Stress Tolerance*, 237–253. https://doi.org/10.1016/B978-0-12-813227-2.00003-5

Rio, D. C., Ares, M., Hannon, G. J., & Nilsen, T. W. (2010). Purification of RNA Using TRIzol (TRI Reagent). *Cold Spring Harbor Protocols*, *2010*(6), pdb.prot5439. https://doi.org/10.1101/PDB.PROT5439

Riyazuddin, R., Verma, R., Singh, K., Nisha, N., Keisham, M., Bhati, K. K., Kim, S. T., & Gupta, R. (2020a). Ethylene: A Master Regulator of Salinity Stress Tolerance in Plants. *Biomolecules 2020, Vol. 10, Page 959*, *10*(6), 959. https://doi.org/10.3390/BIOM10060959

Riyazuddin, R., Verma, R., Singh, K., Nisha, N., Keisham, M., Bhati, K. K., Kim, S. T., & Gupta, R. (2020b). Ethylene: A Master Regulator of Salinity Stress Tolerance in Plants. *Biomolecules*, *10*(6), 1–22. https://doi.org/10.3390/BIOM10060959

Rowe, J. H., Topping, J. F., Liu, J., & Lindsey, K. (2016). Abscisic acid regulates root growth under osmotic stress conditions via an interacting hormonal network with cytokinin, ethylene and auxin. *The New Phytologist*, *211*(1), 225. https://doi.org/10.1111/NPH.13882

Royo, A., & Abió, D. (2003). Salt tolerance in durum wheat cultivars. *Spanish Journal of Agricultural Research*, *1*(3), 27. https://doi.org/10.5424/SJAR/2003013-32

Rudus´•, I., Rudus´•, R., Sasiak, M., Ke, J., ¸pczyn´ski, ¸pczyn´ski, & Sasiak, M. (2012a). Regulation of ethylene biosynthesis at the level of 1-aminocyclopropane-1-carboxylate oxidase (ACO) gene. *Acta Physiologiae Plantarum 2012 35:2*, *35*(2), 295–307. https://doi.org/10.1007/S11738-012-1096-6

Rudus´•, I., Rudus´•, R., Sasiak, M., Ke, J., ¸pczyn´ski, ¸pczyn´ski, & Sasiak, M. (2012b). Regulation of ethylene biosynthesis at the level of 1-aminocyclopropane-1-carboxylate oxidase (ACO) gene. *Acta Physiologiae Plantarum 2012 35:2*, *35*(2), 295–307. https://doi.org/10.1007/S11738-012-1096-6

Rudus´•, I., Rudus´•, R., Sasiak, M., Ke, J., ¸pczyn´ski, ¸pczyn´ski, & Sasiak, M. (2012c). Regulation of ethylene biosynthesis at the level of 1-aminocyclopropane-1-carboxylate oxidase (ACO) gene. *Acta Physiologiae Plantarum 2012 35:2*, *35*(2), 295–307. https://doi.org/10.1007/S11738-012-1096-6

Rudus´•, I., Rudus´•, R., Sasiak, M., Ke, J., ¸pczyn´ski, ¸pczyn´ski, & Sasiak, M. (2012d). Regulation of ethylene biosynthesis at the level of 1-aminocyclopropane-1-carboxylate oxidase (ACO) gene.

Acta Physiologiae Plantarum 2012 35:2, *35*(2), 295–307. https://doi.org/10.1007/S11738-012-1096-6

Saddiq, M. S., Iqbal, S., Afzal, I., Ibrahim, A. M. H., Bakhtavar, M. A., Hafeez, M. B., Jahanzaib, & Maqbool, M. M. (2019). Mitigation of salinity stress in wheat (Triticum aestivum L.) seedlings through physiological seed enhancements. *Journal of Plant Nutrition*, *42*(10), 1192–1204. https://doi.org/10.1080/01904167.2019.1609509

Saddiq, M. S., Iqbal, S., Hafeez, M. B., Ibrahim, A. M. H., Raza, A., Fatima, E. M., Baloch, H., Jahanzaib, Woodrow, P., & Ciarmiello, L. F. (2021). Effect of Salinity Stress on Physiological Changes in Winter and Spring Wheat. *Agronomy 2021, Vol. 11, Page 1193*, *11*(6), 1193. https://doi.org/10.3390/AGRONOMY11061193

Seleiman, M., Alhammad, B. A., Hassan, M. U., Seleiman, M. F., Aslam, M. T., Maqbool, R., Chattha, M. U., Khan, I., Gitari, H. I., Uslu, O. S., Roy, R., & Battaglia, M. L. (n.d.). *Salinity Stress in Wheat: Effects, Mechanisms and Management Strategies*. https://doi.org/10.32604/phyton.2022.017365

Seleiman, M., Aslam, M. T., Alhammad, B. A., Hassan, M. U., Seleiman, M. F., Maqbool, R., Chattha, M. U., Khan, I., Gitari, H. I., Uslu, O. S., Roy, R., & Battaglia, M. L. (n.d.). *Salinity Stress in Wheat: Effects, Mechanisms and Management Strategies Screening of Bio-inoculum for bacterial leaf blight of rice View project DETERMINATION OF NATURALLY GROWING ENDEMIC SAINFOIN SPECIES IN TURKEY/KAHRAMANMARAŞ AND DETERMINATION OF MORPHOLOGICAL CHARACTERISTICS AND FEED QUALITY VALUES View project Salinity Stress in Wheat: Effects, Mechanisms and Management Strategies*. https://doi.org/10.32604/phyton.2022.017365

Seleiman, M. F., Aslam, M. T., Alhammad, B. A., Hassan, M. U., Maqbool, R., Chattha, M. U., Khan, I., Gitari, H. I., Uslu, O. S., Roy, R., & Battaglia, M. L. (2022). Salinity Stress in Wheat: Effects, Mechanisms and Management Strategies. *Phyton-International Journal of Experimental Botany*, *91*(4), 667–694. https://doi.org/10.32604/PHYTON.2022.017365

Siddikee, M. A., Chauhan, P. S., & Sa, T. (2012a). Regulation of Ethylene Biosynthesis Under Salt Stress in Red Pepper (Capsicum annuum L.) by 1-Aminocyclopropane-1-Carboxylic Acid (ACC) Deaminase-producing Halotolerant Bacteria. *Journal of Plant Growth Regulation*, *31*(2), 265–272. https://doi.org/10.1007/S00344-011-9236-6/TABLES/1

Siddikee, M. A., Chauhan, P. S., & Sa, T. (2012b). Regulation of Ethylene Biosynthesis Under Salt Stress in Red Pepper (Capsicum annuum L.) by 1-Aminocyclopropane-1-Carboxylic Acid (ACC) Deaminase-producing Halotolerant Bacteria. *Journal of Plant Growth Regulation*, *31*(2), 265–272. https://doi.org/10.1007/S00344-011-9236-6/TABLES/1

Sornchai, P., van Doorn, W. G., Imsabai, W., Burns, P., & Chanprame, S. (2020). Dendrobium orchids carrying antisense ACC oxidase: small changes in flower morphology and a delay of bud abortion, flower senescence, and abscission of flowers. *Transgenic Research*, *29*(4), 429–442. https://doi.org/10.1007/S11248-020-00209-8/TABLES/2

Su, P., Sui, C., Wang, S., Liu, X., Zhang, G., Sun, H., Wan, K., Yan, J., & Guo, S. (2023). Genome-wide evolutionary analysis of AUX/IAA gene family in wheat identifies a novel gene TaIAA15-1A regulating flowering time by interacting with ARF. *International Journal of Biological Macromolecules*, *227*, 285–296. https://doi.org/10.1016/J.IJBIOMAC.2022.12.175

Tao, J. J., Chen, H. W., Ma, B., Zhang, W. K., Chen, S. Y., & Zhang, J. S. (2015). The Role of Ethylene in Plants Under Salinity Stress. *Frontiers in Plant Science*, *6*(NOVEMBER), 1–12. https://doi.org/10.3389/FPLS.2015.01059

Tu, Y., He, B., Gao, S., Guo, D., Jia, X., Dong, X., & Guo, M. (2019). CtACO1 Overexpression Resulted in the Alteration of the Flavonoids Profile of Safflower. *Molecules 2019, Vol. 24, Page 1128*, *24*(6), 1128. https://doi.org/10.3390/MOLECULES24061128

Ullah, M. A., Abdullah-zawawi, M. R., Zainal-abidin, R. A., Sukiran, N. L., Uddin, M. I., & Zainal, Z. (2022). A Review of Integrative Omic Approaches for Understanding Rice Salt Response Mechanisms. *Plants 2022, Vol. 11, Page 1430*, *11*(11), 1430. https://doi.org/10.3390/PLANTS11111430

Untergasser, A., Nijveen, H., Rao, X., Bisseling, T., Geurts, R., & Leunissen, J. A. M. (2007). Primer3Plus, an enhanced web interface to Primer3. *Nucleic Acids Research*, *35*(suppl_2), W71–W74. https://doi.org/10.1093/NAR/GKM306

Van de Poel, B., Vandenzavel, N., Smet, C., Nicolay, T., Bulens, I., Mellidou, I., Vandoninck, S., Hertog, M. L. A. T. M., Derua, R., Spaepen, S., Vanderleyden, J., Waelkens, E., De Proft, M. P., Nicolai, B. M., & Geeraerd, A. H. (2014). Tissue specific analysis reveals a differential organization and regulation of both ethylene biosynthesis and E8 during climacteric ripening of tomato. *BMC Plant Biology*, *14*(1), 1–15. https://doi.org/10.1186/1471-2229-14-11/FIGURES/11

Wahid, A. (2003). Air pollution impacts on vegetation in Pakistan. *Air Pollution Impacts on Crops and Forests: A Global Assessment*, 189–213.

Wang, K. L. C., Li, H., & Ecker, J. R. (2002). Ethylene Biosynthesis and Signaling Networks. *The Plant Cell*, *14*(suppl_1), S131–S151. https://doi.org/10.1105/TPC.001768

Wei, H., Xue, Y., Chen, P., Hao, P., Wei, F., Sun, L., & Yang, Y. (2021a). Genome-wide identification and functional investigation of 1-aminocyclopropane-1-carboxylic acid oxidase (Aco) genes in cotton. *Plants*, *10*(8), 1699. https://doi.org/10.3390/PLANTS10081699/S1

Wei, H., Xue, Y., Chen, P., Hao, P., Wei, F., Sun, L., & Yang, Y. (2021b). Genome-wide identification and functional investigation of 1-aminocyclopropane-1-carboxylic acid oxidase (Aco) genes in cotton. *Plants*, *10*(8), 1699. https://doi.org/10.3390/PLANTS10081699/S1

Wei, H., Xue, Y., Chen, P., Hao, P., Wei, F., Sun, L., & Yang, Y. (2021c). Genome-wide identification and functional investigation of 1-aminocyclopropane-1-carboxylic acid oxidase (Aco) genes in cotton. *Plants*, *10*(8). https://doi.org/10.3390/PLANTS10081699/S1

Xu, J., Li, Y., Wang, Y., Liu, H., Lei, L., Yang, H., Liu, G., & Ren, D. (2008). Activation of MAPK kinase 9 induces ethylene and camalexin biosynthesis and enhances sensitivity to salt stress in Arabidopsis. *Journal of Biological Chemistry*, *283*(40), 26996–27006. https://doi.org/10.1074/jbc.M801392200

Yadav, S., Modi, P., Dave, A., Vijapura, A., Patel, D., Patel, M., Yadav, S., Modi, P., Dave, A., Vijapura, A., Patel, D., & Patel, M. (2020). Effect of Abiotic Stress on Crops. *Sustainable Crop Production*. https://doi.org/10.5772/INTECHOPEN.88434

Ye, X., Zheng, X., Zhai, D., Song, W., Tan, B., Li, J., & Feng, J. (2017). Expression patterns of ACS and ACO gene families and ethylene production in rachis and berry of grapes. *HortScience*, *52*(3), 413–422. https://doi.org/10.21273/HORTSCI11050-16

Zanetti, M. E., Terrile, M. C., Arce, D., Godoy, A. V., San Segundo, B., & Casalongué, C. (2002). Isolation and characterization of a potato cDNA corresponding to a 1-aminocyclopropane-1-carboxylate (ACC) oxidase gene differentially activated by stress. *Journal of Experimental Botany*, *53*(379), 2455–2457. https://doi.org/10.1093/JXB/

Zapata, P. J., Botella, M. Á., Pretel, M. T., & Serrano, M. (2007). Responses of ethylene biosynthesis to saline stress in seedlings of eight plant species. *Plant Growth Regulation*, *53*(2), 97–106. https://doi.org/10.1007/S10725-007-9207-X/FIGURES/6

Zhang, M., Smith, J. A. C., Harberd, N. P., & Jiang, C. (2016). The regulatory roles of ethylene and reactive oxygen species (ROS) in plant salt stress responses. *Plant Molecular Biology 2016 91:6*, *91*(6), 651–659. https://doi.org/10.1007/S11103-016-0488-1

Zhang, Z., Ren, J.-S., Clifton, I. J., & Schofield, C. J. (2004). Crystal Structure and Mechanistic Implications of 1-Aminocyclopropane-1-Carboxylic Acid Oxidase-The Ethylene-Forming Enzyme. *Chemistry & Biology*, *11*, 1383–1394. https://doi.org/10.1016/j

Zhu, X., Qi, L., Liu, X., Cai, S., Xu, H., Huang, R., Li, J., Wei, X., & Zhang, Z. (2014). The Wheat Ethylene Response Factor Transcription Factor PATHOGEN-INDUCED ERF1 Mediates Host Responses to Both the Necrotrophic Pathogen Rhizoctonia cerealis and Freezing Stresses. *Plant Physiology*, *164*(3), 1499. https://doi.org/10.1104/PP.113.229575

Zou, P., Li, K., Liu, S., He, X., Zhang, X., Xing, R., & Li, P. (2016). Effect of Sulfated Chitooligosaccharides on Wheat Seedlings (Triticum aestivum L.) under Salt Stress. *Journal of Agricultural and Food Chemistry*, *64*(14), 2815–2821. https://doi.org/10.1021/ACS.JAFC.5B05624/ASSET/IMAGES/LARGE/JF-2015-05624T_0001.JPEG

turnitin

Dr. Alvina Gul
Tenured Associate Professor
Deptt of Plant Biotechnology
Atta-ur-Rahman School of Applied
Biosciences (ASAB), NUST, Islamabad

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author	Warda Imtiaz
Assignment title	MS thesis
Submission title	Warda Thesis
File name	Warda Thesis
File size	569.14K
Page count	68
Word count	16,288
Character count	92,526
Submission date	31-Aug-2024 03:21AM (UTC-0700)
Submission ID	2441818700

Dr. Alvina Gul
Tenured Associate Professor
Deptt of Plant Biotechnology
Atta-ur-Rahman School of Applied
Biosciences (ASAB), NUST, Islamabad

Warda Thesis

ORIGINALITY REPORT

13%	7%	10%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	link.springer.com Internet Source	2%
2	www.ncbi.nlm.nih.gov Internet Source	1%
3	www.frontiersin.org Internet Source	1%
4	Mahmoud F. Seleiman, Muhammad Talha Aslam, Bushra Ahmed Alhammad, Muhammad Umair Hassan et al. "Salinity Stress in Wheat: Effects, Mechanisms and Management Strategies", Phyton, 2022 Publication	1%
5	Arlett L. Ibarra-Villarreal, Azucena Gándara-Ledezma, América Dafne Godoy-Flores, Angélica Herrera-Sepúlveda et al. "Salt-tolerant Bacillus species as a promising strategy to mitigate the salinity stress in wheat (Triticum turgidum subsp. durum)", Journal of Arid Environments, 2021 Publication	1%

6	Wahid, A.. "Influence of atmospheric pollutants on agriculture in developing countries: A case study with three new wheat varieties in Pakistan", Science of the Total Environment, The, 20061201 Publication	1 %
7	Submitted to Higher Education Commission Pakistan Student Paper	<1 %
8	Donghua Chen, Xiaoyan Ma, Chunlong Li, Wei Zhang, Guangmin Xia, Mei Wang. "A wheat aminocyclopropane-1-carboxylate oxidase gene, TaACO1, negatively regulates salinity stress in Arabidopsis thaliana", Plant Cell Reports, 2014 Publication	<1 %
9	"Wheat Production in Changing Environments", Springer Science and Business Media LLC, 2019 Publication	<1 %
10	Submitted to Chicago State University Student Paper	<1 %
11	Mehar Fatma, Mohd Asgher, Noushina Iqbal, Faisal Rasheed, Zebus Sehar, Adriano Sofo, Nafees A. Khan. "Ethylene Signaling under Stressful Environments: Analyzing Collaborative Knowledge", Plants, 2022 Publication	<1 %

12	www.intechopen.com Internet Source	<1 %
13	R Ambika Rajendran, Ayyagari Ramlal, Virendra Pal Singh. "Phytohormones in Abiotic Stress", CRC Press, 2024 Publication	<1 %
14	Liu, Mingchun, Julien Pirrello, Christian CHERVIN, Jean-Paul Roustan, and Mondher Bouzayen. "Ethylene control of fruit ripening: revisiting the complex network of transcriptional regulation", PLANT PHYSIOLOGY, 2015. Publication	<1 %
15	"Improving Crop Resistance to Abiotic Stress", Wiley, 2012 Publication	<1 %
16	Hengling Wei, Yujun Xue, Pengyun Chen, Pengbo Hao, Fei Wei, Lu Sun, Yonglin Yang. "Genome-Wide Identification and Functional Investigation of 1-Aminocyclopropane-1-carboxylic Acid Oxidase (ACO) Genes in Cotton", Plants, 2021 Publication	<1 %
17	Submitted to Gordon Institute of Business Science Student Paper	<1 %
18	digitalcommons.usu.edu	

	Internet Source	<1%
19	Tao, Jian-Jun, Hao-Wei Chen, Biao Ma, Wan-Ke Zhang, Shou-Yi Chen, and Jin-Song Zhang. "The Role of Ethylene in Plants Under Salinity Stress", Frontiers in Plant Science, 2015. Publication	<1%
20	hdl.handle.net Internet Source	<1%
21	Sheen Khan, Zebus Sehar, Nidhi, Mohammed Albaqami, Nafees A. Khan. "Ethylene crosstalk with isoprenoid-derived signaling molecules in the context of salinity tolerance", Environmental and Experimental Botany, 2023 Publication	<1%
22	"Genomic Designing for Abiotic Stress Resistant Oilseed Crops", Springer Science and Business Media LLC, 2022 Publication	<1%
23	jjbs.hu.edu.jo Internet Source	<1%
24	Dave, Anand. "Development of Wide Spectrum Biocontrol Actinomycetes Against Fungal Phytopathogens of Cajanus cajan", Maharaja Sayajirao University of Baroda (India), 2023	<1%

	Publication	
25	"Annual Plant Reviews Volume 44", Wiley, 2012 Publication	<1 %
26	Munoz-Robredo, P.. "Ethylene biosynthesis in apricot: Identification of a ripening-related 1-aminocyclopropane-1-carboxylic acid synthase (ACS) gene", Postharvest Biology and Technology, 201201 Publication	<1 %
27	Inderjit, K.M.M. Dakshini, Chester L. Foy. "PRINCIPLES and PRACTICES in PLANT ECOLOGY - Allelochemical Interactions", CRC Press, 2023 Publication	<1 %
28	M. Iqbal R. Khan, Amarjeet Singh, Péter Poór. "Improving Abiotic Stress Tolerance in Plants", CRC Press, 2020 Publication	<1 %
29	bmcgenomics.biomedcentral.com Internet Source	<1 %
30	Amram Eshel, Tom Beeckman. "Plant Roots - The Hidden Half, Fourth Edition", CRC Press, 2019 Publication	<1 %
31	Submitted to University of Nottingham Student Paper	<1 %

32	www.tandfonline.com Internet Source	<1 %
33	vdoc.pub Internet Source	<1 %
34	Yuxin Zhang, Yirong Zhang, Ze Yu, Hanyu Wang, Boya Ping, Yunxiao Liu, Jiakai Liang, Fengwang Ma, Yangjun Zou, Tao Zhao. "Insights into ACO genes across Rosaceae: evolution, expression, and regulatory networks in fruit development", Genes & Genomics, 2024 Publication	<1 %
35	dspace.dtu.ac.in:8080 Internet Source	<1 %
36	Submitted to Curtin University of Technology Student Paper	<1 %
37	Lanfen Wang, Jifeng Zhu, Xiaoming Li, Shumin Wang, Jing Wu. "Salt and drought stress and ABA responses related to bZIP genes from V. radiata and V. angularis", Gene, 2018 Publication	<1 %
38	etheses.whiterose.ac.uk Internet Source	<1 %
39	comptes-rendus.academie-sciences.fr Internet Source	<1 %
	ethesis.inp-toulouse.fr	

40	Internet Source	<1%
41	rnajournal.cshlp.org Internet Source	<1%
42	Bei-ling Fu, Wen-qiu Wang, Xiao-fen Liu, Xue-wu Duan, Andrew C. Allan, Donald Grierson, Xue-ren Yin. "An ethylene-hypersensitive methionine sulfoxide reductase regulated by NAC transcription factors increases methionine pool size and ethylene production during kiwifruit ripening", New Phytologist, 2021 Publication	<1%
43	Sergio Saia, Giandomenico Corrado, Paola Vitaglione, Giuseppe Colla et al. "An Endophytic Fungi-Based Biostimulant Modulates Volatile and Non-Volatile Secondary Metabolites and Yield of Greenhouse Basil (Ocimum basilicum L.) through Variable Mechanisms Dependent on Salinity Stress Level", Pathogens, 2021 Publication	<1%
44	pvj.com.pk Internet Source	<1%
45	rrs.mmu.edu.my Internet Source	<1%

46	George, Dominic Soloman. "Proteomic and Quality Analysis of UV-C and Thermal Treated Cut Chokanan Mango (Mangifera Indica L.) and Josephine Pineapple (Ananas Comosus L. MERR.)", University of Malaya (Malaysia), 2023 Publication	<1 %
47	I. EL-SHARKAWY. "Differential regulation of ACC synthase genes in cold-dependent and -independent ripening in pear fruit", Plant Cell and Environment, 10/2004 Publication	<1 %
48	Jolienolien Pattyn, Johnohn Vaughan-Hirsch, Bramram Van de Poel. "The regulation of ethylene biosynthesis: a complex multilevel control circuitry", New Phytologist, 2020 Publication	<1 %
49	M. Houben, J. Vaughan-Hirsch, J. Pattyn, W. Mou et al. "1-Aminocyclopropane-1-carboxylic acid oxidase determines the fate of ethylene biosynthesis in a tissue-specific way to fine-tune development and stress resilience", Cold Spring Harbor Laboratory, 2024 Publication	<1 %
50	Shrestha, Kumar. "Genetic Mechanisms Underlying Sorghum Plant Resistance to Sugarcane Aphid", Oklahoma State University, 2024	<1 %

	Publication	
51	H. R. Lerner. "Plant Responses to Environmental Stresses - From Phytohormones to Genome Reorganization", CRC Press, 2018 Publication	<1 %
52	patents.google.com Internet Source	<1 %
53	www.chemistry-blog.com Internet Source	<1 %
54	www.ijcmas.com Internet Source	<1 %
55	www.researchsquare.com Internet Source	<1 %
56	Xiao Wang, Juanjuan Zhang, Jing Zhang, Chuanen Zhou, Lu Han. "Genome-wide characterization of AINTEGUMENTA-LIKE family in Medicago truncatula reveals the significant roles of AINTEGUMENTAs in leaf growth", Frontiers in Plant Science, 2022 Publication	<1 %
57	"Management of Phytonematodes: Recent Advances and Future Challenges", Springer Science and Business Media LLC, 2020 Publication	<1 %

58	Ayman EL Sabagh, Mohammad Sohidul Islam, Milan Skalicky, Muhammad Ali Raza et al. "Salinity Stress in Wheat (Triticum aestivum L.) in the Changing Climate: Adaptation and Management Strategies", Frontiers in Agronomy, 2021 Publication	<1 %
59	Srinivasan Damodaran, Kirk L. Parkin. "Fennema's Food Chemistry", CRC Press, 2017 Publication	<1 %

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		