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DEPARTMENT OF BIOLOGY



DIVERSITY IN WHEAT MORPHOLOGY AND PHYSIOLOGY
UNDER SALT STRESS AND ITS BIOINFORMATIC
BACKGROUND

DOCTOR OF PHILOSOPHY, BIOLOGY

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ABSTRACT

DIVERSITY IN WHEAT MORPHOLOGY AND PHYSIOLOGY UNDER SALT STRESS AND ITS BIOINFORMATIC BACKGROUND

PHD THESIS

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(SUPERVISOR: PROF. DR. GÜNCE ŞAHİN)**

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Salinity stress is an important abiotic factor limiting wheat production, especially in arid and semi-arid regions, due to improper irrigation, climate change, global warming, and soil salinity. This study was conducted to evaluate the effects of different levels of salt stress on morphological, physiological, and molecular responses in local wheat cultivars. Within the scope of the study, germination percentage, root length (cm), number of roots (number), coleoptile length (cm), wet and dry seedling weight (g), relative water content (%), chlorophyll content (mg/g), soluble sugars (mg/g), proteins (mg/g), amino acid content (mg/g), proline accumulation and expression levels of SOS1, RAS1, TAS14 genes were analyzed and salinity tolerance was examined.

The results revealed significant genetic differences in salinity tolerance among the tested wheat genotypes. Morphological evaluations revealed that genotypes LW5, LW6 and LW7 were the most resistant cultivars and showed superior tolerance to increasing salt levels with high germination rates, robust root and coleoptile development, while physiological analyses revealed that these resistant genotypes exhibited higher chlorophyll conservation, higher accumulation of soluble sugars and proline and improved osmotic adjustment mechanisms. At the molecular level, SOS1 was identified as the master regulator of ion homeostasis, RAS1 was involved in ABA-mediated stress signaling, and TAS14 was associated with osmotic stress tolerance. Phylogenetic and protein domain analyses confirmed the genetic diversity among these genotypes, highlighting the functional importance of these genes in salt stress adaptation.

These findings suggest the necessity of combining molecular and traditional (classical) breeding strategies to develop high-yielding and salt-tolerant wheat varieties. The results obtained should be supported by field trials to validate the laboratory findings under agronomic conditions, accounting for variables such as environmental factors. Furthermore, future research should focus on the identification of additional stress-related genes and the use of transcriptomic and proteomic approaches to better understand the genetic mechanisms of salinity tolerance.

KEYWORDS: Wheat, Salinity Stress, Morphology, Physiology, Molecular Analysis, Salt Tolerance, Abiotic Stress, Genetic Variation, Sustainable Agriculture.

ÖZET

TUZ STRESİ ALTINDAKİ BUĞDAYDA MORFOLOJİK VE FİZYOLOJİK FARKLILIKLAR VE BİYOİNFORMATİK ARDALANI DOKTORA TEZİ

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Tuzluluk stresi, özellikle kurak ve yarı kurak bölgelerde, yanlış sulama, iklim değişikliği, küresel ısınma, toprak tuzluluğu gibi nedenlerle buğday üretimini sınırlayan önemli bir abiyotik (cansız) faktördür. Bu çalışma, farklı düzeylerdeki tuz stresinin, yerel buğday çeşitlerinde morfolojik, fizyolojik ve moleküler tepkilere etkilerini değerlendirmek için yapılmıştır. Araştırma kapsamında çimlenme yüzdesi, kök uzunluğu (cm), kök sayısı (adet), koleoptil uzunluğu (cm), yaş ve kuru fide ağırlığı (g), relatif su içeriği (%), klorofil içeriği (mg/g), çözünür şekerler (mg/g), proteinler (mg/g), amino asit içeriği (mg/g), prolin birikimi ve SOS1, RAS1, TAS14 genlerinin ekspresyon düzeyleri analiz edilerek tuzluluk toleransı incelenmiştir.

Sonuçlar, test edilen buğday genotiplerinde tuzluluk toleransında önemli genetik farklılıklar olduğunu ortaya koymuştur. Morfolojik değerlendirmelerde, LW5, LW6 ve LW7 genotipleri en dayanıklı çeşitler olarak belirlenmiş olup, yüksek çimlenme oranları, sağlam kök ve koleoptil gelişimleri ile artan tuz düzeylerine karşı üstün dayanıklılık göstermiş; fizyolojik analizler ise dayanıklı bu genotiplerin daha yüksek klorofil korunumu, daha çok çözünür şeker ile prolin birikimi ve gelişmiş ozmotik uyum mekanizmaları sergilediklerini ortaya koymuştur. Moleküler düzeyde, SOS1 iyon homeostazisinin ana düzenleyicisi olarak belirlenirken, RAS1 ABA aracılı stres sinyallemede rol almış ve TAS14 ozmotik stres toleransı ile ilişkilendirilmiştir. Filogenetik ve protein domain analizleri, bu genotipler arasındaki genetik çeşitliliği doğrulayarak, bu genlerin tuz stresine adaptasyondaki işlevsel önemini vurgulamıştır.

Bu bulgular, yüksek verimli ve tuza dayanıklı buğday çeşitleri geliştirmede moleküler ve geleneksel (klasik) ıslah stratejilerinin birleştirilmesinin gerekliliğini ortaya koymaktadır. Elde edilen sonuçlar, çevresel faktörler gibi değişkenleri de göz önünde bulundurmak için laboratuvar sonuçlarının tarımsal koşullarda doğrulanması amacıyla alan denemeleri ile desteklenmelidir. Ayrıca, gelecekteki araştırmalar, tuzluluğa karşı toleransın genetik mekanizmalarını daha iyi anlamak için ek stresle ilişkili genlerin belirlenmesine ve transkriptomik ile proteomik yaklaşımların kullanılmasına odaklanmalıdır.

ANAHTAR KELİMELELER: Buğday, Tuzluluk Stresi, Morfoloji, Fizyoloji, Moleküler Analiz, Tuz Toleransı, Genetik Çeşitlilik, Sürdürülebilir Tarım

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LIST OF ABBREVIATIONS AND SYMBOLS

%	: Percentage
°C	: Degrees Celsius
μL	: Microliter
ABA	: Absciscic Acid
bp	: Base Pairs
C₅H₉O₂	: Proline Acid Concentration
Chl	: Chlorophyll
Cl⁻	: Chloride Ion
CO₂	: Carbon Dioxide
CTAB	: Hexadecyl Trimethyl-Ammonium Bromide
DDI	: Double Distilled Water
DNA	: Deoxyribonucleic Acid
dSm⁻¹	: Decisiemens per meter (unit of electrical conductivity for soil salinity)
EDTA	: Ethylenediaminetetraacetic Acid
g	: Gram
H₂O	: Water
HCl	: Hydrochloric Acid
K₂O	: Potassium Oxide
L	: Liter
LW5	: Libya Wheat - Bushi
LW6	: Libya Wheat - Kharisi
LW7	: Libya Wheat - Jamnia
LW8	: Libya Wheat – Grace
M	: Molar
mL	: Milliliter
mM	: Millimolar
Mw	: Molecular Weight
N	: Nitrogen
NaCl	: Sodium Chloride
NH₄⁺	: Ammonium Ion
nm	: Nanometer
NO₃⁻	: Nitrate Ion
OP	: Osmotic Pressure
PCR	: Polymerase Chain Reaction
PH	: Potential of Hydrogen
PVP	: Polyvinyl Pyrrolidone

RNA	: Ribonucleic Acid
ROS	: Reactive Oxygen Species
rpm	: Revolutions Per Minute
TAE	: Tris-Acetate-EDTA Buffer
TBE	: Tris: Borate-EDTA Buffer
TE	: Tris-EDTA Buffer
TW1	: Türkiye Wheat - Einkorn
TW2	: Türkiye Wheat - Emmer
TW3	: Türkiye Wheat - Bread
TW4	: Türkiye Wheat - Durum
UV	: Ultraviolet
WDS	: Water Deficit Stress



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1. INTRODUCTION

Soil salinity is one of the major issues hindering agricultural productivity in both irrigated and rainfed lands, particularly in arid and semi-arid regions worldwide. Soil salinization is considered a significant threat to global food security, especially where irrigation plays a major role in agriculture. The imbalanced accumulation of salts in the soil reduces fertility, impeding plant growth and ultimately lowering yield. Mineral weathering and seawater intrusion are natural processes that contribute to soil salinity, while over-irrigation, poor drainage, and improper farming practices are human-made causes (Munns & Tester, 2008).

Salinization occurs when the concentration of soluble salts in the root zone reaches levels that inhibit plant growth. The most prevalent soluble salts are Na^+ , Ca^{2+} , Mg^{2+} , Cl^- , and SO_4^{2-} , with trace amounts of K^+ , HCO_3^- , NO_3^- , and B (Rengasamy, 2010). Osmotic stress, ion toxicity, and nutrient imbalance are the primary physiological effects of soil salinity on crops, leading to disruption in various metabolic processes in the plant (Zhu, 2001).

The water balance in the ecosystem (inputs: precipitation and irrigation; outputs: evapotranspiration and drainage) drives the accumulation of salts in agricultural soils. In wet climates, high precipitation and low evaporation cause the leaching of salts, preventing significant accumulation. However, in dry and semi-arid areas, where evaporation rates exceed precipitation rates, salts remain in the soil, gradually accumulating and leading to salinization (Flowers & Colmer, 2008). Climate change, with its rising temperatures and decreasing freshwater availability, exacerbates the challenges to agricultural sustainability (Shrivastava & Kumar, 2015).

Libya's agricultural sector is particularly vulnerable to soil salinity due to its location in arid and semi-arid zones. Low annual rainfall and high temperatures result in high rates of evapotranspiration, which accelerates salt accumulation. According to the FAO, 78% of Libya's agricultural land is rainfed, with only 22% irrigated through methods such as flood irrigation or lining. The continued use of these irrigation practices, combined with uncontrolled evaporation and drainage, has contributed to the widespread salinization of productive lands, reducing crop

productivity and, in the long term, decreasing the amount of arable land (Ghassemi et al., 1995).

Soil salinity mainly hampers agricultural productivity by affecting plants at multiple levels. At the morphological level, plants exposed to salinity stress exhibit stunted growth, reduced leaf expansion, and chlorosis due to toxic levels of salts (Munns et al., 2006). At the physiological level, a high level of salt concentration impairs water uptake, leading to dehydration, stomatal conductance, and consequently restricting photosynthesis and overall biomass accumulation (Parida & Das, 2005). Additionally, salinity induces oxidative stress, leading to the overproduction of reactive oxygen species (ROS) that damage membranes, proteins, and DNA (Ashraf, 2009). To cope with these challenges, plants activate defense mechanisms, including osmoregulatory adjustments, regulation of ion homeostasis, and the production of antioxidant enzymes (Hasegawa et al., 2000).

In addressing the increasing problem of soil salinity, researchers have been trying several approaches to enhance saline tolerance in plants. These include traditional breeding programs, genetic engineering, and agronomic practices. Although traditional breeding focuses on the selection of salt-tolerant cultivars (by, for example, screening for high germination at saline conditions, sodium exclusion efficiency, and osmotic regulation improvements (e.g., Flowers, 2004)), modern molecular biology has made it easier to identify and further manipulate salt-responsive genes for crop improvement. One main approach, which has been widely followed, involves the introduction of relevant genes of different categories—such as ion transport, osmoprotection, and ROS scavenging—that help confer salt tolerance to bread wheat (*Triticum aestivum*) and other crops when overexpressed (Deinlein et al., 2014).

In addition to these genetic advantages are the advantages provided by agronomic practices in improving salt tolerance in soil. One of the common practices is through soil leaching using excess water, flushing salts away from the root zone. Despite its effectiveness, the technique is water-intensive and might be inappropriate for regions with water scarcity (Qadir et al., 2014). Gypsum (CaSO_4) as a soil amendment has been employed to improve soil structure, leading to the displacement of sodium ions, thereby advancing soil sodicity and enhancing water infiltration (Warcence et al., 2002). Biostimulants, among them plant growth regulators, microbial inoculants, and organic amendments, are proposed to enhance

salt stress tolerance by regulating plant hormonal equilibrium and promoting root-microbe interplay (Duarte et al., 2013).

Seed priming protocols could also affect relieving the vulnerability of crops to salt stress. Seed priming involves soaking seeds in solutions of growth hormones, osmoprotectants, beneficial microbes, etc., to improve seedling vigor and salt tolerance. Studies have found that pretreatments of seeds with salicylic acid, gibberellic acid, and proline will significantly improve germination, seedling growth, and antioxidant capacity under saline conditions (Hussain et al., 2016). Thus, an association with a specified salt-tolerant *rhizobacteria* is reported to improve root development and nutrient uptake in wheat and other cereals (Bharti et al., 2014).

Wheat presents a range of forms of global food and nutritional significance. Therefore, the development of salt-tolerant varieties of wheat was necessitated for a certain reason of high-yield doses in adverse environments. Wheat is highly sensitive to salt stress during seed germination, suggesting that this should be a vital focus for breeding and biotechnological intervention (Munns & Gilliam, 2015). The government of Libya has indicated that developing salt-resistant wheat strains will enhance food security for the sovereign country in nearly every wheat-growing region where salt stress coincides with low precipitation (Royo et al., 2008).

Advancements in bioinformatics and genomics of wheat have provided valuable insights into the genetic basis of salt tolerance. With the advancement of sequencing technologies, coupled with genome-wide association studies (GWAS), significant progress has been made in identifying key genes and quantitative trait loci (QTLs) associated with resistance to salinity (Negrão et al., 2017). A major contribution to understanding the gene pathways responsive to salt stress has come from transcriptome and proteomic analysis, which offer valuable insights for precision breeding and genetic modifications (Yang et al., 2020). Integrating the omic technologies with traditional breeding methods holds great promise in accelerating the development of salt-resistant varieties suitable for cultivation in saline areas.

Soil salinity is a significant threat to global agriculture, especially in arid and semi-arid regions, including in Libya. Understanding the mechanisms involved in plant adaptation to salt stress, along with recent advances in genetics, biotechnology, and agronomy, will be crucial for developing effective mitigation

strategies. With ongoing research and technological advancements, enhancing salt tolerance in wheat and other staple crops is becoming increasingly feasible, ensuring more profitable, sustainable agricultural development in the face of challenging environmental conditions.

1.1 Study Plants

Wheat (*Triticum* spp.) forms one of the most widely grown cereal crops around the world and is a staple food for a large portion of the global population. Due to its ability to tolerate various climate conditions and high nutritional value, wheat has become one of the most important food security crops in many countries (Shewry & Hey, 2015). In addition to being a food crop, wheat plays a significant role in several industries, including flour milling, bioethanol production, and animal feed (Curtis et al., 2002). Wheat also holds historical significance, particularly in countries like Türkiye and Libya, where it has been cultivated for centuries. Understanding the characteristics, history, and economic importance of wheat in these regions is critical for assessing its potential resilience to environmental stresses, including soil salinity.

1.1.1 Wheat in Türkiye: Economic, Nutritional Importance, and Geographical Distribution

Wheat is a strategic crop in Türkiye's agricultural sector, representing a significant component of both the economy and food security. In Turkey, seed genetic conservation is practiced by national and well-equipped gene banks in İzmir (the AARI) and in Ankara (CRIFC). It was established in 1972 to protect the PGRs (Zencirci et al., 2022). Wheat (*Triticum* spp.) species' cultivation in Türkiye has a long history as the region is part of the key of the Fertile Crescent, where wheat was domesticated over 10,000 years ago (Heun et al., 1997). Over time, Türkiye has adapted to the climatic changes, developing high-yielding varieties suitable for various conditions. Both *Triticum aestivum* (common wheat/ bread wheat) and *Triticum durum* (durum wheat/macaroni wheat) are cultivated in this country and extensively used for pasta-making as well as bread and other traditional food products (Nesbitt, 2002). In addition to its importance in feeding the population, wheat is a major commercial crop, with flour and pasta being among the country's major export products (FAO, 2021).

Wheat in Türkiye is grown in diverse climates, ranging from temperate coastal areas to arid and semi-arid land within the Central Anatolian plateau. Hence, given the diversity in temperature, precipitation, and soils, Turkish wheat production has been developed from combinations of traditional practices in agriculture and modern techniques. It was due to the extensive application of irrigation, the production of salt-tolerant wheat varieties, and enhanced wheat breeding programs, which have all benefited the sustainability of wheat farming practices throughout the country (Kaya et al., 2002). Several challenges include soil salinity, drought, and climate change effects, notwithstanding developments in wheat production in Türkiye. Research into the adaptability of Turkish wheat genotypes under stressful environmental conditions remains a priority for ensuring long-term food security (Munns & Tester, 2008).

Türkiye's geographical and climatic heterogeneity plays an important role in wheat production. The wheat-growing zones of the country can be divided into three broad regions: the Marmara and Aegean regions, influenced by the Mediterranean climate; the Central Anatolian region, characterized by semi-arid conditions; and the southeastern provinces, which have historically been important wheat crop production areas (FAO, 2021). Central Anatolia is particularly important for wheat production, making it a large proportion of the country's total output. However, rainfed agriculture in this region makes wheat cultivation vulnerable to fluctuations in precipitation and soil salinity (Karaman et al., 2020).

Türkiye has also made great strides in terms of wheat breeding programs, especially in salt tolerance and drought resistance. Research efforts have concentrated on developing wheat genotypes that have the capacity for high-yield cultivation under marginal soils with undesired conditions (Negrão et al., 2017). The integration of modern agronomic practices, such as precision irrigation and soil fertility management, has further contributed to wheat production, especially in saline environments (Tümşavaş & Gülser, 2017).

Soil salinization is a growing problem in wheat-producing regions of Türkiye, especially in irrigated lands, where excessive water use has led to the accumulation of salt in the root zone (Munns & Gilliam, 2015). High sodium (Na^+) and chloride (Cl^-) ions in soil disrupt water uptake in plants, leading to osmotic stress and nutrient imbalances. In wheat plants, salt stress causes reduced germination, stunted seedling growth, and lower grain yield (Flowers et al., 2015).

To address this issue, Turkish researchers are exploring various strategies to improve wheat's salinity tolerance. One such approach is identifying salt-tolerant wheat lines through field tests and molecular tools (Deinlein et al., 2014). Tolerance in certain Turkish wheat varieties has been linked to osmotic adjustment, effective sodium exclusion, and enhanced antioxidant activity (Hussain et al., 2016). Additionally, bioinformatics tools like genome-wide association studies (GWAS) have been used to identify genes associated with salt tolerance in Turkish wheat genotypes, providing a foundation for future breeding programs (Negrão et al., 2017).

Wheat is not only a staple food in Türkiye but also a key sector of the agricultural economy with its derivatives such as flour, macaroni, biscuits, etc... According to the Turkish Statistical Institute (TÜİK), wheat production in 2023 reached 22 million metric tons, marking an 11.4% increase compared to the previous year (TÜİK, 2023). Most of this wheat is used domestically, primarily in bread, which is a cornerstone of the Turkish diet. Wheat is also used to produce pasta, bulgur, and various traditional pastries (Shewry, 2009).

On the international stage, Türkiye is a major exporter of wheat flour, particularly in the Middle East, North Africa, and Central Asia (Cakmak et al., 2010). This sector supports millions of farmers, especially in rural areas where agriculture remains a crucial source of livelihood. However, the international price fluctuation of wheat, climate variability, and growing soil salinity effect make it difficult to maintain stable production of wheat (Karaman et al., 2020). To secure the long-term viability of wheat farming in Türkiye, sustainable agricultural practices, continued research on stress-resistant varieties, and improved water management are essential.

Wheat has a deep cultural and agricultural heritage in Türkiye, and research into breeding salt-tolerant wheat varieties remains crucial for adapting to changing environmental conditions. As agronomic physiology and molecular approaches evolve, Türkiye will continue to play a significant role in global wheat production while ensuring food security for its population. The findings from research on Turkish wheat genotypes under salt-stress conditions will not only benefit local farmers but also contribute to global efforts to improve wheat's resilience to environmental stresses.

Wheat is a key crop in Turkish agriculture and even the economy. Leaving aside Türkiye's place as one of the world's leading nations in producing wheat, it is continuously at the top of the ranks among the bread wheat and durum wheat producers—essential for the bread, plates of pasta, and many other staple foods (FAO, 2021).

The importance of wheat is not even confined to domestic consumption. Türkiye is also one of the major sources of wheat-based products, particularly flour and pasta, to the whole world, making an important share of the agricultural trade balance of the country (TMO, 2022).

At least 6% of the Gross Domestic Product (GDP) in Türkiye is from agricultural production, and a significant part of it comes from wheat. The country manages to produce an average of 19-22 million tons of wheat each year, depending on rain, soil conditions, and agricultural practices (TÜİK, 2023). Wheat cultivation also employs millions of Turkish farmers, especially in central Anatolia, the Thrace region, and some southeastern provinces, where it is the dominant crop (Kaya et al., 2002). Support from the Turkish government for wheat production includes grants and research, as well as irrigation projects that boost yield along with food security.

Flour milling is one of the most important economic aspects of wheat production in Türkiye. The country is the largest exporter of wheat flour in the world, with high-quality products reaching over 160 countries, especially in the Middle East, Africa, and Central Asia (International Grains Council, 2022). Türkiye's geographical location, modern milling infrastructure, and well-developed transport networks give it a competitive advantage in the global wheat market. In addition, durum wheat in Türkiye is key to pasta production, with Turkish products having increasingly received international acclaim for their quality and low price (Cakmak et al., 2010).

Despite these successes, Türkiye's wheat sector faces challenges related to climate change, soil degradation, and fluctuations in global wheat prices. Increasing drought and heat intensity pose a threat to wheat yields, especially in rainfed farming areas. Other problems facing wheat farming include soil salinity due to high irrigation without drainage most times, resulting in reduced productivity of wheat (Tümşavaş & Gülser, 2017). Türkiye invests in research and development programs to develop salt-tolerant and drought-resistant wheat varieties, to promote

sustainable farming practices, and to improve water management systems for solutions to these problems (Negrao et al., 2017).

The Turkish Grain Board (Toprak Mahsulleri Ofisi, TMO) prevents market-stabilizing actions by the government, which regulates grain prices, buys cereals (mainly common and durum wheat, barley, oats) from farmers at guaranteed prices, and manages strategic wheat reserves (TMO, 2022). Such interventions help to protect farmers from the market's volatility, ensuring a dependable supply of wheat for domestic and export uses. But, like every other global disruption, such as the COVID-19 epidemic and geopolitical conflicts, wheat prices fluctuate, which calls for resilient agricultural policies and diversified supply chains (FAO, 2021).

This wheat is the most consumed in Türkiye and constitutes the country's staple food. It is mostly utilized in producing bread, which is the hallmark of Turkish cuisine. On average, a Turkish citizen consumes about 150 kg of wheat in a year, making it one of the highest wheat-consuming countries in the world per capita (TÜİK, 2023). Much of the popular consumption of wheat-based products entails the common flatbreads, which include staple ones like pide, lavash, and yufka, and these show how deeply ingrained wheat is in the culture and diet of the country.

Not only does it play a major role in bread making, but wheat also happens to be the main raw material used in bulgur, a nutrient-rich partially cooked wheat product that has had a place in the diets of individuals in Anatolia for centuries. Bulgur is a prime source of dietary fiber and proteins, as well as being rich in essential vitamins and minerals, mainly vitamin B, Fe, and Mg (Shewry, 2009). Whole wheat products, mainly high in fiber, reduce the risk of cardiovascular diseases and all those blood sugar levels, making wheat the prominent food among foods for keeping healthy (Cakmak et al., 2010).

Another variety of wheat most commonly found in Türkiye is durum wheat. Durum wheat is meant for making pasta and couscous. Durum wheat also excels, as far as higher protein content and higher strength of gluten are concerned, compared to bread wheat, endowing it with good acceptability in the pasta production process. The Turkish pasta has been reputed strongly in world markets mainly because of its good quality and low price; this is very important in reinforcing the economic and nutritional qualities of wheat (International Grains Council, 2022).

Yet, wheat consumption in Türkiye poses challenges in diet diversification and health awareness because of diseases such as obesity and diabetes resulting from unhealthy diets. The awareness of refined wheat products has come up with a challenge to access more whole grain and higher fiber products. Many programs have been developed in the Turkish government and health organizations to help support the changes in people's diets and increase whole grain consumption and fortified wheat products (FAO, 2021).

To ensure the sustainability of wheat production in Türkiye, it is critical to the state's economy, as well as its food security. Changes in climate and environmental degradation have become threats to high-yield wheat production, which in turn calls for building resilient agricultural practices. Genetics research and development on improving wheat for resistance to salinity, drought, and diseases with due consideration for soil health and optimum water use efficiency are pursued by researchers (Negrão et al., 2017). Modern innovations include controlled irrigation systems, improved soil management practices, and precision farming technologies, which have been adopted increasingly for maximizing efficiency in wheat production. Participating in reduced environmental soil fertility would be brought about by sustainable agriculture practices like crop rotation, organic fertilization, and conservation tillage (Tümşavaş & Gülser, 2017).

Wheat is still one of the main products of the Turkish agricultural economy and national diet, contributing to harsh economic conditions through wheat flour and pasta exports. Wheat is economically very strong in international trade, and nutritionally it constitutes the main supply for the majority of macronutrients and micronutrients essential for the Turkish population. All these will thus contribute to ensuring a long-term sustainable approach to issues confronting the wheat sector, such as climate change, soil degradation, and consumer behavior change. For the continued significance of wheat in economic and nutritional terms in Türkiye, investment in agricultural research coupled with policy support and sustainable farming practices would be important.

Wheat production in Türkiye spans a range of agro-ecological areas, which in a country so versatile in climates and soils is, for example, completely covered by Central Anatolia, Southeastern Anatolia, Thrace and Marmara region, Aegean region, and Eastern Anatolian highlands (FAO, 2021). Thus, each of the regions plays an important role in the total wheat production of the country, albeit with

different productivity levels due to rainfall, availability of irrigation, and soil fertility.

About 40% of total wheat production in Türkiye comes from Central Anatolia, and it has the provinces of Konya, Ankara, Afyon, Çorum, Polatlı and Eskişehir under its semi-arid hot summer and cold winter climate (Tümşavaş & Gülser, 2017). Wheat production in this region can be mainly attributed to dryland farming, with a few areas using supplemental irrigation as a result of limited rainfall. However, due to its climatic conditions, Central Anatolia has been a significant zone for wheat production due to its extensive arable land. In contrast, another important wheat field is that of Southeastern Anatolia, which is more favored by climate and has large-scale irrigation projects like the Southeastern Anatolia Project (GAP) in it. Wheat yields in towns such as Şanlıurfa, Diyarbakır, and Mardin have soared since the establishment of the modern irrigation framework (Karaman et al., 2020). Currently, this region is responsible for about 20% of the total wheat produced in the country since both irrigated and rainfed wheat farming play important roles here.

A third essential wheat-producing area in Türkiye is Thrace and the Marmara region, which is found in the northwest part of the country. This area has a temperate climate and has fairly good rainfall distribution, making it even better for the production of both bread wheat and durum wheat. Among the provinces in this region are Tekirdağ, Edirne, and Kırklareli, well known for high wheat productivity and modern agricultural techniques (Kaya et al., 2002). Proximity to big cities like Istanbul and Bursa makes this area a suitable market for wheat and wheat-related products.

The Aegean has the agricultural fame of being primarily tabled for olive and fruit production and does have some wheat growing; more especially, it is in the interior provinces of Uşak, Denizli, and Afyonkarahisar. The Mediterranean zone would be quite similar, with wheat growing in inland valleys and on plateaus, rather than in coastal areas. Through moderate rainfall and fertile soils, which combine to generally give stable yields, wheat farming thrives in both regions.

Lastly, there are the Eastern Anatolian highlands, which include Erzurum, Kars, and Van provinces, which do not qualify as easy environments for wheat growing due to extremely cold and short winters, combined with very limited growing seasons. This region has been particularly beneficial for hardy adapted

cold climate wheat varieties grown successfully in the area and hence adds to the diversity of wheat production in Türkiye (FAO, 2021).

The productivity of wheat in Türkiye varies according to factors such as climate, seed and soil quality, cultivation methods, weeds, and irrigation. Only in the last few decades has Türkiye been highly successful in improving wheat yields through the application of modern agricultural practices by the Green Revolution and the development of improved seed varieties, and mechanization-related organisations, and universities (TMO, 2022).

However, despite this improvement, wheat productivity in Türkiye currently faces several problems, such as drought, soil salinization, and the impacts of changing climate. The average yield of wheat in Türkiye has been reported to be between 2.5 and 3.5 tons per ha, subject to the conditions of the regions and farming systems in the country (FAO, 2021). For example, irrigated wheat farming in Southeastern Anatolia and Thrace can produce higher yields of over 4 tons per hectare, while dryland wheat farmers in Central Anatolia harvest less, particularly because of lower rainfall and water availability (Negrão et al., 2017).

Improved wheat varieties are probably among the most important criteria for the production of wheat in Türkiye. The Turkish government and the research institutes have funded many wheat breeding programs to produce very high-yield, pest-resistant, and drought-tolerant varieties adaptable to the various agro-ecological zones. The establishment of salt-tolerating wheat varieties is highly applicable in regions affected by salinization of soils, like the irrigated plains of Southeastern Anatolia (Cakmak et al., 2010).

Rising temperatures, modified precipitation patterns, and frequently occurring droughts could lead to reduced wheat yields in the vulnerable regions (Munns & Gilliam, 2015). To these causes, Turkish farmers have embraced conservation agriculture in terms of reduced tillage, crop rotation, and organic soil amendments to improve soil health and enhance water retention (Tümşavaş & Gülser, 2017).

Government policies and agricultural subsidies are also very critical in the maintenance of wheat productivity. The Turkish Grain Board is providing financial support to the farmers that ensure the stability of the production of wheat as well as the mitigation of market risks. Alongside it, state-backed research initiatives

develop innovative farming techniques and improve wheat adaptability towards environmental stresses (TMO, 2022).

As Türkiye develops ways to transform production into wheat under very changing environmental and economic challenges, recipes of plans are being spread to increase productivity and sustainability. With improvements such as precision agriculture related to satellite-guided irrigation and soil moisture monitoring, farmers reduce their wastage of water and waste resources (FAO, 2021). Furthermore, there are also advances made in digital farming tools, such as those used for real-time monitoring of wheat crops for the improved management of pests, diseases, and nutrient needs.

Biotechnology and genetic research in wheat improvement will acquire more emphasis. Current investigations are concentrating on identifying and incorporating important genes for drought and salinity tolerance (Negrão et al., 2017). The new molecular breeding techniques and genomic selection methods will be beneficial for the rapid establishment and proper development of climate-resilient wheat varieties in Türkiye.

Also, the most promising will be organic and regenerative wheat farming. Increased consumers' interest in sustainable food has put Turkish farmers into organic wheat to take advantage of soil health and biodiversity, as well as significantly reduced agricultural chemicals (Cakmak et al., 2010). This sustainability-driven wheat production would be in line with most agricultural trends worldwide and hence open doors for high-value exports.

Production of wheat in Türkiye immediately depends on soil fertility, biodiversity, geographical conditions, climate conditions, seeds and seeding time, and improved agricultural practices. Although the advancement in wheat productivity continues to be huge over these years, there are still needs to be problems such as climate change, water unavailability, and soil deterioration, which call for further study and innovativeness. After all, technology will only keep improving the future of wheat genetics and sustainable farming methods so that Türkiye will remain in the position of a leading wheat producer and fulfill its food security for future generations.

1.1.2 Wheat in Libya: Economic, Nutritional Importance, and Geographical Distribution

Hectare after hectare, farmed wheat has cut across Libya's history, shaped not only by the country's idiosyncratic climatic conditions but also by the historical developments in agricultural practice and socio-economic conditions. Libyan wheat production faces serious challenges: predominating arid and semi-arid grounds, high temperatures, and insufficient supplies of water with soil salinity (FAO, 2021). However, wheat brings solace to the nation's food security and sustainability economically. Wheat cultivation has undergone various changes in Libya, thanks to the several civilizations that have occupied the land, among them the ancient Berbers, the Romans, and the Arabs.

Modern attempts to improve wheat productivity within Libya are based on expanding irrigation systems, developing salt-tolerant varieties, and practicing environmentally sustainable agricultural techniques (Tadesse et al., 2017). Knowledge of wheat cultivation history in Libya can reflect different ways that agriculture has managed to adapt over time in arid areas and give insight into future improvements in the country's wheat production system.

Wheat is probably the most significant crop in Libya's agricultural economy and food security. Despite the arid and semi-arid climate of the country, wheat has remained an important staple crop because the Libyan people rely heavily on it for their daily subsistence. With the increase in demand for wheat, Libya also imports it, beyond what is produced domestically (FAO, 2021). Wheat-based products such as bread, pasta, and couscous are the basic foods in Libya, making the crop a very important aspect of national food security (Tadesse et al., 2017).

Historically, Libya has had limited success achieving self-sufficiency in wheat production owing to environmental, water, and land constraints. To increase its local production, the country has embarked on big agricultural schemes such as irrigated wheat farming initiatives. Yet it is reported that Libya imports 90% of its wheat almost entirely from international markets, that is, the EU, the United States, and Ukraine (FAO, 2021). This heavy dependence on imports renders Libya vulnerable to fluctuations in the price of wheat at the global level and also creates a possibility of disruption from geopolitical factors in major wheat-exporting regions (Vandewalle, 2012).

Assistance to farmers through subsidies and investment in modern irrigation techniques and research on salt-tolerant wheat varieties are some of the strategies the Libyan government has adopted to enhance food security regarding wheat

dependency. Initiatives aimed at improvement in wheat storage facilities and those directed at managing the supply chain have also been included to minimize post-harvest losses and guarantee stable supplies of wheat products for the people (Ghoneim, 2015).

Agriculture is another major source of employment for poor rural communities besides oil exports. Agriculture, especially wheat production, continues to offer job opportunities to thousands of Libyans living in fertile regions such as Jabal Akhdar and the coastal plains (Mattingly, 2003). Rural and urban economies thrive by emulating wheat farming.

Wheat growing benefits small-scale farmers as well as support from large-scale government farmers. Employment is generated in areas such as those employed in wheat farming, transportation, milling, baking, and distribution, among others (Wright, 1982). The development of a resilient wheat sector has high potential for reducing dependency on imported food products, thus contributing positively to economic diversification efforts in Libya. Sustainable farming practices, coupled with improved irrigation systems and investment in agricultural technologies, would strengthen domestic wheat production and achieve food sovereignty and economic stability (FAO, 2021). Nonetheless, some infrastructural and policy changes need to be made to achieve the full potential that Libya's wheat industry can reach (Tadesse et al., 2017).

As the country has little production of wheat, the country, like many others, relies greatly on imports from different countries to feed. It makes or reacts quickly to international conditions in trade agreements regarding wheat imports since wheat importation is from several countries, including Ukraine, Russia, Canada, and the European Union (FAO, 2021). Such imports are essential to the great domestic demand since local production covers only a very small part of the total consumption of wheat (Vandewalle, 2012).

However, fluctuations in the international price of wheat, the price of transport, and risks brought about by trade disruptions are some major risks to food security in Libya. Political instability and conflicts, especially in the key wheat-exporting regions like the Black Sea area, have on several occasions caused disruptions in the supply chain, impacting the affordability and availability of wheat-based products in Libya (Ghoneim, 2015). As a response to these threats, the Libyan government diversified its strategies of importing wheat and investing in

local production initiatives, thereby reducing dependence on foreign wheat markets (Tadesse et al., 2017).

Wheat forms part of the basic staples in Libya, which is an important source of carbohydrates, protein, and micronutrients. Wheat products such as bread, pasta, and couscous are consumed daily by every section of the population in Libya. The consumption of wheat is therefore significant for the fulfillment of energy and nutrition needs (Cakmak et al., 2010).

The consumed wheat product in Libya is bread. Bread is mostly khubz, flat, and bazeen, but there are many varieties of this kind. Wheat is also found in pasta, the basic food of a nation that had historical links with Italy. It also includes couscous, one of the most popular dishes prepared with durum wheat semolina all over North Africa, including Libya (FAO, 2021). Wheat is a good source of dietary fiber that keeps the gut healthy while also preventing long-term diseases, especially diabetes and cardiovascular disorders (Shewry, 2009). Important vitamins and minerals provided by whole wheat include the B vitamins, Fe, Zn, and Mg that help with overall health and well-being (Cakmak et al., 2010). However, most refined wheat flour, which is popularly used in processed foods, is nutritionally less rich. Such conditions have led to the increasing prevalence of diet-related health problems in Libya, such as obesity and diabetes (Ghoneim, 2015).

Addressing these issues, health and nutrition initiatives in Libya are concentrating on promoting whole grains and improving the biofortification of wheat. Biofortified wheat varieties enriched with essential micronutrients such as zinc and iron have been introduced in those countries as an initiative to combat nutrient deficiencies and improve public health (Cakmak et al., 2010). Public awareness campaigns with a focus on the benefits of whole wheat products and balanced diets are in place to encourage healthy eating habits among Libyans (FAO, 2021).

The wheat sector in Libya is beleaguered with a myriad of challenges such as climate change, water scarcity, soil degradation, and economic depression. Even with all these factors, the reality is that the arid environment within Libya makes large-scale rain-fed wheat farming almost impracticable, as such requires heavy investments in irrigation and soil management technologies (Tadesse et al., 2017). Besides, the increasing effect of desertification and salinity on agricultural lands would make efforts to improve wheat productivity harder (Mattingly, 2003). It is

possible, notwithstanding these many challenges, to positively influence wheat production in Libya by the adoption of sustainable agriculture. The research and development of salt-tolerant wheat varieties and the promotion of water-efficient irrigation technologies and soil conservation methods would enable overcoming environmental constraints that inhibit increased production of wheat within the country (FAO, 2021).

Increase control-environment agriculture, such as greenhouse wheat growing, hydroponic cultivation, etc., for maximizing the possibility of increasing wheat production in the country while minimizing water consumption (Ghoneim, 2015). International cooperation and investment in agricultural research will essentially strengthen Libya's wheat sector. In this context, partnerships with organizations such as FAO, ICARDA, and regional agricultural institutions would provide the required exchange of knowledge, access to improved seed varieties, and technical assistance for sustainable wheat farming (Tadesse et al., 2017). Moreover, future policy reforms that would support smallholder farmers in increasing their market access and improving the storage and distribution infrastructure of marketed goods will recognize long-term food security and economic resilience.

Therefore, wheat remains one of Libya's economic lifelines and a very critical nutritional resource for Libyans. Self-sufficiency in wheat production is faced with many hurdles, but efforts continue to improve local production, diversify trade partnerships, and promote healthier patterns of wheat consumption. This may eventually lead to better food security and economically sustainable development. Blending modern agricultural innovations with traditional knowledge would help strengthen the wheat sector domestically, thereby lessening dependence on imports for wheat, which ultimately places the nation in a more sustainable and resilient food system.

The ecological and climatic settings of the country make a substantial contribution to the wheat production activities of Libya in areas that are suitable for cultivation purposes. Agricultural regions in Libya are located primarily along the Mediterranean coast, where the crop is also grown in some densely populated desert areas that have an irrigation network. The most prominent areas producing wheat include the Jabal Akhdar area to the northeast, the coastal plains surrounding Tripoli and Misrata, and their inland farming in the Fezzan region (FAO, 2021). Compared

to the vast arid regions dominating the landscape, these regions have relatively better soil fertility and availability of water.

Situated in Jabal Akhdar—the most Mediterranean part of Libya—the region is the most productive for wheat. Its moderate rainfall and relatively cooler temperatures favor wheat growing better than in the dry interior. The area supports different irrigation methods, both traditional and modern, which help sustain wheat farming activities throughout the season. Reliance on supplemental irrigation is left high, however, because of periodic droughts (Tadesse et al., 2017). The coastal plains of Libya, especially around Tripoli, Misrata, and Benghazi, are also significant contributors to the total amount of wheat produced in the country. Irrigated with groundwater and controlled agricultural practices that boost the quality of output, these areas receive somewhat less rain than Jabal Akhdar, but the proximity of urban centers ensures that it is economically feasible to farm wheat here (Mattingly, 2003). While concerns have been increasing about depletion, they have been integral to ensuring continued wheat harvests in these coastal areas.

On the contrary, it has been observed that Fezzan in southern Libya must have a more adverse environment for wheat cultivation due to very dry conditions and a limited amount of water. By using underground water reserves from the Nubian Sandstone Aquifer System, however, large-scale irrigated wheat farms have been established for agricultural projects in Fezzan, thus enabling the formation of such projects. The distribution of resources has been complemented by high-tech irrigation techniques such as the use of center-pivot systems and hydroponic farming for growing wheat in these desert areas (FAO, 2021). They have been generally successful, though the depletion of groundwater reserves and the costs of maintaining irrigation infrastructure provide a real threat to the sustainability of wheat agriculture in Fezzan.

Several environmental and agronomic factors, such as soil salinity, water scarcity, the use of outdated agricultural practices, and climate change, limit wheat productivity in Libya. Wheat production is significantly lower across the entire country than the world average, hence requiring research and intervention for continued improvement in production efficiency (FAO, 2021). Salinity of soil is among the most troublesome problems affecting wheat production in Libya. Excessive groundwater abstraction for irrigation in the coastal and inland irrigated areas increased soil salinity levels to the extent of affecting wheat production.

Stress due to salinity affects the rate of germination; it stunts the growth of plants and generally lowers the yield of grains. To this end, Libyan agricultural researchers are exploiting the option of using salt-tolerant varieties of wheat with improved soil management practices to alleviate the salinity impact on crop production (Tadesse et al., 2017). Libya, like most arid or semi-arid countries, is fully based on groundwater, and the country's aquifers are already overexploited, extracting more groundwater than is recharged, leading to groundwater surface decline. Consequently, the Great Man-Made River (GMMR) is now regarded as a temporary solution, needing complementary water management strategies, such as investment in water-saving irrigation techniques (FAO, 2021). Over and above, the productivity of wheat in Libya was inhibited by obsolete farming methods and limited access to modern agricultural technology. Farmers still practice traditional ploughing, sowing, and harvesting, which have demonstrated inefficiency as compared to mechanization. The gap continues to widen as there is limited access to quality seeds, fertilizers, and pest control measures. There is a need for investment in agricultural extension programs and farmer education initiatives to improve farming practices and productivity in wheat.

Quite clearly, climate change adversely and significantly threatens productivity for wheat in Libya. Recurring droughts coupled with rising temperatures and changing precipitation patterns have increasingly made wheat cultivation much more strenuous. Evapotranspiration is enhanced by rising temperatures and therefore results in greater losses in soil and crop water. Adaptation to the impacts of climate change and the drought-resistant traits of wheat should be researched (Tadesse et al., 2017). With integrated resilient agricultural strategies, Libya can be able to enhance its wheat productivity even in these harsh environmental conditions. The average wheat yield in Libya is between 1.0 and 1.5 metric tons per hectare, far below the accepted global average of between 3.5 and 4.5 metric tons per hectare (FAO, 2021). The low yield levels have necessitated immediate interventions to enhance efficiency in wheat production. Thus, Libya is currently financing agricultural research and development programs in genetic improvement, soil conservation, and better water management for increased efficiency in yield. Genetic improvement of wheat through its cultivation is one of the best ways to improve productivity. Such wheat will be developed to have better drought, salinity, or extreme temperature tolerance capability. These

farmers will be integrated, and these wheat varieties will increase wheat production and reduce dependency on imports in Libya (FAO, 2021).

Precision agriculture technologies based on satellite imaging and remote sensing would also be used to improve land use and crop health monitoring. Various innovations in water management are being prioritized to sustain wheat farming in Libya. The application of strategies such as drip irrigation, deficit irrigation, and rainwater harvesting has the potential to raise the level of water efficiency and provide a steady supply of wheat. It also opens new opportunities to research the growing possibilities of hydroponic and greenhouse wheat cultivation as an avenue for more production with less water consumption (Tadesse et al., 2017). Policymakers and other stakeholders in agriculture are making efforts to develop comprehensive strategies for production targeting wheat within the context of long-term food security goals in Libya. They include strengthening agricultural infrastructure, improving supply chain efficiency, and providing cash support for farmers, among others. A resilient wheat sector in Libya is now going to enable the country to match demand and consumption domestically against external forces in the wheat prices (FAO, 2021).

Wheat cultivation is confined to an arid environment in Libya; however, important wheat-growing areas like Jabal Akhdar, coastal plains, and Fezzan still contribute to national production. However, while breaking new ground in wheat production, productivity is generally below average due to soil salinity, water scarcity, outdated farming practices, and climate change. These challenges require a multi-faceted approach that combines advanced agricultural research, better irrigation techniques, and good land management practices. Prospects for wheat farming in Libya hinge on the successful adoption of drought- and salt-tolerant wheat varieties, modernized farming techniques, and efficient water use strategies. By investing in agricultural innovation and policy reforms, Libya will then be able to bolster its wheat sector, lessen its dependence on imports, and enhance food security for its growing population.

1.2 Salinity and Its Effects on Plants

1.2.1 Salt Stress and Its Sources

1.2.1.1 Soil Salinity and Irrigation Water

Soil salinity is a limiting factor in agricultural production, especially in arid and semi-arid regions, where evapotranspiration rates are high and precipitation is scant. This encompasses the concentration of soluble salts to levels that negatively impact plant growth and productivity (Munns & Tester, 2008). Natural geological formations, irrigation water with elevated levels of salts, inadequate drainage, and the use of saline groundwater for irrigation are their main sources (Qadir et al., 2014). Salinity stress disrupts plant growth by altering soil structure, reducing water availability to plants, and causing ion toxicity, which leads to retarded growth and low crop yields. Naturally supplied irrigation adds to how far a soil stays affected by salinity and enables plant growth. When there is not enough rainfall in any given year to flush soluble salts from the root zone, irrigation management practices, in particular, do not allow salt accumulation (FAO, 2021). The water quality, irrigation frequency, and drainage efficiency are key factors in managing the levels of soil salinity. Salinity not only causes soil degradation but also leads to water waste, making long-term sustainability difficult to achieve on agricultural lands (Rengasamy, 2010).

Both natural and man-made causes contribute to soil salinity. The primary natural sources of salts are the weathering of parent rocks, oceanic salt beds, and underground salt deposits (Shahid et al., 2018). In coastal regions, seawater intrusion toward estuarine waters also contributes to the salinization of soils, for which an important factor is over-abstraction from the groundwater. Man-made factors include poor irrigation practices, the application of an excessive number of districts, and land-use changes that have disrupted the natural water balance (Ghassemi et al., 1995). Irrigation with saline water is among the foremost causes of soil salinity. When water is evaporated with salt accumulation in it, the salts that are left behind are stored in the soil profile. Poor drainage and bad irrigation systems can lead to waterlogging and, in turn, prevent salt leaching with marked salt accumulation near the root zone (Pitman & Läuchli, 2002). Such conditions degrade the soil and make it less productive with time. High salinity kills plants by impeding water absorption and disrupting the nutrient balance mechanism.

Salinity ultimately leads to osmotic stress, the main cause of water deficit in plants, even if moisture is available in the soil (Munns, 2002). Furthermore, excessive accumulation of sodium (Na^+) and chloride (Cl^-) ions results in the competition for essential nutrients, ultimately leading to deficiencies and

morphological disorders in the plants. Among the usual salt-stress symptoms are yellowing leaves, stunted growth, and a decline in seed germination rates (Parida & Das, 2005). Wheat, a plant species with moderate salinity tolerance, demonstrates poor germination, lower biomass accumulation, and delayed growth under high salinity stress (Zhu, 2001). Salinity-stimulated oxidative stress in the plant since salt stress imposes the production of reactive oxygen species, which causes damage to cell structures, thereby leading to plant death (Mittler, 2002). Soil affected by salinity adjacent is detrimental to microbial communities, causing a reduction in soil biodiversity, thereby enforcing damage to beneficial microbial interactions necessary for nutrient cycling and plant growth (Ruppel et al., 2013). The efficient use and management of irrigation water are crucial to decreasing soil salinity while subsequently making agricultural practices sustainable. Various irrigation methods have been developed to control salt accumulation and check soil health:

1. Leach and Drainage Improvement: Allowing excess water to move through the soil beyond crop requirements helps leach away salts accumulated and held in the root zone. The establishment of adequate drainage facilities—encompassing sub-surface drains and raised beds—will ensure the removal of salts or excess water from the system (Corwin et al., 2007).

2. Use of Good Quality Water: Therefore, selecting irrigation water with very low salinity levels minimizes the possibility of salt accumulation. It is also desirable to mix saline water with water of lower salinity and/or treated wastewater to maintain soil quality (Rhoades et al., 1992).

3. Drip and sprinkler irrigation: These methods directly deliver water to the rhizosphere, thus reducing the deposition of salts over the soil. In the case of drip irrigation, it enhances water-use efficiency while minimizing salt crystallization (Skaggs et al., 2006).

4. Crop Rotations and Salt-Tolerant Crops: The growth of salt-tolerant crops, such as barley and some wheat genotypes, in rotation with sensitive crops is likely to improve soil structure and render it less susceptible to saline stress (Munns et al., 2016).

5. Soil Amendments and Organic Matter Addition: Incorporating organic matter, gypsum, and biochar into the farmland helps in improving the soil structure to facilitate the leaching of salts (Tejada et al., 2006). Organic amendments also

create a favourable environment for beneficial microbial activity, leading to increased soil fertility and soil health.

Soil salinity problems are expected to be intensified by climate change, particularly in regions that are already water-stressed and warm (Lesk et al., 2016). Still, rising sea levels heighten the prospect of seawater mingling with fresh water used for irrigation and of high evaporation rates concentrating inorganic salts in the soil. Thus, sustainability of water resources, land conservation, and progress in genetic engineering for salt-tolerant crops are needed to mitigate the crucial impact of climate change on soil salinity. Advances in bioinformatics and genomics are informed by studying plant reactions to salt stress. These tools identify salt-resistant genes using CRISPR-Cas9 technology, thus providing seldom analysis of salt-tolerant genes for intelligent breeding (Zhang et al., 2020). Integrating the traditional agronomic system with technological innovations will be the way forward for agricultural productivity within the sphere of salt-affected regions.

1.2.1.2 Sources of salinity

Soil salinity, caused by a combination of natural and anthropogenic factors, has increased salt levels in soil and water bodies. These factors do affect the productive performance of agriculture in particular, soil fertility, and overall environmental sustainability. Therefore, an understanding of the soil salinity origin is critical to achieving sustainable crop production and ecological balance (Qadir et al., 2014).

1. Loss of Water

One of the major contributors to the saline soil is the wasting of water by evaporation and transpiration. Evaporation occurs when temperatures in arid and semi-arid zones are high, leading to even higher rates of evaporation of water. These processes make the water form at the surface of the soil so that it evaporates and leaves behind salts in the soil through a process known as "evaporative concentration," which causes salt accumulation in the free-soil surface (Rengasamy 2010). Additionally, a lot of water is lost in the form of transpiration from the plants, thereby increasing the salt concentration in the root zone further.

Poor water-retention capacity in sandy soils will escalate the process, where most salts remain following drainage of water (Shahid et al., 2018). Effective soil-

moisture management practices, such as mulching and conservation tillage, will help to avoid water loss, hence halting the increase in salt due to such loss.

2. Salts Polluting Soil and Water from Seawater Intrusion

One of the sources of soil and water salinity is the intrusion of seawater into the coastal aquifers. This occurs when overpumping lowers the water table, while the sea forces itself in. As a result, irrigation water becomes saline, further salinizing the soil (Ghassemi et al., 1995). Such processes emerge as a threat in the Mediterranean coastal region of Libya due to heavy groundwater exploitation (FAO, 2021). Careful extraction of groundwater, groundwater recharge tactics, and the introduction of desalinated water for irrigation could go a long way in slowing down, if not completely stalling, seawater intrusion and thus the salinization of further agricultural lands (Pitman and Läuchli, 2002).

3. Increase in Saline-Irrigation

The growth of crops using saline water for irrigation is one of the primary causes of soil salinity, thereby causing the soil to accumulate salts. In a nutshell, using even low or moderate-rank salinity levels in irrigation waters can result in the mixing of salts with soil over time. Droughts, desertification, and intensive agricultural activities make the problem even worse (Mark, 1983). Continuous irrigation without a proper drainage system causes water lodging, which prevents the leaching of salts, leading to their accumulation in the root zone (Corwin et al., 2007). This necessitates the implementation of effective irrigation strategies like drips, scheduled irrigation, and improved drainage facilities to mitigate the menace of salinity irrigation (Rhoades et al., 1992).

4. Wastewater Draining from Upper Land Areas to Low-Elevation Areas

Wastewater drainage with dissolved salts from uplands to the down terrain contributed significantly to soil salinity, especially in agricultural and urban areas. The waters typically contain high concentrations of salts, fertilizers, and other contaminants, depositing in the lowlands' soils (Ghassemi et al., 1995). Unprotected irrigation systems and mismanagement of wastewater further aggravate the threat to soil productivity (Shahid et al., 2018). There could be a hope to stop the accumulation in opposing the salt load through pre-wastewater acts like wetland management or buffer zones, which could limit the entire seepage of salt into the agricultural system (Qadir et al., 2014).

5. Salt-Laden Sea Spray Transformed and Deposited by Wind and Rain

Moreover, in the coastal plains, salt-laden sea spray effectively carried with the wind can bear down with a mixed background of some rainwater deposition to salinize the soil. The mechanism at work is one in which coastal winds are supposed to carry minute salt particles that settle on top of the soil or layer themselves on the rainwater (Pitman & Läuchli, 2002). This natural activity will enhance the salt content of soil, particularly along the seashores, for some time (FAO, 2021). The establishment of windbreaks, growing salt-resistant plant species, and the use of soil additives, such as gypsum, are prevention practices used against the effect of sea salt deposition (Tejada et al., 2006).

6. Dissolution of Accumulated Salts and Their Redistribution

In some areas, natural salt deposition within soils dissolves and moves to other parts of the soil due to water movement. This presumably happens when salt that has accumulated from rainfall or irrigation water dissolves, leading to transport from one area to another, causing secondary salinization (Al-Hilal, 2020). These processes are more catastrophic to poorly drained soil and typically in lower elevations. As a management effort, a comprehensive program of controlled drainage, controlled irrigation, and soil amendment techniques would reduce the extent of redistribution of dissolved salts caused by the previous pressure of salinization of the soil (Qadir et al., 2014). Understanding the sources of soil salinity enables the development of suitable measures for its control. Loss of water, seawater intrusion, saline irrigation water, wastewater runoff, wind-driven salt deposition, and salt dissolution all culminate in high salinity levels in the soil. Implementing sustainable agriculture practices, efficient irrigation technologies, and land-use planning can help lower the soil salinity problem and improve soil health. Addressing soil salinity requires a holistic approach combining existing scientific research, policy support, and farmer education, while this should be done for sustainable agriculture.

1.2.1.3 Saline Soil

Within various scenarios of dry environments, a high degree of salinity conditions can manifest itself when exceptionally high temperatures and low humidity result in evaporation occurring at a much more rapid rate than rainfall does (Mark, 1983). This creates a situation within which salt deposits are formed, and saline concentration is highly concentrated on the soil, mainly in the surface

layer where roots are. Saline soils also accompany a set of much more soluble and insoluble salts that impede the normal growth of vegetation, particularly when used for crop farming. This is much evident in arid and semi-arid climates, which have considerably escalated the process because the rates of evaporation are intensified by very high temperatures and low humidities, and, thus, they lead to great deposition of some salts in the upper soil layers (Mark, 1983). Subsequently, soil fertility declines, meaning it becomes harder for plants to take up water and essential nutrients and, thereby, limits agricultural productivity (Munns & Tester, 2008). Salinity is widely present near areas with high salinity water bodies, such as in coastal areas, where seawater intrusion affects groundwater quality. Within this region, as salt water is continuously flushing the profile into the soil, more salts are becoming so contained via destabilization of soil structure and function (Ghassemi et al., 1995). The uppermost soil layer, where most plant root systems find themselves, is much affected by salt, as these accumulate there due to capillary forces and heaves from evaporative processes (Pitman & Låuchli, 1996).

Several factors underlie the formation of saline soils, including environmental impacts and anthropogenic actions. Such influences stem from the forces of nature, such as salt depositions on the surface of land, water sprinkling, or winds transporting the precipitated salts to the land; and probably due to direct actions of man, such as irrigation with saline waters, improper drainage, the use of excessive amounts of chemical fertilizers, and overall climatic conditions that favour an inappropriate accumulation of salts. In regions where precipitation is not enough to leach out the excess salts from the root zone, salinity will continue to rise, depriving the soils of their ability to nurture plant life (Rengasamy, 2010).

1. Natural Formation of Salts and Salt Weathering: Some soils are naturally richer in salts, which may be attributed to the disintegration of parent rock and the deposition of saline minerals across geologic history. High evaporation rates allow these salts to accumulate close to the depth of the soil, thereby exacerbating the problems resulting from salinity (Shahid et al., 2018).

2. Irrigation with Saline Water: In many areas where groundwater largely comprises high-level salts, farmers sometimes have to switch their reliance to such water for watering the land. Continuous watering and lack of any provision for allowing for adequate drainage cause the gradual buildup of salts in the soil, becoming incapable of eliminating the salt load positively (FAO, 2021). Also,

because an insufficient quantity of water is supplied by inefficient irrigation methods, the resultant waterlogging has the propensity to further intensify salinity buildup (Corwin et al., 2007).

3. Seawater Infiltration and Coastal Salinity: Coastal areas are particularly at risk for seawater intrusion due to excessive extraction of groundwater, which lowers the water table, allowing the salty seawater to filter into the freshwater reserves. Saline-like waters and soils are completely transformed, leading to land degradation on an unprecedented level in those areas (Pitman & Läuchli, 2002).

4. Overuse of Fertilizer and Chemical Accumulation: Soil salinity may also result from the excessive application of artificial fertilizer in the form of an oversupply of sodic, chloride, and sulphate ions in an already-salted afflicted soil. The accumulation of salts is slow and progressive, which further decreases soil fertility and affects plant health (Tejada et al., 2006).

Saline soils have been detrimental to plant growth and development due to several factors of concern. Among other things, the high salt concentration in the root zone interferes with the osmotic balance by making it too difficult for the plants to intensively absorb water, even when the water is readily available from the soil. This phenomenon, known as osmotic stress, renders the plants incapacitated in growth, leaf drying, and diminished yield (Munns, 2002). High amounts of Na^+ and Cl^- ions in saline soils induce the said imbalances, thereby checking the uptake of other vital nutrients. The ions compete for the binding sites with such necessary chemical entities as K^+ , Ca^{2+} , and Mg^{2+} , which support physiological functioning in plants. As a result, plants become deficient in essential nutrients, leading to various physiological disorders, including chlorosis, necrosis, and reduced photosynthetic efficiency (Parida & Das, 2005).

Oxidative damage is also caused by salt stress in plants due to increased production of reactive oxygen species (ROS). These most reactive molecules attack cells, degrade proteins, destabilize cell membranes, and destabilize the often-large network of enzymatic activities available to the cell (Mittler, 2002). The plants attempt to rectify all negative effects by instigating the energy-wasting antioxidant defence system; however, long-term exposure to excessive salinity levels may outpace the plant's defence systems, eventually causing plant death (Ruppel et al., 2013).

Management to maintain productivity in saline soils may involve several techniques in agriculture, engineering, and biological mitigation in salt buildup stabilization of the soil. Consequently, such mitigation would involve:

1. Leaching and Drainage Improvement: Leaching needs to be applied to an excess of water to aid the flushing away of accumulated soil salts beneath the plant root area. The method is efficient, where drainage is adequate to prevent waterlogging. There exist opportunities with the installation of sub-surface drainage to drain away saline water from saturated soil (Corwin et al. 2007).

2. Soil amendments and organic matter addition: An application of gypsum (calcium sulfate) helps to displace sodium ions in saline soil, thus improving soil structure while decreasing sodicity. Organic soil derivatives, such as compost and manure, enhance soil biological activities, improve water holding capacity, and alleviate soil salinity (Tejada et al., 2006).

3. Use of Salt-Tolerant Crops and Crop Rotation: Growing crops such as barley and certain wheat genotypes that can thrive well in a saline environment adds to their so-called sustainability attributes in terms of agriculture. Introducing legumes and deep-rooted plants into crop rotations to improve soil structure that enhances salt leaching (Munns et al., 2016).

4. Efficient Irrigation Practices: The water used can be used for cropping only with fewer losses on the soil surface by the adoption of improved water management strategies like drip and subsurface irrigation. These are the systems that bring the water directly to the roots of the plant(s), thus maintaining soil moisture and avoiding the buildup of salts (Skaggs et al., 2006).

5. Afforestation and Windbreak Establishment: Planting salt-tolerant forest species in saline-affected areas aims to stabilize soil, reduce wind erosion, and prevent further salt deposition from atmospheric pathways. Windbreaks usually offer additional protection to farmlands from salt-laden dust storms (FAO, 2021).

The prospects of genetics, genomics, bioinformatics, and related tools may be employed to devise solutions to manage saline soils for stable productivity. Understanding the genetic architecture underlying salt tolerance in plants for the development of stress-tolerant crop varieties through marker-assisted breeding together with gene editing using the CRISPR system, in abiotic stress, is one area of accomplishment coming from the study of genetics (Zencirci et al., 2021). Furthermore, microbial biotechnological strategies, for example, the employment

of plant-growth-promoting bacteria (PGPB), may prove useful in developing salt-tolerant plant cultures with a reinforced soil microbial population (Ruppel et al., 2013).

Given this setup, saline soils contribute to drastically reducing agricultural productivity. This comes as no exception in arid and coastal regions where the evaporation rate surpasses precipitation. Apart from obstructing plant growth, the soluble salt accumulation results in obscurity and partial removal of nutrient intake, and hinders total crop yield. Effective management strategies, such as soil amendments and crop irrigation improvement, must be accurately adapted to mitigate the impact of soil salinity on agricultural land. Constant research in the genetics of plants and soil microbiology will help create sustainable resolutions to these saline soil problems.

1.2.1.4 The harmful effect of salinity on the soil

The salts do affect the soil properties and water movements in the soil. With an increase in sodium ions (Na) in the soil solution, the soil cation-exchangeable sodium concentration rises. When the adsorbed sodium content on the clay granules becomes 15% or higher of the cation exchange capacity of the soil, then the mass soil structure is altered, thus hampering soil composition due to (Waisel 1972). Salts do affect water relations in the soil. Again, a higher concentration of dissolved salt from the soil solution increases the osmotic potential of the soil solution, making it more negative, ultimately leading to a decrease in soil water potential.

The lower the soil water potential, the less the difference between the soil water potential and the plant root water potential ends up being, for which the water absorption ability of the root decreases (Al-Hilal, 2020). The irrigation water used is projected as a salty source for the soil since this water is not clean but mixed with some salts based on the source; up to 1000 cubic g can be added upon another reference. Whenever water is used for irrigation, there is a high risk of evaporation of water into the soil, and salts remain within the soil, leading to an accumulation of the salts therein, especially where drainage facilities are not good. Moreover, the farmers are using no irrigation practices of leaching to remove the accumulated salts in the soil from the root zones (Carter, 1975). The accumulation of salts in the soil becomes a major problem in agriculture, particularly in desert and semi-desert regions, due to the high rate of evaporation of water from the soil. Also, there is not

enough rainfall to wash the accumulated salts from the root zones to contribute hugely to their accumulation into the soil (Al-Hilal, 2020).

1.2.2 Global and Local Classification of Saline Lands

1.2.2.1 Widening phenomenon of salinity

About 800 million ha of the world's land, as reported by the (FAO, 2005), have been confronted with salt degradation, with 397 million ha going saline (the table shows this) and the rest 434 million ha in soda; that is, slightly above 6% of the area concerned. All sodic lands are natural; salinity is almost natural, too, except that a significant part of the newly cultivated land has been reclaimed. All this land turned saline due to denudation caused by man to the vegetation cover or undergrowth (Munns 2005). Data from FAO (2005) confirm that, out of the 1,500 million hectares of dry arable land, 30 million (2%) belong to secondary salinity, and of the 320 million ha of irrigated land, 45 million ha, or 20%, are salinized, although such lands are only 15% of the total cultivated land. The yield of irrigated lands is two or more times that of unirrigated lands; hence, it suffices to produce about 30% of the world's food (Munns, 2005). Tables give an indication of the global distribution of saline and sodic lands as reported by Lee (Szabolca, 1989).

Table 1.1. Regional distribution of areas affected by salinity (in million ha)

Regions	Total area	Saline soils		Sodic soils	
	(million ha)	(million ha)	(%)	(million ha)	(%)
Africa	1899.1	38.7	2.0	33.5	1.8
Asia / The Pacific / Australia	3107.2	195.1	6.3	248.6	8s
Europe	2010.8	6.7	0.3	72.7	3.6
Latin America	2038.6	60.5	0.3	72.7	3.6
Near East	1801.9	91.5	5.1	14.1	0.8
North America	1923.7	4.6	0.2	14.5	0.8
Total	12781.3	397.1	3.1	434.3	3.4

The map also shows the geographical distribution of soils affected by salinity worldwide, according to Sparks (1995).

1.2.2.2 Global Classification of Saline Lands

Saline land classification is instrumental in understanding the properties, management, and impact on productivity of agriculture. Saline soils are classified

based on preselected concentrations of soluble salts and the percentage of total exchangeable sodium present in the soil solution. This classification enables a researcher and farmer to devise a strategic framework for soil reclamation according to specified land use (Shainberg, 2023).

Categories of Saline Lands

Usually, classifications through their physicochemical properties lead to saline lands into three major groups:

1. Saline Lands

They bear high concentrations of soluble salts, which essentially check the growth of most crop plants. The exchangeable Na in them is not in a position to adequately change the soil structure and properties. The exchangeable Na in the soil is below 15%, hence, it has no mechanism of becoming sodic. Saline lands are often seen where evaporation exceeds precipitation, consequently causing salt accumulation in the topsoil.

The following are the characteristics of saline lands:

- Soil pH value: Less than 8.5
- SAR value: Less than 15%
- EC value: More than 4 mmol/cm at 25°C

Through osmotic stress, excess salt in the soil inhibits water uptake in crop plants. During leaching, excessive salt concentration destroys cellular metabolism, affecting nutrient absorption, causing stunted growth, and low crop output. The major reclamation strategies for desired saline lands are mostly based on leaching through good irrigation practices and other practices, such as the application of gypsum (Qadir et al., 2014).

2. Saline-Alkaline Lands

Salt-alkaline lands are equal to the much-dreaded saline lands with significant salt content and high sodium content. These soils are substantially finer barriers to agriculture, as crop health is put at a higher risk by both salinity stress and the soil dispersion initiated by sodium (Shainberg, 2023). Sodium with concentrations over 15% in these soils causes fine clay particles to swell and disperse, resulting in very low soil permeability and poor aeration.

Characteristics of saline-alkali soils include:

- The soils have a high pH value ranging from more than 8.5.

- From a fifth to one-half of the soil consists of Na and is over 15% of the SAR.

- High K values result in a low conductivity of more than 4 mmol₊cm⁻¹ at 25°C.

Comprehensive soil reclamation efforts comprising gypsum or H₂SO₄ application to lower the soil Na concentration may significantly contribute to an improvement in soil structure. Adequate water or irrigation management should, therefore, be devised to restrain such lands from further salinization and alkalization (Pitman & Läuchli, 2002).

3. Non-Saline Alkaline Lands

Non-saline alkaline lands are those soils with high exchangeable Na levels and low water-solution dissolved salt concentrations. These soils thus exhibit two acute problems because of Na toxicity, destroying plant growth even in cases where there is no high salinity. This degradation causes well-aerated soils to compact largely and prevents meaningful water and root penetration (Al-Hilal, 2020).

Characteristics of non-saline alkaline lands include:

- Soil pH value: Greater than 8.5
- SAR value: More than 15%
- EC value: Less than 4 mS cm⁻¹ at 25°C

Reclamation of non-saline alkaline soils is done by applying gypsum to replace Na with Ca, which enhances soil structure and permeability. Turning to deep plowing and the inclusion of organic matter will improve soil aeration and microbial activity (Qadir et al., 2014).

The key properties of different saline soils in a comparison table are given below (Shainberg, 2023):

Table 1.2. The key physicochemical properties of different saline soil types

Soil Type	pH	SAR	EC
Saline Soil	< 8.5	< 15%	> 4 mmol ₊ /cm at 25°C
Saline-Alkaline Soil	> 8.5	> 15%	> 4 mmol ₊ /cm at 25°C
Non-Saline Alkaline	> 8.5	> 15%	< 4 mmol ₊ /cm at 25°C

American Classification of Soil Salinity

Salinity classification based on soil EC and SAR has been developed by the USDA. This classification system is useful in evaluating soil suitability for

agriculture and arranging the necessary soil reclamation strategies (Rhoades et al., 1992).

According to USDA classification:

- Non-saline soils: $EC < 2$ dS/m (deci-Siemens per meter)
- Slightly saline soils: $EC = 2-4$ dS/m
- Moderately saline soils: $EC = 4-8$ dS/m
- Strongly saline soils: $EC = 8-16$ dS/m
- Extremely saline soils: $EC > 16$ dS/m

High-scale measures should indeed be applied to manage areas characterized by high salinity to sustain agriculture. Leaching, appropriate irrigation scheduling, and a portfolio of salt-tolerant crops are fundamental in the management of salinity in affected regions (FAO, 2021).

Saline soils are found in varying extents, all over the globe. According to the FAO, some 397 million ha of soil are rated as saline, this amounting to about 3.1% of the total land area. Major concentrations of saline lands are located:

- Asia and the Pacific: 195.1 million ha (6.3% of total land area)
- Near East: 91.5 million ha (5.1% of total land area)
- Latin America: 60.5 million ha (3.0% of total land area)
- Africa: 38.7 million ha (2.0% of total land area)
- Europe: 6.7 million ha (0.3% of total land area)
- North America: 4.6 million ha (0.2% of total land area)

Worldwide prevalence of saline lands dictates the adoption of sustainable agricultural practices and soil management techniques to combat salinity-induced degradation (FAO, 2021).

The segregation of lands among saline lands provides a framework for understanding what impacts they have upon any soil degradation and agricultural productivity. By classifying saline soils, they would be separated into saline, saline-alkaline, and non-saline alkaline types, allowing researchers and policymakers to push forth the right kind of strategies to reclaim soil. Global stakeholderism in Asiatic saline soils through better irrigation practices, soil amendments, and cultivation of salt-tolerant crops is unequivocally fundamental to food security and environmental sustainability.

1.2.3 Wheat Growth Under Salt Stress

Soil salinity is one of the most important environmental constraints for wheat (*Triticum aestivum* L.) production, mainly in arid and semi-arid areas where such salt accumulation in the soil reduces the overall productivity and growth of plants. Salinized agricultural lands have been an increasing problem, resulting from poor irrigation management, high evaporation rates, climate change, and soil degradation arguments which have decreased available land for cultivation. Since it is one of the staple food crops for mankind, the understanding of wheat's response to salinity stress is critical to global food security and sustainable agricultural practices. This study examines salinity effects on wheat germination, growth, and physiological processes while also pointing out some strategies capable of improving salt tolerance in wheat (Dolatabadian et al., 2011).

High soil salinity results in osmotic unbalance that hinders water uptake and thus develops physiological drought-like conditions in plants. The availability of water in roots becomes limited, which restricts cell expansion, stunted growth, and poor crop establishment. High concentration of salts in soil primarily reduces wheat yield by impairing the physiological functions, since plant development is impaired. Salinity also inhibits the uptake of water, increases the uptake of excess Na and Cl in the tissues of plants, affects the absorption of essential nutrients, mainly K and Ca, and further causes disturbance in metabolism.

The Food and Agriculture Organization (FAO) has released reports stating that nearly 20.0% of irrigated lands across the globe face the effects of salinity and that there is a significant decrease in wheat productivity due to the same. Such a growing challenge indicates the absolute necessity for the development of salt-tolerant varieties of wheat along with agronomic practices to help mitigate the negative impacts of salinity in soil upon crop yields (Ashraf & Harris, 2004).

Salinity severely affects the seedlings of wheat. During germination, osmotic stress and ionic stress make it very difficult to absorb water by preventing the hydration of seeds. Such delayed germination could, however, result from failure of absorption of water or absorption of a small quantity, or in extreme cases, even completely preventing germination. Besides, salinity modifies those enzyme activities normally needed for the breakdown of carbohydrates and proteins, which have been stored in the seed, and this could affect the metabolism of seeds as well as their early growth. Studies have found that the salt-tolerant wheat genotypes showed a better percentage germination rate when grown under salinity conditions

compared with the salt-susceptible cultivars. High salt concentration germination capability is often a result of osmotic adjustment and induction of the stress-responsive pathway that allows seeds to withstand adverse situations. Seed priming techniques with osmoprotectants such as proline, glycine betaine, and polyamines have been shown to increase germination percentages by stabilizing cellular structures and improving water retention during saline conditions (Al-Hilal, 2020). It (salinity) not only affects germination but also has an effect on root and shoot development and biomass accumulation, as well as on the whole vigor of plants. The curtailment in root formation is a major challenge for wheat under salt stress. The presence of Na salts disrupted root architecture by interfering with K uptake necessary for cell division and root elongation. Consequently, salt-affected wheat plants have been shorter and thinner in roots, thus, with reduced ability to absorb water and nutrients. The same trend applies to shoots, whereby plants have shown reduced leaf expansion, lower tiller production, and shorter internodes. This reduction in the vegetative stature compromises photosynthetic efficiency and aggravates the effects produced by salinity on biomass production in wheat (Ali et al., 2004).

So, besides the production hindrance, salinity accelerates leaf senescence due to oxidative stress and a change in hormonal balance. Accumulation beyond a certain threshold of salts leads to the production of ROS, which are responsible for cellular injuries to lipids, proteins, and DNA. Plants have protective mechanisms against oxidative stress, mainly antioxidant SOD, CAT, and POD. Thus, in salt-sensitive wheat varieties, the antioxidative capacity is often insufficient to prevent damage to cellular structure or functions, leading to yellowing leaves earlier, reduced chlorophyll content, and consequently lower photosynthetic activity. Salt stress also leads to the degradation of chlorophyll pigments, and this in turn limits the carbon assimilation, with a decreased net result in productivity in the plants as well as grain yields (Aly, 1979).

Plants of the wheat family employ various physiological strategies for tolerating various salt stresses, such as osmotic adjustment, ion homeostasis, and stress hormone regulation. Essentially, osmotic adjustment, referring to the overall mechanism of osmotic adjustment, is the most important survival strategy; it represents the accumulation of compounds or compatible solutes such as proline, glycine betaine, and soluble sugars intended for turgor retention and prevention of

water loss, all of which take place in the cytoplasm or cytoplasmic surroundings. These nutrients play a very important role in the stabilization of proteins and membranes while improving the ability of the plant to live under high concentrations of salts. Research has proven that salt-tolerant wheat genotypes have the trait of proline accumulation, which enables them to maintain metabolic functions under osmotic stress (Barlow & Foulkes, 1976).

Ion homeostasis is indeed another one of the adaptive responses by which wheat plants actively regulate sodium and chloride ion uptake and compartmentalization to avoid toxic impacts. The SOS (Salt Overly Sensitive) signaling pathway is the most important sodium exclusion mechanism; one of its components is the SOS1 gene that encodes a plasma membrane Na^+/H^+ antiporter for exporting excess sodium from root cells. With this mechanism, a K^+/Na^+ ratio favorable to cellular function and enzyme activity is preserved. And further, Na^+ sequestration into vacuoles through tonoplast transporters protects the cytoplasm from ion toxicity. Regulation of these mechanistic transport systems is essential for internal cellular integrity and ensures continued growth conditions under saline exposure (Dolatabadian et al., 2011).

The hormonal regulation of the components of salt stress response is ABA, which accumulates due to osmotic stress, plays a crucial role in stomatal closure as well as stress signaling, and increases the levels of ABA, thus reducing water loss through transpiration. The drawback of prolonged stomatal closure is that it limits CO_2 uptake, which can have adverse effects on photosynthesis. Salinity, ethylene, and jasmonic acid also have a role in promoting stress-responsive gene expression and antioxidant defense mechanisms towards salinity tolerance. This hormonal coaction determines how far they can adapt to saline environments (Downton, 1977). Thus, the situation becomes challenging with increasing salinity problems in the soil. Such efforts to increase the salinity tolerance of wheat must be based on genetic, physiological, and agronomic bases. Therefore, breeding programs emphasizing salt-tolerant genotype selection have defined most essential traits associated with resilience-such as deep rooting, K^+/Na^+ high-selectivity, and efficient osmoprotectant accumulation. Molecular breeding and developments in genetic engineering towards transgenics were, hence, very useful in developing several transgenic wheat lines that had overexpressed stress tolerance genes such as NHX (Na^+/H^+ antiporter), and SOS1, improving Na exclusion and enhanced

plant performance under saline conditions (Bhawat & Bhatia, 1993). Besides genetic approaches towards breeding salinity tolerance into varieties, agronomic management practices can make a substantial contribution towards alleviating salinity effects. These include soil amendments, organic, and biofertilizers for improving soil structure and reducing Na accumulation. Proper irrigation scheduling, coupled with alternative developed sources of water such as brackish water-induced controlled salinity levels, for use, will go a long way toward maintaining soil quality and reducing degradation. Silicon, K, and Ca supplements showed an increase in wheat resistance due to reduced ion toxicity and improved nutrient uptake (Ashraf & Harris, 2004).

1.2.3.1 Effect of Salt Stress on Plant Growth and Biochemical Processes

1.2.3.1.1 Effect of Salinity on Plants

Soil salinity also poses a major constraint to plant growth, interfering with germination, water absorption, and basic plant metabolism. High levels of soluble salts in the soil-water decrease the osmotic potential, thereby reducing the absorption of water by plant roots because ions are exogenously present near the root surface even on adequate soil moisture (Munns & Tester, 2008). Abundant Na and Cl ions come in the way of nutrient wiring and can cause nutrient deficiency, which, in turn, can bring about some physiological disorders (Parida & Das, 2005). Poor crop productivity and weak growth are thus the consequences, and in extreme cases, crop failure. As such, plants are categorized based on their response to salt stress and the levels of tolerance they exhibit to salinity.

Salt-sensitive plants resist any growth reduction in low salinity, which could be as low as 2-3 g/L (Rhodes & Lovejoy, 1990). Crucial processes in these plants are reduced to a level that yields much lower productivity when the plants are grown under saline stress. Examples include bean (*Phaseolus vulgaris*), melon (*Cucumis melo*), onion (*Allium cepa*), citrus (*Citrus* spp.), and apricots (*Prunus armeniaca*). Salt-tolerant plants can show somewhat effective tolerance to an increased salt concentration of about 3.5 g/L (all around them); they appear to grow healthy and give yield when grown in soil that has a salt content of >3 g/L, carrot (*Daucus carota*), peach (*Prunus persica*), and broad bean (*Vicia faba*) are some examples.

Salt-tolerant plants are tolerant to a higher concentration of salt, up to 10 g/L, and have a wonderful array of adaptations that provide a successful solution in terms of biological osmosis, ion localization, and increased antioxidant activity that ameliorates stress. The examples include tomatoes (*Solanum lycopersicum*), corn (*Zea mays*), bread wheat, and cultivated barley. Highly salt-tolerant plants possess superior adaptive mechanisms to survive in high-salinity surroundings, allowing them to withstand salt concentrations up to 18 g/L. Such plants include cotton (*Gossypium* spp.), sugar beet (*Beta vulgaris*), and date palm (*Phoenix dactylifera*), showing variation in root architecture and excretion for getting rid of extra salts and keeping internal homeostasis, respectively (Gupta & Huang, 2014).

Salinity stress brings about numerous physiological and biochemical disorders in plants. The prime among them is osmotic stress, which causes the lowered absorption of water into plant cells due to the lowered osmotic potential of the soil solution. Thus, a membrane boke occurs, and water absorption is slowed. Neutralized ions cause dehydration because the plant loses water so rapidly through leaf stomata that it flows back, producing wilting, after which growth is slowed (Munns & Tester, 2008). Soils under the salinity conditions boost Na or Cl content higher and higher due to continued uptake of these ions, leading to their translocation across the plant. Excessive levels of the two elements in the vacuole result in cytotoxicity, reducing enzyme activity, thereby leading to chlorosis, while K, Mg, and Ca have difficulties in being absorbed within the plant due to the prevailing effect of Na ion (Parida & Das, 2005; Munns, 2002). Salinity brings yet another concern that whichever essential element is not taken can be easily leached out by irrigation water. When this happens, the situation reaches a dead end. An example is the nutrient K⁺. Salinity stress continues to enhance the usurpation of ROS, which in essence rip cellular structures, proteins, and lipids. Actions of antioxidant defence in all plants are activated in countering oxidative stress brought on by salt, whose prolonged occurrence leads to the offsetting of the protective measures that allow plant productivity and life (Mittler, 2002).

The understanding of plant response to salinity is imperative for one to develop strategies to improve plant resilience in saline soils. The utilization of salt-tolerant cultivars and soil management can maintain agricultural productivity in saline environments. Henceforth, the development of genetic and biochemical mechanisms for salt tolerance will add to the plants' adaptation to salinity stress.

Modern biotechnological techniques such as genetic engineering and molecular marker-assisted breeding offer a great scope to develop crops that can withstand high levels of salinity without hampering the plants' growth and yield (Qadir et al., 2014). Soil amendments, good irrigation practices like drip or furrow irrigation, and rotation with salt-tolerant species are effective practices to minimize the impact of salinity on agriculture.

1.2.3.1.2 Damages of Salinity to Plants

High salinity levels cause significant damage to plants, affecting their physiological and cellular function. In the soil, the high salt concentration hinders water absorption, nutrient uptake, and metabolic activities, thereby acting as an obstacle to growth and productivity. The severity of the impacts of salinity varies according to the species of plants, concentration, and duration of the exposure to salinity. As the stress from salt increases, plants struggle to adjust the cellular homeostasis, leading to great physiological and biochemical disturbance (Munns, 1993).

At the cellular level, salinity will damage many important biological functions. A primary action is the inhibition of all enzymatic activities contributing to the transformation of nutrients in plant cells. High salinity results in a marked increase in the rates of cellular respiration, resulting in excess energy utilization. This shift renders an energy imbalance, disrupting the synthesis of essential biomolecules required for growth and development (Lazof & Bernstein, 1999). Also, high salt concentrations can detrimentally affect the activities of the meristematic cells, leading to a significant reduction in cell elongation. The structural integrity is hence impaired, contributing to tissue disruption and other impairments of cellular growth (Cramer, 1992).

Salinity enhances oxidative stress through the rapid formation of deleterious free radicals. These free radicals damage vital biomolecules, including proteins, lipids, sugar, and nucleic acids, and are involved in disrupting cellular homeostasis. Further, the interaction of free radicals with cellular components disrupts the usual metabolic pathways, resulting in an exaggeration of growth inhibition (Xiong et al., 2002). In the severe cases, under oxidative damage conditions, compounds of programmed cell death rather than repair processes are activated and due to the accumulation of ROS in cells. Yet another excruciating salinity enhances oxidative

stress through the rapid formation of deleterious free radicals. These free radicals damage vital biomolecules, including proteins, lipids, sugar, and nucleic acids, and are involved in disrupting cellular homeostasis. Further, the interaction of free radicals with cellular components disrupts the usual metabolic pathways, affecting an exaggeration of growth inhibition (Xiong et al., 2002).

In the severe cases, under oxidative damage conditions, compounds of programmed cell death rather than repair processes are activated due to the accumulation of ROS in cells. The issue of salinity in present scenarios is significantly reduced water content in plant tissues. The osmotic imbalance generated by the high salt concentration inhibits the absorption of water by roots to rehydrate at the cellular level. Counting on the loss of water absorption, plants may start to wilt, lack turgor pressure, and finally result in desiccation (Munns, 2002). Salt stress severely restricts plant uptake of crucial mineral nutrients, leading to imbalances of nutrient supply. In most cases, excess Na and Cl ions displace essential macronutrients K, Ca, and Mg, causing nutrient imbalances that in turn impair plant metabolism (Munns, 1993).

From a genetic perspective, sustained exposure to high salinity levels generates genotoxic effects. Intra-cytosolic Na ion accumulation disrupts DNA stability, leading to mutations, amongst other point mutations or strand breaks. Severe ionic stress can induce the breakdown of whole DNA, leading to cell death (Al-Sahhoki & Fagai, 2014). This is particularly important in seedlings and developing plant tissues because genetic materials are vulnerable to damage by salts. Knowledge of critical cellular targets of salt injury is invaluable in devising efficient strategies to counter these injuries. Modern breeding and transgenic approaches focus on the introduction of traits that sustain ionic equilibrium, antioxidant activity, and stress pathways to make plants more resilient. Furthermore, amendment of the soil, improved irrigation practices, crop rotation with salt-tolerant species, and wise use of fertilizers can be proposed for sustainable agricultural practices, thus neutralizing salt threats to plant ecology and productivity (Qadir et al., 2014).

1.2.3.1.3 Effect on the Level of Tissues and Organs

Salinity stress affects plant tissues in a variety of ways, leading to significant physiological and structural alterations, including the hormonal balance of plant

tissues that regulate growth and enzymatic activities; the disruption of these activities under the saline condition, which disrupts hormonal balance by lowering cytokinin levels in leaves, thus weakening and retarding plant growth (Kingsbury et al., 1984; Ziska, 1990; Vankova et al., 2011).

This grows to exacerbate plant development as a result of the hormones' interplay, further slowing down shoot elongation. Among the conspicuous effects of salinity is the early aging of plant leaves. Salinity causes an accumulation of toxic elements such as Na and Cl in leaf tissue cells, resulting in leaf yellowing, necrosis, and the appearance of dead spots (Munns et al., 1988). These signs indicate cell damage and reduced photosynthetic activity, eventually leading to premature senescence and decreased crop yields. The accumulation of toxic ions in the leaves affects the transpiration efficiency of the plants, thereby aggravating plant dehydration. The major effect of salinity on plant organs is the reduced efficiency in photosynthesis. Salinity limits the effectiveness of stomatal function, leading to partial or complete stomatal closure that, in addition to exercise, limits CO₂ uptake and ultimately results in decreased photosynthetic rates (Downton, 1977; Walker et al., 1981; Srethivasulu Reddy et al., 1998). Insofar as it leads to reduced compensation through photosynthesis, there is the limitation of energy slack with obvious implications for growth and the attenuation of biomass accumulation. The decrease in photosynthesis efficiency also leads directly to lower leaf area as salt restricts leaf expansion and consequent leaf development (Ali et al., 2004). The smaller leaf area has the direct consequence of resulting in a lower rate of photosynthetic output, thus decreasing plant productivity (Bhawat & Bhatia, 1993).

There is putative visible leaf damage caused by the build-up of excessive sodium and chloride ions after injury by salt. These ions interfere with the cellular membranes, leading to ion efflux, chlorophyll degradation, and inevitable tissue necrosis (Garg & Gupta, 1995). The resultant damage further affects light-capturing efficiency and weakens plant resistance. As an instance, continued exposure to salinity manifests into long-term toxic symptoms, which include severe yellowing and mild root growth, and greatly depressed seedling germination rates.

Protracted exposure to salinity for several months will start damaging plant reproductive organs. In cases of prolonged salinity stress, it continues to lead to both flower and fruit drop post-setting, hence significantly reducing the potential yield (Munns et al., 2002). When physiological stress is triggered by salinity, it

disrupts reproductive development interfering with pollination, fruiting, and seed maturation, ultimately reducing the plant's ability to reproduce. That reduction in reproductive success demonstrates the long-term manifestation of salinity on cropping systems.

Nonetheless, salinization similarly affects a general plant morphology of responsiveness, leaf density, leaf count reduction, and ultimately both wet and dry biomass accrual (Sadak et al., 2015). This decline in biomass is not only reflected in reduced yields but also forage fodder production, making salinity a serious concern in highly biomass-dependent agricultural systems. Opportunities to mitigate these effects involve methods such as soil amendment, management of irrigation, and growing salt-tolerant cultivars that could maintain their productivity under saline conditions.

Understanding how salinity affects tissues and organs is critical to designing effective control measures. Studies on plant adaptation could provide answers through either enhanced ion separation or regulation of stress-responsive genes that eventually result in enhanced tolerance to salinity in any crop.

Recent innovations in genetic engineering and molecular breeding technology are bringing hope to the development of salt-tolerant lines with the promise of nurturing the persistent growth of such cultivars in saline environments.

1.2.3.1.4 Effect on the Level of Biochemical Processes

1- The effect of salt stress on the carbohydrate content of plants

The salts can either enhance or reduce the carbohydrate content of the plant. Some studies suggest that salt reduces carbohydrates, as Aly (1979) mentioned that total carbohydrates in the stems and leaves in grape (*Vitis vinifera*), pomegranate (*Punica granatum*), orange (*Citrus sinensis*), navel orange (*Citrus sinensis* var. *navel*), and guava (*Psidium guajava*) decreased as the salts increased in irrigation water. Likewise, Gorham et al. (1981) noticed a decrease in monosaccharide content during the treatment of carrot plants with salts. Some examples from studies conducted by Hanson and Hitz (1982) and Morgan (1992) demonstrate that plants facing stress conditions usually result in poor synthesis of sugars, sugars insoluble in very small quantities.

Zidan (1991) confirmed that wheat exposed to salinity suffers a stark decrease in carbohydrate content. As reported by Hathout (1996) during research

into the effect of various sodium chloride salt concentrations on common wheat (*Triticum aestivum*), an increased salt concentration (3200 and 1600 ppm) decreased the carbohydrate content, while a lower salinity rate of 800 ppm had a significant increase.

Two genotypes of faba bean (*Vicia faba*) (103 and 153) showed a rise in carbohydrate content with increasing salinity, as opposed to the results in the Crosby variety, indicating its decline instead. In the case of a salt-stressed tomato plant, the concentration of sucrose was higher than in those treated with NaCl salt since the sucrose phosphate building enzyme is active while the invertase enzyme is decreased (Zhifang et al., 1998). The future levels of sucrose are severely raised in the cotyledons of the *Chenopodium guinoa* wild plant treated with sodium chloride salt compared to their untreated counterparts (Prado et al., 2000). According to Gadallah (1999), the accumulation of soluble sugars and proline represents a useful defence mechanism against salt-stress conditions in Polysaccharides also prevent cellular molecules like enzymes from interfering with their structure and function by balancing the osmotic pressure (OP) of the cytosol with the vacuole and the external environment (Sminoff et al., 1989; Rhodes, 1987).

2- Effect of salt stress on plant protein content

Shimose (1957) reported that the introduction of salt into the soil resulted in decreased levels of total nitrogen in barley, kidney beans, and maize. On the other hand, studies by Nieman (1965), Gates et al. (1966), Uprety & Sarin (1975), and Shams El-Din et al. (1992) indicated that the range of salt-induced protein reductions in the studied plants was not statistically different. The studies conducted by Radi et al. (2014), Abd-El-Ghaggar et al. (1998), and Borzouei et al. (2012) showed that there is a decrease in the total soluble protein content in wheat and beans when the plants are exposed to salt stress. Whereas Hamada & Khuleaf (1995) and Francios et al. (1988) stressed that exposure of the wheat plant to salt stress leads to an increase in the soluble protein fraction.

Ashraf and Harris (2004) further showed that proteins accumulated under stress conditions (salinity) are not a good indicator of salt resistance in plants and therefore cannot be a valid indicator or guide.

3- Effect of salt stress on plant amino acid content

Barlow et al. (1976) and Jashi and Naik (1980) observed increased free amino acid content in maize and sugarcane (*Saccharum officinarum*) plants under

saline conditions as well as the cations, which indirectly serve as a toxicant for the plants since these nitrogenous substances also have a direct antagonistic effect on plant growth. Also refer to the following excerpt: "According to Chandra (1982), changes in enzyme activities are likely to occur in plants grown in saline media."

Stavarek & Rains (1976) and Nieman & Shannon (1976) suggested that nitrogenous compounds surge and amino acids grow with increasing salinity concentration levels. The investigation brought on by Mansou (2000) determined that amino acids, including alanine, glutamate, valine, arginine, glycine, serine, leucine, proline, and asparagine accumulate in plants that are exposed to salt stress.

4- Effect of salt stress on plant content of proline

Salinity is an environmental factor that is causing adverse effects on the growth and development of plants by disturbing cellular metabolism and cellular structures (Stewart 1966; Stewart and Lee 1974). Effects of salinity on cellular systems initiated an increase in the synthesis of Na⁺ ions, proline, and saccharose. (Levitt, 1980; Greenway and Munns, 1980). In high salt-stress environments, plants increase the concentration of special compounds, one of which is proline (Stewart et al., 1966). According to Stewart and Lee (1974), the role of proline is osmotic regulation during salt stress, and the leaves of plants grown in a saline medium at concentrations based on the osmotic potential of the environment.

The result of this experiment done by Guerrier (1997) on separating tomato leaves, which were exposed to 12 g/l of NaCl salt for six hours, indicated a significant increase in glutaminase activity, and the accumulation of proline content in the leaves of the salinity-sensitive variety treated with a NaCl salt in comparison with the leaves of the resistant variety was more. In salinity-sensitive tomato varieties, increased proline accumulates only when treated with NaCl salt and progresses to its greatest accumulation over the remaining 27 hours. It was found that the accumulation of proline in salinity takes place after cell damage; it only appears as a delayed response and remains at this high level even after the return of osmotic pressure in stressed tissues to their normal state (Trotel et al., 1996; Delauney & Verma, 1993). In light of the above-discussed points, the accumulation of proline in salt-stressed plants has been validated previously as it helps plants perform certain functions. The following points elaborate on its necessity. Salinity stress tends to be reduced when proline is available because proline allows everyone to absorb the largest amounts of water or food available in nature (Yong et al. 1998).

Proline formation prevents the breakdown of proteins of the plant, thus preventing their degradation, for its presence helps strengthen the bonds between the amino acid molecules constituting the protein, especially at the very start of plant growth, as protein breakdown at this critical stage would then expose the plant to aging and death (Lehninger, 1982).

Proline guards the plant by regulating the osmotic pressure of the cellular system (Hare and Cress, 1997; Verbruggen and Hermans, 2008). Protecting plants from free ions and reactive oxygen radicals by scavenging them and guarding them against oxidative stress (Stewart and Lee, 1974; Noctor and Foyer, 1998).

1.3 The Study Hypothesis and Objectives

1.3.1 Problem of the Study

The high soil salinity is an essential abiotic stressor destroying the production of wheat worldwide. The plants' physiology and morphology change as a result of high soil salts, which are the most affected processes, like germination, seedling growth, and final yield (Munns & Tester, 2008). The soil salinity issues are most prominent due to bad irrigation practices, rising global temperature with climate change, and prolonged chemical fertilizers, resulting in the accumulation of toxic salts in agricultural lands (Shrivastava & Kumar, 2015). Body development is a very critical need for securing food and continuity in food production.

The detrimental impacts of salinity stress on plants may be numerous, such as osmotic stress, ion toxicity, and nutrient imbalance. With a high concentration of NaCl, water absorption is interfered with by reduced osmotic potential in the soil solution, making plants not have enough water to absorb (Zhu, 2001). Osmotic stress causes dehydration, cell turgor loss, and net loss in growth due to cell expansion inhibition. Moreover, in this scenario, Na⁺ and Cl⁻ ions could accumulate within plant tissues and cause ion toxicity and interference with key physiological processes like photosynthesis and protein synthesis (Parida & Das, 2005). Further, the uptake of nutrients adds a negative effect as the excess Na⁺ and Cl⁻ ions compete with principal nutrients like K, Ca, and Mg, resulting in their deficiencies and impairing plant metabolism and the action of enzymes (Munns et al., 2006).

Salinity stress is a key stressor of first significance during the early growth periods in wheat, affecting germination rates and seedling vigor. Studies have reported various phenotypic and physiological changes due to low to moderate salt

concentrations (3-100 mmol NaCl), e.g., changes to root and shoot development, chlorophyll synthesis, and total biomass accumulation (Flowers et al., 2015). At higher salt levels (150-200 mmol NaCl), the toxic effects become much more defined and cause a significant reduction in growth and, sometimes, complete death (Deinlein et al., 2014). Hence, genetic studies are needed so that these genotypes that exhibit salt resistance can be identified. The same resistance can be conjugated to plant breeding for the development of high-yielding, salt-resistant wheat varieties.

One key aspect regarding salinity tolerance in wheat is the identification and characterization of genes contributing to salt-stress resistance. Such genes have opened survival opportunities for certain plant species, which lack them in other cases, thereby making them highly prone to salt-induced damage (Roy et al., 2014). Variability in the salt tolerance genes found in various wheat species suggests that comparing the nucleotide sequence and the amino acid composition could provide clues about the resistance to salt at the molecular level. Bioinformatics techniques such as BLAST analysis allow researchers to demonstrate consistency in such genes between plant families in formation and evolutionary patterns in adaptation to salinity (Negrão et al., 2017).

Agronomic and physiological evaluations of wheat grown under conditions of high salinity are also capable of pointing out tolerant genotypes. The evaluation of traits, like germination percentage, root length, shoot length, biomass weight, chlorophyll content, soluble sugars, and proline content, would enable the assessment of salt tolerance in different wheat varieties (Hussain et al., 2016). Estimates of heritability and genetic variance on salt tolerance traits under controlled laboratory conditions can help prioritize promising genotypes pending more substantial breeding programs aimed at enhancing wheat tolerance toward salinity and drought (Qadir et al., 2014).

Given that soil salinity is rather widespread and has caused a lot of adverse effects on wheat cultivation, this study is one in which salinity can be analyzed for its influence on germination, growth, and physiological responses in different wheat genotypes.

The integration of molecular, bioinformatics, and agronomic evaluations aims at finding salt-tolerant wheat genotypes that could be utilized as gene banks for developing new cultivars that might thrive under saline conditions. Therefore,

the outcomes of this study stand as partial reflections of global efforts to enhance crop productivity in salt-ravaged areas and render sustainable wheat production in harsh environmental contexts.

1.3.2 The Study Hypotheses

Soil salinity is an abiotic stress factor that highly influences the growth and productivity of plants, especially that of wheat, a major staple food grain of the world. Accumulation of salt in the root area interrupts various physiological processes of plants, leading to poor germination, stunted seedling growth, and a decrease in overall yield (Munns & Tester, 2008). The intensity of salinity stress imposed depends upon the salt concentration in the atmosphere; higher salinity imbibes toxic stress leading to death, yet lower salt concentration might allow for physiological adjustments (Zhu, 2001). This study examines the effects of various salt concentrations on the growth and development of wheat with a particular reference to morphological and physiological changes brought by salinity stress.

Comprehending the genetic background of salinity tolerance in wheat is very much needed for developing wheat varieties suitable to thrive in saline environments. Genetic variation in salt-tolerance genes will decide whether a genotype of wheat is to be considered resistant or susceptible to salinity stress. Comparative genomic studies and in silico analyses, such as BLAST and preparation of a phylogenetic tree, could provide evolutionary patterns of salinity tolerance genes and their probable resemblance found in other plant organisms (Flowers et al., 2015). This study aims to investigate the effects of salt stress on wheat at multi-level presentation-germination, seedling growth, physiological response, and genetic variation in salt tolerance genes.

- Salinity has a detrimental effect on the germination and growth of seedlings.
- Low and medium salt concentrations (3-100 mmol) of NaCl cause phenotypic and physiological (biochemical) changes.
- The toxic effect of NaCl appears on plants exposed to salt concentrations (150-200 mmol), which may cause plant death.
- High concentrations of NaCl (100-200 mmol) used in wheat plants affect the germination rate and the growth and development of seedlings.
- Some plants do not have a salinity resistance gene because they are not resistant to salinity.

- There may be a variation in the salinity resistance genes between different types of wheat, whether through a comparison of nucleotide sequences or an examination of the amino acids composing the proteins.
- The salinity resistance gene in wheat varieties may exhibit variations, and these differences can be identified using the NCBI program.
- The salinity tolerance gene in wheat may share similarities with genes in other plants, which can be confirmed through BLAST analysis.
- By making a phylogenetic tree and comparing different genotypes of salinity tolerance genes in wheat, we may be able to see how these genotypes evolved and how genetic diversity exists among them.

1.3.3 Objectives of the Study

Environmental challenges, of which soil salinity is the most critical, are impacting wheat's performance owing to its interference with some basic physiological and biochemical processes in the said crop. Wheat's salt tolerance varies among genotypes.

Some possess adaptive mechanisms contributing to their survival under salinity. The mechanisms involve ion homeostasis, osmotic adjustment, and antioxidant defense, as well as changing with time those morphological traits like root length and seedling biomass (Munns & Tester, 2008). Understanding salinity on wheat with development stages is of paramount importance to identify tolerant varieties and formulate successful breeding strategies. This study only purposes to evaluate wheat seedlings concerning the various growth and physiological aspects under stress to assess salt resistance levels according to different salt concentrations.

The molecular and bioinformatics approaches, apart from phenotyping, vastly help in unwrapping the genetic bases of salinity tolerance. Comparative analyses of genomes and sequences, as well as phylogenetic research, assist in tracing genetic variations that have enabled salt stress adaptation (Negrão et al., 2017). In an interdisciplinary approach, agronomic, physiological, and molecular assessments have been integrated to pinpoint how distinct wheat genotypes face salinity stress. Based on these findings, this study is projected to make valuable contributions to the improvement of wheat breeding programs in terms of improved variation in tallies of assessments of the salinity tolerance to offer support for

sustainable agricultural procedures, thus beyond those regions where most influenced by soil salinity.

- Investigate the impact of salinity on the germination percentage of wheat grains throughout the experiment period (10 days).
- Examine the effect of salinity on various growth parameters, including:
 - Root length.
 - Root numbers.
 - Coleoptile length.
 - Fresh weight of seedlings.
 - Dry weight of seedlings.
 - Percentage of relative water content in the seedlings.
- Study the influence of salinity on several physiological processes, including:
 - Estimation of chlorophyll content (A, B and A+B) in seedling extracts.
 - Determination of the soluble sugar content in the seedling extracts.
 - Determination of the soluble protein content in the seedling extracts.
 - Determination of the total free amino acid content in the seedling extracts.
 - Determination of proline content in seedling powder.
- The study examines molecular studies using bioinformatics applications with the following objectives:
 - Agronomic evaluation of the possible tolerance to salinity of wheat genotypes.
 - Estimation of genetic parameters for salinity and drought, such as heritability, genetic variation, and phenotype, under lab conditions.
 - Selection of the best drought-tolerant and salinity-tolerant wheat genotypes as a nucleus for breeding programs by further hybridization.

1.3.4 Importance of the Study

Salinity in soil is among the most common issues that affect agricultural production, mostly in arid and semi-arid areas, where water deficiency has significantly aggravated the issue. Wheat, being a major food crop, is very prone to the impact caused by salt stress, hence deeply affecting potential yield and

threatening food security (Munns and Tester, 2008). Agricultural lands are getting saltier, so it is imperative to understand the effects of salt stress on wheat plant growth and development. The report is valuable because it comprehensively explores wheat responses to salinity at the morphological, physiological, and molecular levels, whereby key tolerance characteristics can be identified. Comparing various salt-tolerant wheat genotypes may allow the identification of genetic and biochemical traits that play a role in the enhancement of salt tolerance, thus improving the selection of tolerant wheat varieties for cultivation in salt-affected soils.

In places such as Libya, agricultural lands are mainly classified under the arid and semi-arid zone, leading to an extreme concern about salinity (Ghassemi et al., 1995). As long as wheat is the principal component of the country's food supply, soil salinity cannot be ignored if reliable crop yield is to be kept. A close examination of many physiological traits, such as chlorophyll content, proline accumulation, and soluble protein concentration, will explain the behavior of wheat plants against salinity stress.

Moreover, this contribution will supply an elucidation of the genetic underpinnings of salinity tolerance through bioinformatic analysis. The study provided in this study will serve as a base for transforming agronomic data into genomic data, thus encouraging improved breeding strategies for enhancing salinity resistance in wheat. In the field of biotechnology, on the scientific forefront in modern agriculture, this research will go further to indicate the employment of computational tools to evaluate genetic variations in salt-tolerant wheat genotypes. Such techniques as BLAST analysis and construction of phylogenetic trees shall be applied to delineate evolutionary relationships existing between wheat and related species that are salt tolerant, providing crucial insight into genetic adaptation mechanisms (Negrão et al., 2017). The findings from this research will, in turn, become of paramount value not just to wheat breeders and agronomists but also to policymakers and other agricultural stakeholders engaged in equitable management of crop production in the salt-affected areas. As such, the determination of superior salt-tolerant wheat genotypes is an important stepping stone in mitigating detrimental effects due to soil salinity in global food security.

1.3.5 Contribution of the Study

The research proceeds to widen the area of study in plant stress physiology and agricultural biotechnology by presenting a multidimensional examination of wheat's reaction to salt stress. Unlike the past research endeavors that kept a focus solely on field traits or molecular marking, this study anticipated the merging of both research areas; that is, its findings should offer newer meanings and clearer viewpoints leading to an overall understanding of the capacity of wheat for salt resistance. At a conclusive level, the study demonstrates the effects of various salt stress forces upon wheat seedlings in terms of the effects upon seed germination, seedling growth, and other physiological changes. These could easily come forward to apply in breeding programs summoned to instigate wheat support in situations plagued by salinity ailments. Besides salinity-related testing, this research employs advanced bioinformatics to dissect genetic variations of salt-tolerant wheat genotypes. BLAST analysis and phylogenetic tree construction as tools selected for this study have singled out certain genetic markers that could be associated with salt tolerance.

Such knowledge paves the way for further discovery on a rapidly growing subject in facilitating crop genetic improvements for stress resilience. Furthermore, this study provides a genetic contrast to evolutionary patterns in genes responsible for salinity stress, thus providing new insights on plant adaptability to salinity conditions (Flowers et al., 2015). The study spells scientific expansion but also has huge implications for sustainable agriculture and food security. Identifying salt-tolerant wheat genotypes would be an impetus for breeding programs to devise novel wheat genotypes standing sturdily in saline environments. This would indeed be great for countries like Libya, where wheat production is so vital for national food security, and the successful installation of this proposition virtually betrays its defiance to the adjustment of soil salinity. The entire findings from this study may very well serve to shape agricultural policies and guide future research work to make salt-stress crop resilience better. This research also propels developments in wheat breeding strategies while providing proper management of genetic resources and ensuring sustainable agricultural practices, considering the demands of climate change and environmental stressors.

1.3.6 Previous Studies and Research

Wheat, one of the most crucial crops for sustaining the growing global population, plays a significant role in human and animal nutrition, as well as the paper industry. A decrease in wheat production leads to rising bread prices and the cost of food products made from wheat (Süzer, 2004).

Changes that occur in plants under the influence of biotic and abiotic environmental factors are referred to as stress. Stress has adverse effects on plant growth and development, leading to a decrease in quality and yield, as well as the death of plant organs. The extent of damage caused by stress factors depends on the genetic adaptation of the plant to its environment (Singh and Dubey, 1995).

Seed germination is the most fundamental and vital stage of the plant growth cycle. Numerous studies on various plant species have shown that salinity has a detrimental impact on seed germination (Essa, 2002; Mutlu and Bozcuk, 2007; Çarpıcı, 2009; Xu et al., 2011; Khodarahmpour et al., 2012). Salinity affects seed germination in various ways, including altering the osmotic potential of the germination medium, which affects seed water absorption (Khan and Weber, 2006), causing toxicity that changes nucleic acid metabolism enzyme activity (Gomes-Filho et al., 2008), altering protein metabolism (Yupsains et al., 1994; Dantas et al., 2007), disrupting hormonal balance (Khan and Rizvi, 1994), and reducing the binding rate of the plant (Promila and Kumar, 2000; Othman et al., 2006).

Arfan et al. (2007) found that the application of a 150 mM NaCl concentration to wheat resulted in a decrease in plant biomass, dry weight, and leaf quantity, leading to a reduction in cereal yield. Sayar et al. (2010) examined the effect of salt and drought stresses on durum wheat development, using two durum wheat varieties (BD290273 and Omrabia) that were tolerant to salinity and drought. They observed adverse effects of NaCl on germination and shoot growth.

Kumar et al. (2012) studied the impact of salinity on germination, growth, yield, and yield components in eight wheat genotypes (KRL1-4, K8434, K88, K9644, K9465, K9006, HD 2733, and HD 2329) at different salinity levels (3, 6, 9, and 12 dSm⁻¹). They found that the lowest salt concentration (3 dSm⁻¹) did not affect germination, growth, or yield parameters, while higher salt concentrations reduced germination, growth, and yield. K9644 and K9465 genotypes were sensitive to salt stress, whereas K9006, K8434, KRL1-4, K88, and HD2733 genotypes exhibited resistance to higher salt concentrations.

Similar results have been obtained in studies on other plant species. A study on tomato reported a reduction in germination rate due to a high salt concentration (150 mM NaCl) (Foolad and Lin, 1997, 1998). Another study on the tomato showed an adverse relationship between salinity and germination percentage (Kaveh et al., 2011). Similarly, Cuartero and Fernandez-Munoz (1999) found that the time required for germination increased with an increase in salt concentration in the same plant. Neamatollahi et al. (2009) reported that an increase in NaCl concentration reduced the germination percentage of *Foeniculum vulgare*. In a study by Lombardi and Lupi (2006), the application of 400 and 500 mM NaCl for 10 days reduced germination by 38% and 40%, respectively, in barley (*Hordeum secalinum*). According to these studies, an increase in salinity concentration leads to a decrease in germination rate, mainly due to ion imbalance, osmotic disturbance, and reduced water uptake by seeds.

One of the most significant effects of salt stress on plants is the reduction in growth rate. Salinity affects plant growth in various ways. The initial response to salinity during the early stages of development is due to the osmotic effect of the salt-containing soil solution. This effect is similar to the effect caused by water stress (Munns, 2002). The impact of salinity on plant growth depends on the duration of exposure to salinity. According to Munns (2002), in the first few seconds and minutes of exposure, plants lose water from their cells, causing them to shrink. While the cells return to their original size hours later, salt stress reduces the rate of cell expansion, resulting in decreased root and leaf growth. After a few days, the cell division phases are affected. Weeks later, vegetative growth changes, and months later, these changes occur during the reproductive period. Munns (2005) has examined the effects of salinity on plants in two different processes. One is the rapid process caused by osmotic stress, and the other is the slow process resulting from salt accumulation in the leaves, known as salt-specific stress. These processes ultimately lead to leaf death, a decrease in total photosynthetic green area, and a decrease in yield (Munns, 2002).

Plants exposed to high salt concentrations for an extended period accumulate Na⁺ and Cl⁻ ions, leading to leaf damage and death. The damage and death of leaves are related to the high salt concentration that accumulates in vacuoles. Many studies have reported that plants are more sensitive to salt stress during germination and early vegetative growth stages (Munns, 2002, 2005).

According to a study by Khatun and Flowers (1995), exposure to salinity (10 mM NaCl) resulted in a 38% reduction in seed setting rate in rice (*Oryza sativa*). Another study on seepweed (*Suaeda salsa*) found that shoot diameter, branch length, number of branches, and plant height were significantly affected by salt stress (Guan et al., 2011). Additionally, a study on *Glycine max* by Dolatabadian et al. (2011) reported a significant reduction in shoot and root weight, plant height, and leaf count due to salt stress, while leaf area was not affected. According to a study by Kaydan and Yağmur (2006), an 8 dSm⁻¹ salt dose applied to wheat reduced seedling emergence rate, photosynthetic pigment content, and above and below-ground dry weights.

In a study by Moud and Maghsoudi (2008) on durum wheat, the effects of two different salt concentrations (9 and 15 dSm⁻¹ NaCl) on coleoptile and root development were investigated. The research revealed that salt stress reduced coleoptile and root development.

El-Lethy et al. (2013) examined the effects of potassium application on pasta wheat varieties under saline conditions using three different concentrations of NaCl (40, 80, and 120 mM NaCl) and two doses of potassium (25 and 150 mg K₂O/kg). The results of the study showed significant reductions in dry and fresh weight as well as plant height, particularly in plants treated with 120 mM NaCl.

Akhtar et al. (2015) conducted a study to examine the impact of salt stress on wheat yield, physiological characteristics, and growth development. In this study, plants were exposed to three different salt concentrations (0, 25, and 50 mM NaCl). The research revealed that all plants irrigated with the highest salt concentration (50 mM NaCl) died.

The decrease in photosynthesis in plants under salt conditions is attributed to a reduction in water potential. Photosynthesis is constrained by the accumulation of high Na⁺ and Cl⁻ ions in chloroplasts. The salt-induced changes in ion concentrations within plant tissues have been associated with reduced photosynthetic rates and disrupted carbon metabolism, which ultimately affect plant productivity and yield. There is a positive relationship between photosynthesis and yield.

Fisarakis et al. (2001) reported that salt stress leads to a reduction in photosynthesis. The effect of salinity on plants depends on factors such as salt concentration, plant species, and genotype. Some studies suggest that low salt

concentrations can stimulate photosynthesis. For instance, Parida et al. (2004) demonstrated that low salt concentrations increase stomatal conductance in small-flowered mangrove (*Bruguiera parviflora*). Salt stress can accelerate plant aging, alter enzyme activity, and change the cytoplasmic structure. Reduced stomatal conductance under salt stress can result in a limitation of the carboxylation cycle due to CO₂ restriction.

Romero-Aranda et al. (2001) stated that an increase in salt levels in the roots leads to a decrease in leaf water potential, affecting various growth stages in most plants. The osmotic effects of salinity reduce water uptake from the soil by increasing dissolved concentrations in the root zone.

Studies have shown that salinity can lead to nutrient imbalances in plants. It negatively affects nutrient uptake, availability, transport, and distribution within plant tissues. Salt stress reduces nutrient accumulation in plants, particularly nitrogen (N) uptake. This decrease in N uptake can be due to interactions between Cl⁻ and nitrate (NO₃⁻) ions or Na⁺ and ammonium (NH₄⁺) ions. High soil salinity can inhibit phosphorus (P) uptake, primarily due to competition with other ions and low P solubility. Salt stress can also lead to ion toxicity in plant tissues, disrupting ion balance and affecting various physiological processes, including photosynthesis and nutrient uptake.

2. MATERIALS and METHOD

The morphological and physiological experiments in this study utilized Petri dishes (9 cm in diameter) lined with filter paper, where 10 seeds were placed in each dish. Two types of salt were used for the experiment: NaCl and CaCl₂. These salts were chosen to simulate the impact of salinity on seed germination and growth, which is a common issue in agricultural areas affected by soil salinization. The effects of these salts on the seeds' morphology and physiology were closely monitored to understand the degree of salt tolerance in the wheat genotypes under study.

2.1 Materials

Plants used in the study are as follows (Figure 2.1):



Figure 2.1. Plant samples used in that study

- Four types of wheat from Türkiye were identified and classified by the Department of Biology, Faculty of Science, Bolu Abant Izzet Baysal University— Türkiye (Table 2.1) (Figure 2.2):
 - Einkorn Wheat (*Triticum monococcum* spp. *monococcum*) (TW1)
 - Emmer Wheat (*Triticum dicoccum* ssp. *dicoccum* Schrank.) (TW2)
 - Bread Wheat (*Triticum aestivum* L.) (TW3)
 - Durum Wheat (*Triticum turgidum* L. var. *durum*) (TW4)

Table 2.1. Types of wheat landraces from Türkiye

Wheat landraces	Code	Source	Registration Date	Latin Name	GPS Coordinates
Einkorn	TW1	Seben Güneyce Village	2021	<i>Triticum monococcum</i> spp. <i>monococcum</i>	40°21'46"N 31°31'6"E
Emmer	TW2	Sinop Durağan Gölgerişi	2020	<i>Triticum dicoccum</i> ssp. <i>dicoccum</i> Schrank	41°26'15"N 35°22'54"E
Bread	TW3	Bahri Dağdaş Uluslararası Tar. Araş. Enst. Müd. Çumra Konya	01-04- 2004 and 2016	<i>Triticum aestivum</i> L.	37°34'15.0"N 32°43'38.7"E
Durum	TW4	Bahri Dağdaş Uluslararası Tar. Araş. Enst. Müd. Karapınar Konya	06-05-1997 and 2016	<i>Triticum turgidum</i> L. var. <i>durum</i>	37°43'57.5"N 33°31'36.0"E

TW: Turkish wheat



Figure 2.2. Types of wheat from Türkiye

- Four types of wheat from Libya were identified and classified by the Agricultural Research Center, Misurata - Libya (Table 2.2) (Figure 2.3):
 - Bushi - Common Wheat (*Triticum aestivum* L.) (LW5)

- Kharisi - Common Wheat (*Triticum aestivum* L.) (LW6)
- Jamnia - Common Wheat (*Triticum aestivum* L.) (LW7)
- Grace - Durum Wheat (*Triticum turgidum* L.) (LW8)

Table 2.2. Types of wheat from Libya

Wheat landraces	Code	Source	The Presence and Color of the Awns	Farming System	Latin Name	GPS Coordinates
Bushi	LW5	Valley of Atba Fezzan	Black awned	Continuous and supplemental irrigation	<i>Triticum aestivum</i> L.	26°11'28"N 13°23'14"E
Kharisi	LW6	Valley of Atba Fezzan	Without awn	Continuous and supplemental irrigation	<i>Triticum aestivum</i> L.	26°11'28"N 13°23'14"E
Jamnia	LW7	Jfara Plain Libya	Without awn	Dry and supplementary irrigation	<i>Triticum aestivum</i> L.	32°35'58"N 11°41'39"E
Grace	LW8	Al Jabal al Akhdar, Libya	Black awned	Rainfed agriculture	<i>Triticum turgidum</i> L.	32°16'39"N 21°44'06"E

LW: Libyan wheat



Figure 2.3. Types of wheat from Libya

Soft wheats are often grown under rainfed conditions along the coastal strip, in semi-desert areas, or under irrigation in desert oases. Varieties cultivated in semi-desert regions (with low rainfall ranging from 50-150 mm) are characterized by their high resistance to drought, early maturity, and high number of offspring, though their productivity is relatively low. These varieties are well suited to areas with limited seasonal rainfall and high temperatures in the final stages of growth. Hard wheat, on the other hand, is cultivated in the Green Mountain areas, where rainfall ranges from 400 to 600 mm.

2.1.1 Primers Used in the Study

A list of primers used in this study with their sequences (Table 2.3).

Table 2.3. A list of primers used in this study

Primer	Sequence	Annealing temperature	Reference
SOS1	F: CAAGGATTTTGCTCCCTCAG	56°C	Wu et al., 1996; Shi et al., 2000
	R: CTTCTGGTTCGCTTCCACAT		
AtHKT1	F: AGTAGACCTCTCTACACTTTCC	55°C	Rus et al., 2001
	R: TTACTTCCTCAATCCATGGGAG		
TSW12	F: CAATATGGAAATGGTTAGCAAAA	52°C	Torres-Schumann et al., 1992
	R: TCGATACAAGACCCCCAAAA		
NHX1	F: ATTCTTCACCAGCACCGTTC	58°C	Sottosanto et al., 2007; Barragán et al., 2012
	R: TCAGATCCAGCAGCATTGAC		
HKT1	F: CATCTCCTTCTTCGGTTTGG	58°C	Hamamoto et al., 2015
	R: GATCATCCCTTCGTTGTTCG		
TAS14	F: AGATGGCACAATACGGCAAT	54°C	Godoy et al., 1994
	R: TCATAAACACGGGACACCA		
ASR2	F: TGTGTGAAATGCCAAACCAT	55°C	Frankel et al., 2006
	R: ATCCAAACGGGCTCAAAGTAA		
RAS1	F: CACCATGCCAAACACTAGCAGCTC	62°C	Ren et al., 2010
	R: TTAAATCGCTTGCGGCGGAG		

2.1.2 Plant Materials

Eight genotypes, consisting of four from Türkiye, -Einkorn (*Triticum monococcum* spp. *monococcum*), emmer (*Triticum dicoccum* ssp. *dicoccum* Schrank), common/bread wheat (*Triticum aestivum* L.), and durum wheat (*Triticum turgidum* L. var. *durum*)- and four from Libya, including Fritesa, Pushy, and Kheresy (common wheat, *Triticum aestivum* L.), and Al-Neamah durum (*Triticum turgidum* L.) from the Misurata Agricultural Research Station, Libya.

2.1.3 Molecular Analysis Materials

- CTAB buffer
- Microfuge tubes
- Mortar and pestle
- Liquid nitrogen
- Microfuge
- Absolute ethanol (ice cold)
- 70 % ethanol (ice cold)
- 7.5 M ammonium acetate
- 55°C water bath
- Chloroform: Isoamyl alcohol (24:1)
- Sterile water
- Agarose
- 6x loading Buffer
- 1x TBE solution
- Agarose gel electrophoresis system
- Ethidium bromide solution

2.1.4 Buffers

CTAB buffer (100 ml): 2.0 g CTAB, 10.0 ml 1 M Tris pH 8.0, 4.0 ml 0.5 M EDTA pH 8.0, 28.0 ml 5 M NaCl, 40.0 ml H₂O, 1 g PVP 40,000), Adjust all to pH 5.0 with HCl and make up to 100 ml with H₂O.

1 M Tris HCl pH 8.0: Dissolve 121.1 g of Tris base in 800 ml of H₂O. Adjust the pH to 8.0 by adding 42 ml of concentrated HCl. Allow the solution to cool to room temperature before making any final pH adjustments. Adjust the volume to 1 L with H₂O. Sterilize the solution by autoclaving. 5 M NaCl: 292.2 g NaCl, 700 ml

H₂O, dissolve and bring the volume up to 1 L with additional H₂O. 5x TBE buffer: 54 g Tris base, 27.5 g boric acid, 20 ml of 0.5M EDTA (pH 8.0). Make up to 1 L with water. To prepare a 0.5x working solution, dilute the 5x stock buffer by a factor of 10. 1% Agarose gel: 1 g agarose dissolved in 100 ml TBE.

TE buffer: A typical recipe for making 1X TE buffer is:

10 mM Tris is brought to pH 8.0 with HCl, 1 mM EDTA, 1 ml of 1 M Tris base (pH 8.0), and 0.2 ml EDTA (0.5 M) are mixed and made up with double-distilled water up to 100 ml. Add microliter amounts of high molarity HCl to lower the pH to 8.

EDTA: To prepare EDTA at 0.5 M (pH 8.0): Add 186.1 g of disodium EDTA•2H₂O to 800 mL of H₂O. Stir vigorously on a magnetic stirrer. Adjust the pH to 8.0 with NaOH (~20 g of NaOH pellets). Dispense into aliquots and sterilize by autoclaving. The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to ~8.0 by the addition of NaOH.

50x TAE Electrophoresis Running Buffer: 242 g Tris free base, 18.61 g disodium EDTA, 57.1 ml glacial acetic acid, and DDI H₂O to 1 litre. Add the Tris free base and EDTA to approximately 700 ml DDI H₂O and stir until the Tris and EDTA are dissolved. Add the acetic acid and adjust the volume to 1 litre. The 1x TAE solution is 40 mM Tris, 20 mM acetate, and 1 mM EDTA and typically has a pH around 8.6. The pH is generally not adjusted.

Gel Loading Dye, Blue (6X): Gel Loading Dye, Blue (6X) is a bromophenol blue-based loading dye offering convenient gel loading and sharp bands.

2.2 Methods

- **Seed Preparation:** Wheat seeds were washed with distilled water and sterilized with bleach to prevent fungal and bacterial growth during germination (Singh, 2020). Each treatment was represented by three Petri dishes (replicates).
- **Solutions Used in Cultivation:**
 - Distilled Water: Used as a control.
 - Salt Solutions: Used at different osmotic potentials (-1, -3, -5, -7, -13 and -15 bars) (Türkoglu et al., 2022) (Table 2.4).

Table 2.4. Salt Solutions are used at different osmotic potentials

Osmotic potentials (Bar)	mMolar (mM)	Sodium chloride weight (g/L)
-1	30.25	1.768
-3	90.83	5.308
-5	153.99	8.999
-7	212.17	12.399
-13	398.97	23.316
-15	460.35	26.903

- **Experiment Execution:** The experiment was conducted in a single phase:
 - **Laboratory Cultivation:** Common wheat seeds were sown in Petri dishes using a random distribution method (Figure 2.4). The treatments were as follows:
 - Common wheat grains were sown individually, receiving only 2 mL of distilled water every two days (used as a control).
 - Common wheat seeds were sown individually and received 2 mL of salt solutions with varying osmotic potentials (-1, -3, -5, -7, -13 and -15 bars) (Türkoglu et al., 2022).
 - Three replicates were used for each treatment.



Figure 2.4. Laboratory cultivation

- **Treatment Methods, Location, Duration of Cultivation, and Measured Criteria:**

Laboratory Cultivation:

- Cultivation Duration: Ten days.
- Growth Medium Used: Petri dishes with filter paper.
- Solutions Used: Control, -1, -3, -5, -7, -13 and -15 bars (Türkoglu et al., 2022) (Figure 2.5).



Figure 2.5. Solutions used

- **Estimation of Germination Percentage:** The germinated seeds were counted daily from the first day to the tenth day. The percentage of grain and seed germination was calculated daily based on the method described by Bonner & Galston (1952) using the following formula:

$$\text{As Germination percentage} = \frac{\text{germinated seeds number}}{\text{total number of seeds}} \times 100$$

2.2.1 Methods for Evaluating Morphological Growth Parameters

Root Length, Number of Roots, and Coleoptile Length:

After ten days, the number of roots, root lengths, and coleoptile lengths of all seedlings were measured using a ruler (in cm). The average number of roots, root lengths, and coleoptile lengths for each petri dish were then calculated (Figure 2.6).



Figure 2.6. Evaluating morphological growth parameters

Relative Water Content, Fresh and Dry Weight of Seedlings

Seedlings from all treatments were washed with distilled water, dried on filter paper, and their fresh weight was measured using a precision balance (in g). The seedlings were then transferred to perforated paper bags and placed in an oven at 70°C for 48 hours to obtain dry weight. The relative water content of the seedlings was then calculated according to the following equation, as mentioned by Elsayed et al. (2021):

$$\text{Water content percentage} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100$$

2.2.2 Quantitative Estimation of Chlorophyll Content (A, B)

Chlorophyll was extracted as described by Lichtenthaler (1987). A 0.5-g sample of fresh leaves was ground in a porcelain mortar for 5 min with 10 mL of 80% ethanol. The mixture was then transferred to glass tubes and heated in a water bath at boiling temperature for 15 minutes. The absorbance of the solution was measured spectrophotometrically at 665 and 649 nanometers, using 80% ethanol as the reference. Chlorophyll A and B content was then determined according to the following equation (Lichtenthaler, 1987):

$$\text{Chl A} = (13.95 * A_{665.0}) - (6.88 * A_{649})$$

$$\text{Chl B} = (24.96 * A_{649.0}) - (7.32 * A_{665})$$

2.2.3 Quantitative Estimation of Some Physiological Metabolites

The concentrations of specific metabolites with osmotic potential were determined in seedling extracts, including:

- Soluble Sugars (mg/g of fresh weight)
- Soluble Proteins (mg/g of fresh weight)

- Amino Acids (mg/g of fresh weight)
- Proline (mg/g of dry weight)

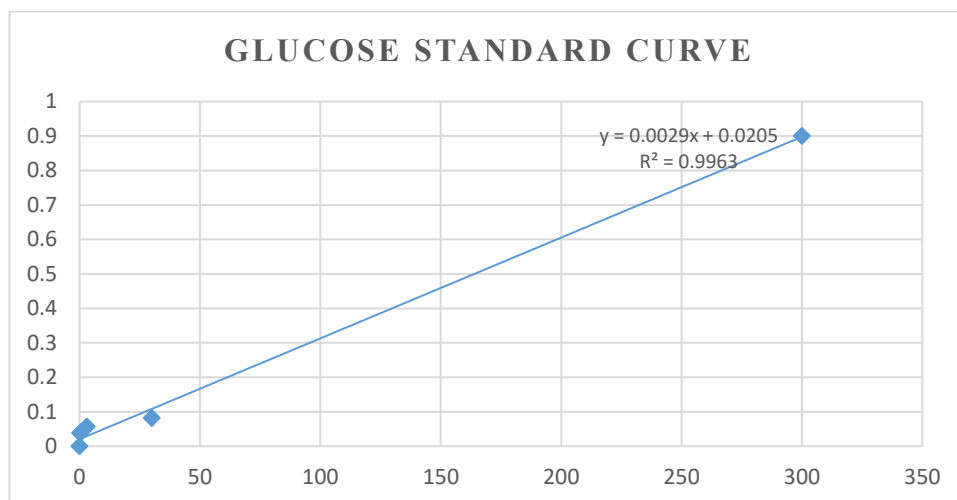


Figure 2.7. Glucose standard curve

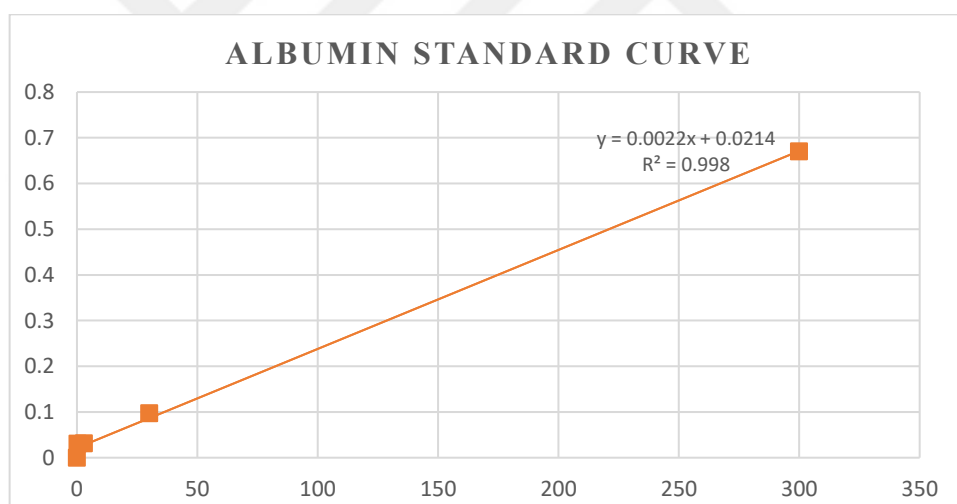


Figure 2.8. Albumin standard curve

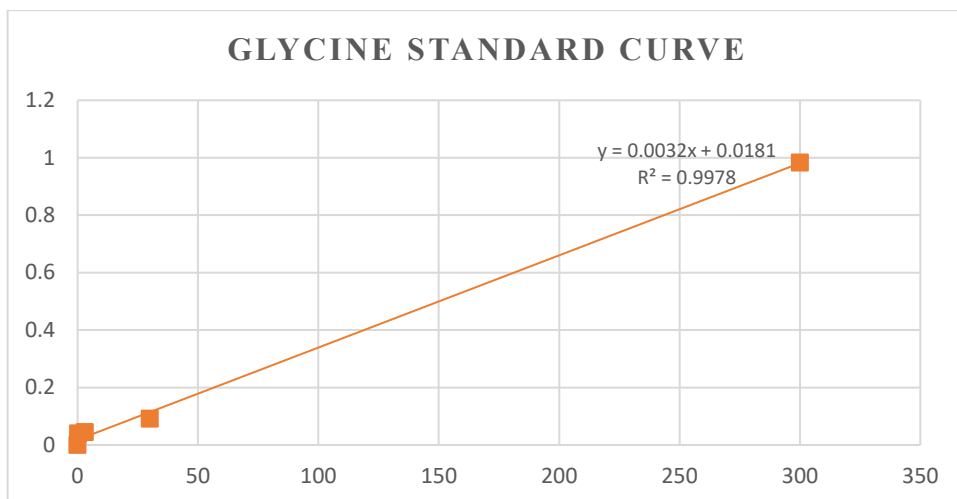


Figure 2.9. Glycine standard curve

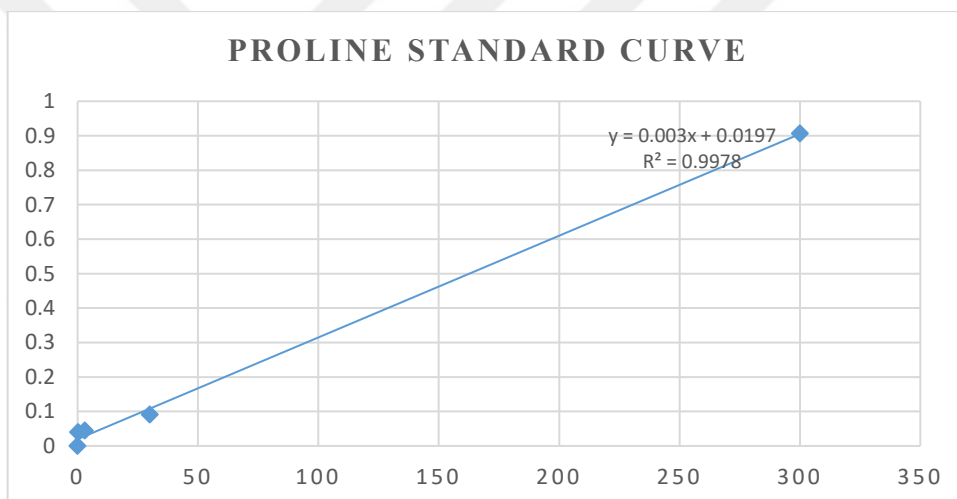


Figure 2.10. Proline standard curve

Methods:

2.2.3.1 Estimation of Soluble Sugars

The quantity of soluble sugars was determined using the method described by Dubois et al. (1956) as follows:

- Plant extract was prepared from wheat seedlings by grinding 0.5 grams of fresh seedlings in a porcelain mortar, followed by the addition of 10 mL of distilled water. The mixture was centrifuged at 4000 rpm for 15 minutes.
- 0.05 mL of the previously prepared plant extract was placed in a test tube and diluted to 2 mL with distilled water.

- 1 mL of 5% phenol solution was added.
- 5 mL of concentrated sulfuric acid was gradually added drop by drop until a golden color appeared.
- The tubes were left undisturbed for 10 minutes, then shaken before being placed in a water bath at a temperature of 25-30°C for 20 minutes.
- Readings were recorded using a spectrophotometer at a wavelength of 490 nanometers.
- Sugars were estimated using the standard glucose curve.

2.2.3.2 Estimation of Soluble Proteins (Biuret Assay)

This method was carried out as described by Ali et al. (2005):

- Biuret reagent was prepared by dissolving 3 grams of cupric sulfate pentahydrate and 9 g of Na and K tartarate in 500 mL of 0.2 M Na hydroxide. Then, 5 g of K iodide was added, and the solution was mixed well and made up to a liter with 0.2 M Na hydroxide solution, followed by thorough mixing.
- Standard solution was prepared by dissolving 0.5 g of BSA in 100 mL of distilled water.
- A standard curve was prepared.

Procedure:

- In each test tube, 2 mL of albumin (according to the concentrations prepared for the standard curve as mentioned above) was placed, and 3 mL of Biuret reagent (3 replicates for each concentration) was added.
- A control was prepared by adding 2 mL of distilled water to a test tube and then adding 3 mL of Biuret reagent.
- All tubes were placed in a water bath at 37°C for 10 minutes.
- UV/Visible spectrophotometer was set up to a wavelength of 540 nanometers after calibrating with the control.
- Three replicates were conducted for each sample, and the quantity of soluble proteins was determined.

2.2.3.3 Estimation of Total Free Amino Acids

The quantity of total free amino acids was determined as described by Lee and Takahashi (1966):

- Ninhydrin solution was prepared by dissolving 0.25 g of ninhydrin in 100 mL of ethyl alcohol.
- Regular Streit's solution was prepared by dissolving 8 g of Na hydroxide in 100 mL of distilled water, and then 10 mL of citric acid was dissolved in 50 mL of Na hydroxide solution and made up to 100 mL with distilled water.
- Chloride reagent was prepared by dissolving 16 mg of it in 10 mL of the regular Streit's solution. Then, 10 mL of ninhydrin solution was added, mixed well, and a red color appeared. Diluent was prepared by mixing equal volumes of distilled water and 95% ethyl alcohol.

Procedure:

- In each test tube, 1 mL of chloride reagent, 0.2 mL of plant extract, and 0.8 mL of distilled water were mixed well, and each tube was covered with marble.
- The tubes were placed in a boiling water bath for 20 minutes.
- The tubes were removed from the water bath, and 5 mL of diluent solution was added to each tube. The tubes were mixed well, and a violet-blue color appeared.
- Readings were taken using a spectrophotometer at a wavelength of 570 nanometers within 15 minutes of the tubes being removed from the water bath. A standard curve was used to estimate the amino acid concentrations.

2.2.3.4 Estimation of Proline Content

The concentration of proline was determined using the method described by Bates et al. (1973):

- Acid ninhydrin solution was prepared by dissolving 1.25 g in 30 mL of glacial acetic acid and 20 mL of phosphoric acid, then heating the mixture to dissolve it.
- A 3% solution of sulfosalicylic acid was prepared by dissolving 3 grams of it in 100 mL of distilled water.

Procedure:

- 0.1 grams of dried seedling powder were ground in 10 mL of sulfosalicylic acid solution. The mixture was then transferred to a centrifuge tube and subjected to centrifugation at 4000 rpm for 15 minutes.
- 2 mL of the supernatant was mixed with 2 mL of acid ninhydrin solution and 2 mL of glacial acetic acid. The mixture was boiled for one hour in a boiling water bath.
- The samples were cooled rapidly in an ice bath.
- To each sample, 4 mL of toluene solvent was added, and the samples were mixed for two minutes.
- The solution was allowed to settle, and readings were taken using a spectrophotometer at a wavelength of 520 nanometers, with toluene as a blank.
- The readings were converted to concentration using a proline standard curve.

2.2.4 DNA Extraction

Total genomic DNA was extracted seeds when they germinated and had coleoptile shoot and or first leaf through the coleoptile following the method described by Doyle et al. (1990) with some modification.

The coleoptile shoots and leaves were lyophilized by freezing drying and then stored at -80°C until use. The tissue samples were ground to a powder by adding liquid Nitrogen. Genomic DNA extraction was performed using 0.1 g bulked tissue obtained from equal weights of freeze-dried leaf samples from individual plants within an accession. The bulked tissues were placed in 2 ml Eppendorf tubes. DNA was isolated using the cetyltrimethylammonium bromide (CTAB) method. The extracted DNA was resuspended in IX TE.

2.2.5 Procedure for DNA Extraction

1- Grind 100 mg of plant tissue to a fine paste in approximately 500 µl of CTAB buffer. Transfer CTAB/plant extract mixture to a microfuge tube.

2- Incubate the CTAB/plant extract mixture for about 15 min at 55 °C in a recirculating water bath.

3- After incubation, spin the CTAB/plant extract mixture at 12000 g for 5 min to spin down cell debris. Transfer the supernatant to clean microfuge tubes.

4- To each tube add 250 µl of Chloroform: Iso Amyl Alcohol (24:1) and mix the solution by inversion. After mixing, spin the tubes at 13000 rpm for 1 min.

5- Transfer the upper aqueous phase only (contains the DNA) to a clean microfuge tube.

6- Invert the tubes slowly several times to precipitate the DNA. Generally, the DNA can be seen to precipitate out of solution. Alternatively, the tubes can be placed for 1 hr at -20 °C after the addition of ethanol to precipitate the DNA. Following precipitation, the DNA can be pipetted off by slowly rotating/spinning a tip in the cold solution. The precipitated DNA sticks to the pipette and is visible as a clear, thick precipitate. To wash the DNA, transfer the precipitate into a microfuge tube containing 500 µl of ice-cold 70 % ethanol and slowly invert the tube. (Alternatively, the precipitate can be isolated by spinning the tube at 13000 rpm for a minute to form a pellet. Remove the supernatant and wash the DNA pellet by adding two changes of ice cold 70 % ethanol).

7-After the wash, spin the DNA into a pellet by centrifuging at 13000 rpm for 1 min. Remove all the supernatant and allow the DNA pellet to dry (approximately 15 min). Do not allow the DNA to over-dry, or it will be hard to re-dissolve.

8-Resuspend the DNA in sterile DNase-free water (approximately 50-400 µl H₂O; the amount of water needed to dissolve the DNA can vary, based on how much is isolated). RNase A (10 µg/ml) can be added to the water before dissolving the DNA to remove any RNA in the preparation (10 µl RNase A in 10 mL H₂O).

9-After resuspension, the DNA is incubated at 65 °C for 20 min to destroy any DNases that may be present and stored at 4°C.

10-Agarose gel electrophoresis of the DNA will show the integrity of the DNA, while spectrophotometry will indicate concentration and cleanliness.

2.2.6 DNA Quantification

To determine the quality and amount of isolated DNA, samples of the isolated DNA 5 µl was run on a 1.0 % agarose gel in TAE buffer (0.04 M Tris-acetate; 1 mM EDTA, pH 8) as outlined by Sambrook et al. (1989).

After staining the gel with ethidium bromide for 15 minutes, a 100-base-pair marker (1 kb MassRuler DNA ladder mix) was used to determine the quantity. DNA quality was determined on a white/UV-transilluminator and photographed.

2.2.7 PCR Amplification of Target Gene

A thermal cycler machine (DNA Engine® (2720) thermal cyclers) and 96-well PCR plates were used to perform polymerase chain reaction (PCR). Under the following conditions: 94°C for 5 min, then Denaturation at 94°C for 1 min, annealing (54°C - 64°C for 1 min based on the sequence of primers) and elongation at 72°C for 1 min, followed by using 35, followed by a final extension at 72°C for 10 min. The PCR products were separated in a 1.2% agarose gel prepared in IX TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.3) with incorporated ethidium bromide.

Amplification products were visualized with a UV light box (254 nm wavelength) and photographed on a digital gel documentation system.

2.2.8 Sequencing of Salt Tolerance Genes (SOS1, RAS, and TAS14) from Wheat

Each amplicon gene obtained from specific PCR was separated in a 1.2 % agarose gel and stained with ethidium bromide for running buffer.

PCR products were purified using the QIAquick PCR Product extraction kit. (Qiagen, Valencia). Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer) was used for the sequence reaction, and then it was purified using a Centrisep spin column. DNA sequences were obtained by Applied Biosystems 3130 genetic analyzer (HITACHI, Japan), and a BLAST® analysis (Altschulet al., 1990) was initially performed to establish sequence identity to GenBank accessions. The phylogenetic tree was created by the MegAlign module of LasergeneDNASar version 12.1 Thompson et al., 1994), and Phylogenetic analyses were done using maximum likelihood, neighbour joining, and maximum parsimony in MEGA11 (Tamura et al., 2021).

2.2.9 Computational Analysis (BLAST) and Construction of Phylogenetic Tree

The salt tolerance gene(s) sequences obtained from different genotypes were compared directly with those in GenBank by BLAST search ([http:// blast.ncbi.nlm.nih. gov/Blast.cgi](http://blast.ncbi.nlm.nih.gov/Blast.cgi)). Translation of nucleotide sequences to amino acid was performed with the ExPASy program. Construction of a phylogenetic tree for the sequences of nucleotides and amino acids was done using the MEGA11 sequence alignment program (Tamura et al., 2021).

2.2.10 Statistical Analysis

The experimental design used was a randomized complete block with three replications. SPSS software v. 25 was used to analyze the variance mediated one-way analysis of variance, at 5% probability (Snedecor and Cochran 1989).

3. RESULTS

3.1 Morphology Analysis

3.1.1 Estimation of Germination Percentage (%)

The ANOVA test showed that germination percentage was significantly influenced by wheat landrace cultivar and osmotic potential ($P \leq 0.05$), with a highly significant interaction between these two factors. The results (Table 3.1 and Figure 3.1) showed that germination percentage varied significantly among the genotypes at different levels of osmotic stress applied. The Duncan test revealed significant differences in the responses of genotypes to varying osmotic potential levels.

Among the landraces studied, TW2, and particularly LW5 and LW7, showed the highest resistance to salinity by maintaining the highest germination percentages across all stress levels. These cultivars exhibited a very high germination rate under severe stress at an osmotic potential of -15 bar. LW5 and LW7 continued to withstand this stress, registering 63% and 78.33% germination, respectively. In contrast, LW8 exhibited poor to very negligible germination at mild to severe osmotic pressures (-1 to -13 bar). Other landraces, such as TW3 and TW4, showed moderate tolerance, with a sharp decline in germination percentage as osmotic potential decreased. Our findings suggest great variation in salinity tolerance among wheat landraces, indicating significant potential for breeding salt-tolerant wheat cultivars, as evidenced by TW2, LW5 and LW7. The statistical results strongly support the agricultural application of these findings.

Table 3.1. The effects of varying osmotic potential levels, induced by different salt stress concentrations, on the germination percentage of wheat landraces

Wheat landraces	Germination percentage (%) at different osmotic potentials						
	0 Bar	-1 Bar	-3 Bar	-5 Bar	-7 Bar	-13 Bar	-15 Bar
TW1	90.00 ^{abcdef} ±8.31	100.00 ^a ±0.00	100.00 ^a ±0.00	80.00 ^{ghijk} ±0.00	79.67 ^{ghijk} ±8.09	80.00 ^{ghijk} ±0.00	41.00 ^{pq} ±10.29
TW2	100.00 ^a ±0.00	100.00 ^a ±0.00	100.00 ^a ±0.00	100.00 ^a ±0.00	99.33 ^a ±2.54	87.67 ^{cdefgh} ±17.56	86.33 ^{efghi} ±29.65
TW3	87.67 ^{cdefgh} ±12.23	89.00 ^{bcdefg} ±3.05	92.67 ^{abcde} ±6.40	71.67 ^{kl} ±14.16	59.33 ^{mn} ±17.01	3.33 ^t ±4.80	0.00 ^t ±0.00
TW4	76.33 ^{jk} ±26.06	83.00 ^{fghij} ±23.95	86.67 ^{defgh} ±30.10	66.33 ^{lm} ±26.72	40.33 ^{pq} ±19.91	26.33 ^{rs} ±11.89	3.00 ^t ±4.66
LW5	99.33 ^a ±2.54	100.00 ^a ±0.00	100.00 ^a ±0.00	99.67 ^a ±1.83	96.33 ^{abcd} ±8.09	77.00 ^{ijk} ±26.02	63.00 ^m ±28.91
LW6	94.33 ^{abcde} ±8.58	97.67 ^{ab} ±6.26	93.33 ^{abcde} ±10.28	87.00 ^{defgh} ±14.42	83.00 ^{fghij} ±23.95	58.67 ^{mn} ±25.96	51.33 ^{no} ±25.02
LW7	98.67 ^{ab} ±4.34	97.33 ^{abc} ±8.28	95.33 ^{abcde} ±14.56	97.67 ^{ab} ±7.28	97.00 ^{abc} ±9.52	81.00 ^{fghijk} ±29.75	78.33 ^{hijk} ±27.80
LW8	48.33 ^{op} ±21.19	54.00 ^{no} ±33.49	46.67 ^{op} ±21.87	34.00 ^{qr} ±22.68	24.00 ^s ±13.80	7.33 ^t ±6.40	2.67 ^t ±6.40

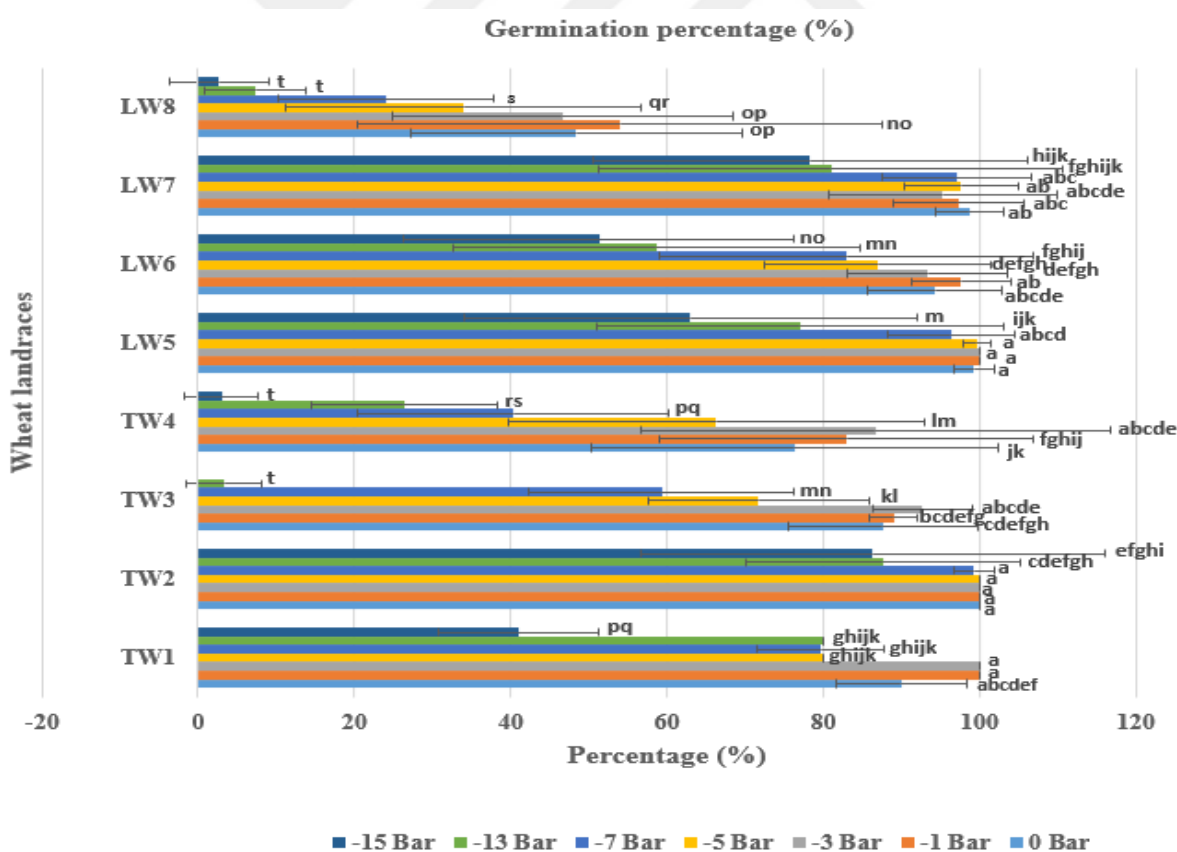


Figure 3.1. Impact of osmotic potentials on germination percentage in wheat landraces

3.1.2 Root Length (cm)

The root length of wheat landrace cultivars under different osmotic potential levels was significantly affected by the species, osmotic potentials, and their interaction, as indicated by the ANOVA analysis ($P \leq 0.05$). The results (**Table 3.2 and Figure 3.2**) reveal notable differences between the landraces and stress levels.

At 0 Bar (control), the longest root length was observed in LW7 (16.63 cm), significantly outperforming the other cultivars. TW2 followed with a root length of 12.69 cm, while LW8 and TW3 exhibited shorter root lengths of 6.49 cm and 5.86 cm, respectively. As osmotic stress increased, the root lengths of all genotypes decreased progressively.

At -1 Bar, LW7 and TW2 maintained relatively higher root lengths, while TW3 and LW5 showed moderate reductions. At -5 Bar, a sharp decline in root length was observed across all cultivars, with values ranging between 3.18 cm (LW7) and 1.10 cm (TW3). Under the most severe osmotic stresses (-13 Bar and -15 Bar), most cultivars exhibited almost negligible root growth. However, LW5 and LW6 displayed some tolerance under these conditions, maintaining minimal root lengths (0.68 cm and 0.54 cm at -13 Bar, respectively). Among the tested wheat landraces, LW7 consistently demonstrated the highest tolerance to osmotic stress, retaining longer roots under all stress levels compared to the other cultivars. Conversely, LW6 and LW5 were the most sensitive, with root lengths declining drastically as stress increased.

Table 3.2. The effects of varying osmotic potential levels, induced by different salt stress concentrations, on the root length of wheat landrace cultivars

Wheat landraces	Root length (cm) at different osmotic potentials						
	0 Bar	-1 Bar	-3 Bar	-5 Bar	-7 Bar	-13 Bar	-15 Bar
TW1	8.02 ^{ef} ±1.80	7.23 ^{fg} ±1.28	4.44 ^{klm} ±1.10	3.68 ^{lmno} ±1.30	1.86 ^{pqrs} ±0.82	-	-
TW2	12.69 ^b ±2.74	12.52 ^b ±1.42	9.47 ^{cd} ±1.26	4.27 ^{klmn} ±1.43	2.51 ^{opqr} ±1.04	-	-
TW3	5.86 ^{hi} ±1.03	5.32 ^{hijk} ±0.80	3.23 ^{mno} ±0.56	1.10 ^{stu} ±0.75	0.78 ^{stu} ±0.51	-	-
TW4	10.48 ^c ±4.46	9.29 ^{cde} ±3.45	6.06 ^{ghi} ±1.17	2.73 ^{opqr} ±1.19	1.01 ^{stu} ±0.94	-	-
LW5	8.28 ^{def} ±1.06	5.53 ^{hijk} ±0.85	4.98 ^{ijkl} ±0.80	3.37 ^{mno} ±0.56	2.68 ^{opqr} ±0.39	0.68 ^{stu} ±0.21	0.28 ^u ±0.15
LW6	8.88 ^{de} ±2.80	5.68 ^{hij} ±1.12	5.18 ^{hijk} ±1.33	2.92 ^{nopqr} ±0.68	2.83 ^{opqr} ±0.81	0.54 ^{stu} ±0.26	0.26 ^u ±0.13

LW7	16.63 ^a ±1.78	9.18 ^{d^e} ±1.48	5.59 ^{hijk} ±0.86	3.18 ^{mnpq} ±0.48	2.97 ^{nopq} ±0.82	0.64 ^{stu} ±0.21	0.41 ^{tu} ±0.12
LW8	6.49 ^{gh} ±3.04	6.44 ^{gh} ±2.56	2.99 ^{nopq} ±0.98	1.72 ^{qrst} ±0.97	1.54 ^{rstu} ±1.03	-	-

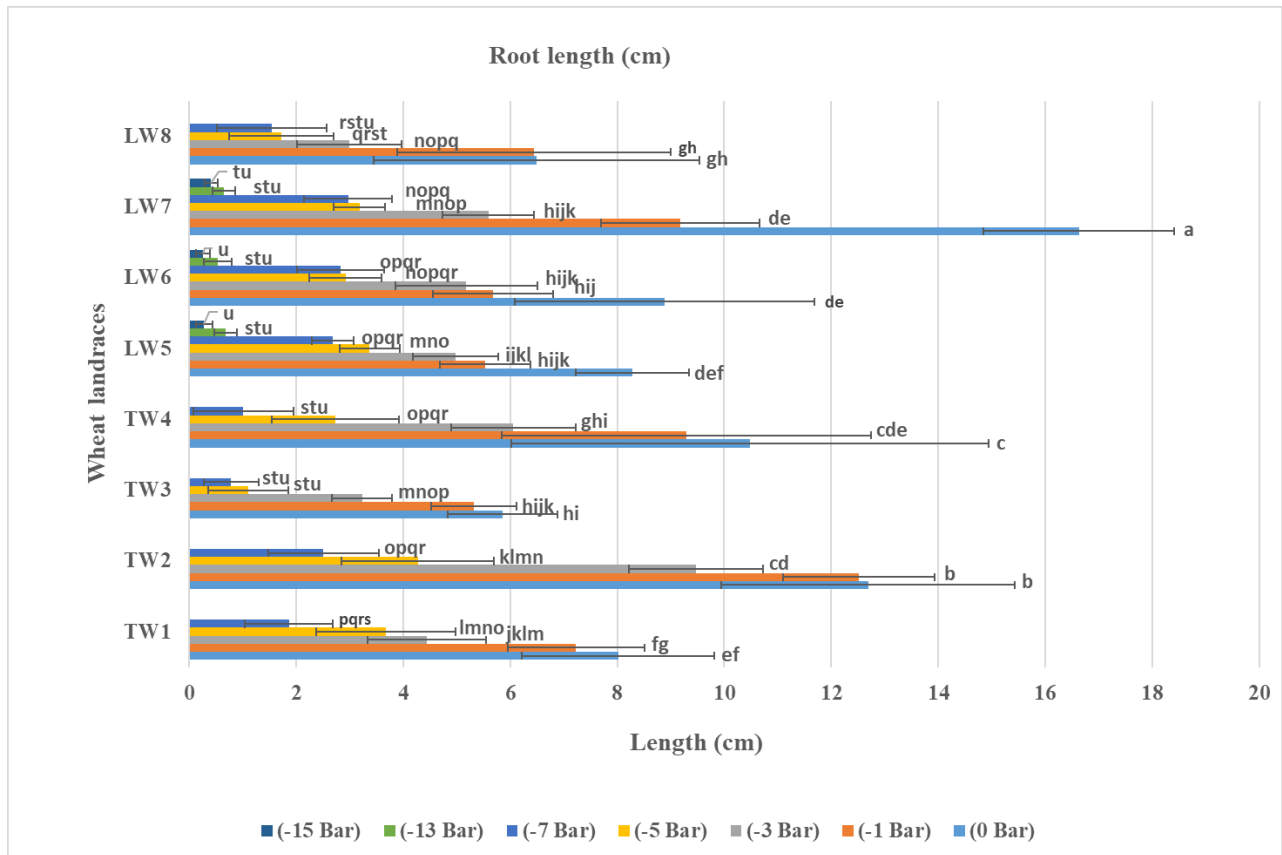


Figure 3.2. Impact of osmotic potentials on root length

3.1.3 Root Numbers (n)

The number of root wheat landrace cultivars under different osmotic potential levels was significantly influenced by the species, osmotic potentials, and their interaction, as demonstrated by the ANOVA analysis ($P \leq 0.05$). The results (Table 3.3 and Figure 3.3) highlight significant variations among the landraces and stress levels. At 0 Bar (control), the highest root numbers were observed in LW8 (5.67 ± 1.16), significantly surpassing the other cultivars. TW2 and LW5 followed with root numbers of 5.42 ± 0.52 and 5.42 ± 0.52 for both, while TW3 and TW4 had lower root numbers at 4.58 ± 0.67 and 4.83 ± 0.39 , respectively. As osmotic stress increased, the root numbers of all cultivars generally decreased. At -1 Bar, LW8 and LW5 maintained relatively higher root numbers, while TW3 and TW4 exhibited moderate reductions. At -5 Bar, a significant decline in root

numbers was observed across all cultivars, with values ranging from 4.09 ± 1.22 (LW8) and 2.83 ± 1.03 (TW3). Under the most severe osmotic stresses (-13 Bar and -15 Bar), most cultivars showed minimal or no root development. However, LW5 and LW6 displayed some resilience under these conditions, maintaining minimal root numbers (3.00 ± 0.00 and 1.83 ± 0.84 at -13 Bar, respectively). Among the tested wheat landraces, LW7, LW5, and LW6 consistently demonstrated the highest tolerance to osmotic stress, retaining higher root numbers across various stress levels compared to the other cultivars. These findings emphasize the importance of selecting stress-tolerant genotypes for cultivation in regions prone to salinity and osmotic stress.

Table 3.3. The effects of different levels of osmotic potential, induced by various salt stress concentrations, on the root numbers of wheat landraces

Wheat landraces	Root numbers (n) at different osmotic potentials						
	0 Bar	-1 Bar	-3 Bar	-5 Bar	-7 Bar	-13 Bar	-15 Bar
TW1	4.75 ^{bcdefgh} ±0.62	4.75 ^{bcdefgh} ±0.62	4.92 ^{bcdef} ±0.29	4.67 ^{cdefgh} ±0.65	3.55 ^{ij} ±0.82	-	-
TW2	5.42 ^{ab} ±0.52	5.08 ^{abcde} ±0.29	4.83 ^{bcdefg} ±0.58	4.33 ^{fgh} ±0.99	3.58 ^{ij} ±0.90	-	-
TW3	4.58 ^{defgh} ±0.67	4.58 ^{defgh} ±0.52	4.42 ^{efgh} ±0.67	2.83 ^{kl} ±1.03	2.33 ^{lm} ±0.89	-	-
TW4	4.83 ^{bcdefg} ±0.39	4.17 ^{ghi} ±0.94	4.17 ^{ghi} ±0.94	3.25 ^{jk} ±0.62	2.67 ^{kl} ±0.49	-	-
LW5	5.42 ^{ab} ±0.52	5.25 ^{abcd} ±0.45	5.42 ^{ab} ±0.52	5.08 ^{abcde} ±0.29	4.67 ^{cdefgh} ±0.49	3.00 ^{jk} ±0.00	1.33 ^{no} ±0.65
LW6	5.33 ^{abc} ±0.49	5.25 ^{abcd} ±0.45	5.00 ^{abcdef} ±0.00	4.33 ^{fgh} ±0.78	4.83 ^{bcdefg} ±0.94	1.83 ^{mn} ±0.84	1.17 ^o ±0.39
LW7	5.17 ^{abcd} ±0.39	5.25 ^{abcd} ±0.62	5.17 ^{abcd} ±0.58	4.75 ^{bcdefgh} ±0.87	5.00 ^{abcdef} ±0.60	3.00 ^{jk} ±0.00	1.83 ^{mn} ±0.72
LW8	5.67 ^a ±1.16	5.33 ^{abc} ±0.99	4.58 ^{defgh} ±1.24	4.09 ^{hi} ±1.22	2.80 ^{kl} ±0.79	-	-

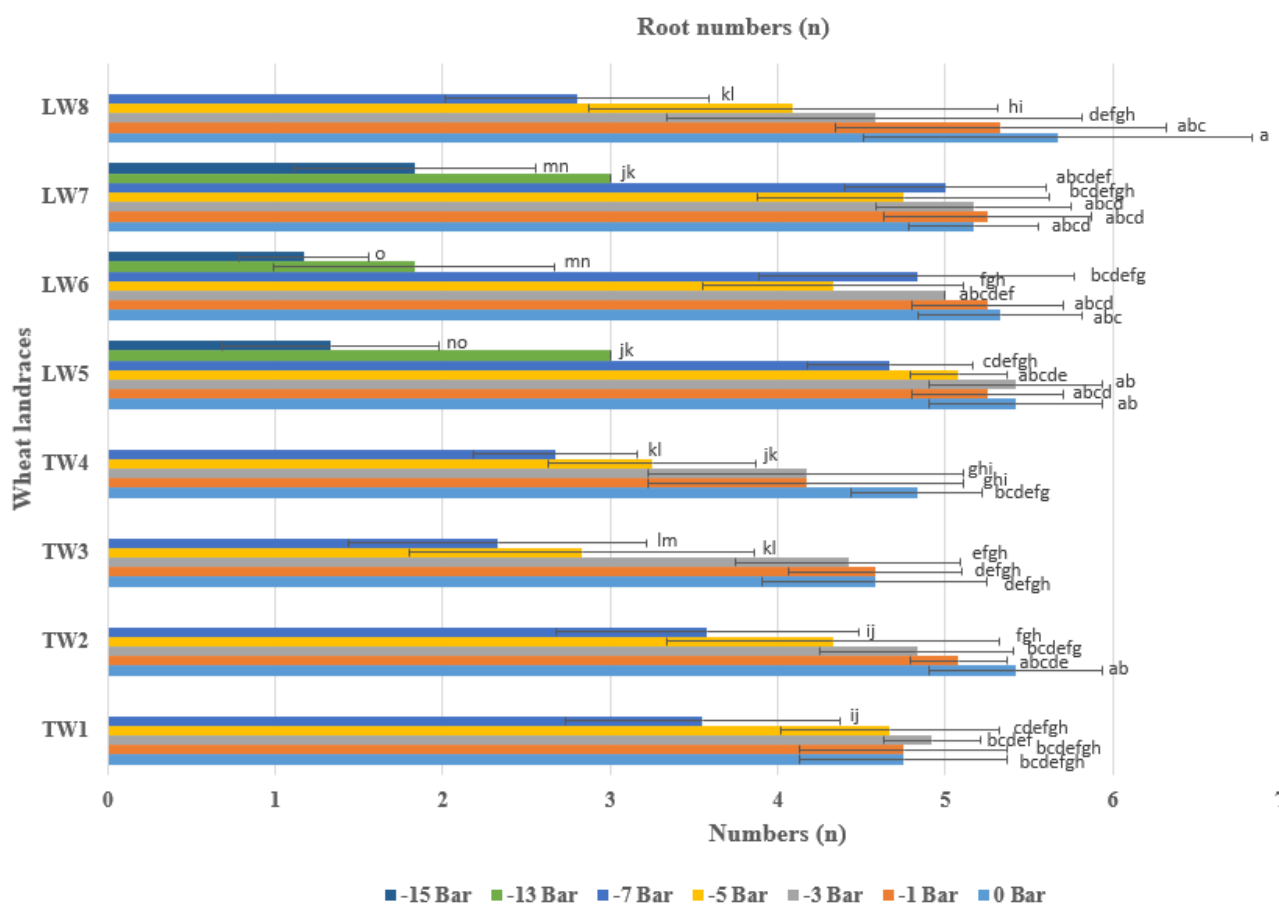


Figure 3.3. Impact of osmotic potentials on root numbers

3.1.4 Coleoptile Length (cm)

The coleoptile length of wheat landrace cultivars under different osmotic potential levels was significantly influenced by the species, osmotic potentials, and their interaction, as demonstrated by the ANOVA analysis ($P \leq 0.05$). The results (**Table 3.4 and Figure 3.4**) highlight significant variations among the landraces and stress levels. At 0 Bar (control), the longest coleoptile length was observed in LW7 (13.00 ± 1.06 cm), significantly outperforming the other cultivars. LW5 and LW6 followed with coleoptile lengths of 12.34 ± 0.59 cm and 11.71 ± 1.41 cm, respectively, while TW3 and LW8 exhibited shorter coleoptile lengths at 5.16 ± 1.08 cm and 7.74 ± 4.60 cm, respectively. As osmotic stress increased, the coleoptile lengths of all cultivars decreased progressively. At -1 Bar, LW7 and LW5 maintained relatively longer coleoptile lengths, while TW3 and TW4 showed moderate reductions. At -5 Bar, a sharp decline in coleoptile length was observed across all cultivars, with values ranging from 4.22 ± 0.52 cm (LW7) and 1.48 ± 0.56 cm (LW8).

Under the most severe osmotic stresses (-13 Bar and -15 Bar), most cultivars exhibited almost negligible coleoptile growth. LW5 and LW6 displayed some tolerance under these conditions, maintaining minimal coleoptile lengths (0.14 ± 0.05 cm and 0.10 ± 0.00 cm at -13 Bar, respectively).

Among the tested wheat landraces, LW7, LW5 and LW6 consistently demonstrated the highest tolerance to osmotic stress, retaining longer coleoptile lengths under all stress levels compared to the other cultivars. In contrast, TW3 and LW8 were the most sensitive, with coleoptile lengths declining drastically under increasing stress. These findings emphasize the importance of selecting stress-tolerant genotypes for cultivation in regions prone to salinity and osmotic stress.

Table 3.4. The effects of different levels of osmotic potential, induced by various salt stress concentrations, on the coleoptile length of wheat landraces

Wheat landraces	Coleoptile length (cm) at different osmotic potentials						
	0 Bar	-1 Bar	-3 Bar	-5 Bar	-7 Bar	-13 Bar	-15 Bar
TW1	8.37 ^d ±1.09	8.10 ^d ±0.85	5.42 ^{fg} ±0.96	3.43 ⁱ ±0.74	1.67 ^k ±0.50	-	-
TW2	10.08 ^c ±1.32	9.78 ^c ±1.14	6.59 ^{ef} ±1.24	2.94 ^{ij} ±0.87	1.02 ^{kl} ±0.59	-	-
TW3	5.16 ^g ±1.08	4.98 ^{gh} ±0.74	3.43 ⁱ ±0.57	2.03 ^{jk} ±1.10	1.33 ^{kl} ±0.74	-	-
TW4	8.45 ^d ±1.89	8.07 ^d ±1.45	4.86 ^{gh} ±1.03	2.05 ^{jk} ±0.84	1.61 ^k ±0.79	-	-
LW5	12.34 ^{ab} ±0.59	10.48 ^c ±1.02	7.63 ^{de} ±0.70	5.15 ^g ±0.59	3.59 ⁱ ±0.46	0.14 ^l ±0.05	-
LW6	11.71 ^b ±1.41	8.30 ^d ±1.60	6.48 ^{ef} ±1.20	3.20 ^{ij} ±0.59	3.28 ^{ij} ±0.55	0.10 ^l ±0.00	-
LW7	13.00 ^a ±1.06	11.84 ^{ab} ±0.77	7.58 ^{de} ±1.26	4.22 ^{ghi} ±0.52	3.88 ^{hi} ±0.70	0.14 ^l ±0.07	-
LW8	7.74 ^{de} ±4.60	7.19 ^{de} ±2.82	3.25 ^{ij} ±1.23	1.48 ^k ±0.56	1.01 ^{kl} ±0.62	-	-

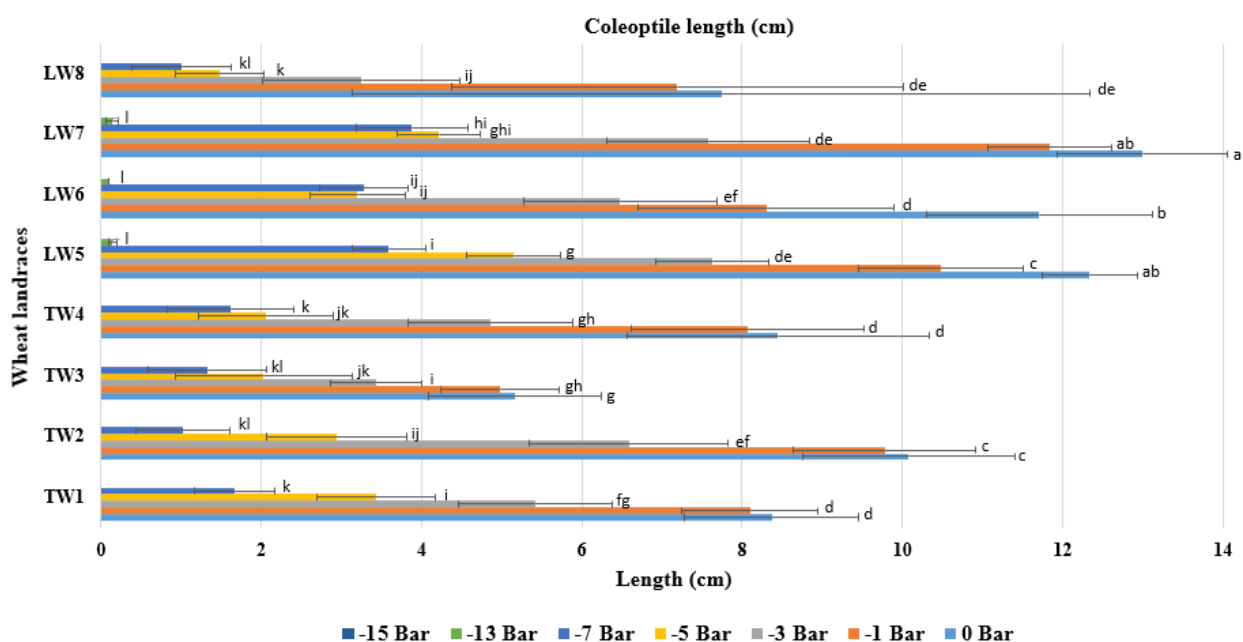


Figure 3.4. Impact of osmotic potentials on coleoptile length

3.1.5 Fresh Weight of Seedlings (g)

The fresh weight of seedlings in wheat landrace cultivars under different osmotic potential levels was significantly influenced by the species, osmotic potentials, and their interaction, as demonstrated by the ANOVA analysis ($P \leq 0.05$). The results (**Table 3.5** and **Figure 3.5**) highlight significant variations among the landraces and stress levels.

At 0 Bar (control), the highest fresh weight was observed in LW5 and LW6 (0.201 ± 0.021 g and 0.201 ± 0.043 g, respectively), significantly outperforming the other cultivars. LW7 and LW8 followed with fresh weights of 0.199 ± 0.031 g and 0.181 ± 0.071 g, respectively, while TW1 exhibited the lowest fresh weight at 0.101 ± 0.016 g. As osmotic stress increased, the fresh weights of all cultivars decreased progressively. At -1 Bar, LW5 and LW8 maintained relatively higher fresh weights, while TW1 and TW3 showed moderate reductions. At -5 Bar, a sharp decline in fresh weight was observed across all genotypes, with values ranging from 0.127 ± 0.021 g (LW5) and 0.076 ± 0.016 g (TW1). Under the most severe osmotic stresses (-13 Bar and -15 Bar), most genotypes exhibited almost negligible fresh weight. However, LW5 and LW6 displayed some tolerance under these conditions, maintaining minimal fresh weights (0.066 ± 0.013 g and 0.058 ± 0.010 g at -13 Bar, respectively).

Among the tested wheat landraces, LW5, LW6 and LW7 consistently demonstrated the highest tolerance to osmotic stress, retaining higher fresh weights under various stress levels compared to the other cultivars. These findings emphasize the importance of selecting stress-tolerant cultivars for cultivation in regions prone to salinity and osmotic stress.

Table 3.5. The effects of different levels of osmotic potential, induced by various salt stress concentrations, on the fresh weight of seedlings of wheat landraces

Wheat landraces	Fresh weight of seedlings (g) at different osmotic potentials						
	0 Bar	-1 Bar	-3 Bar	-5 Bar	-7 Bar	-13 Bar	-15 Bar
TW1	0.101 ^{ijklmnop} ±0.016	0.105 ^{ijklmno} ±0.023	0.084 ^{nopqrs} ±0.016	0.076 ^{pqrstu} ±0.016	0.065 ^{rstuv} ±0.014	-	-
TW2	0.111 ^{ghiklm} ±0.023	0.148 ^{cde} ±0.040	0.127 ^{efghi} ±0.026	0.081 ^{opqrst} ±0.019	0.064 ^{rstuv} ±0.015	-	-
TW3	0.122 ^{fghij} ±0.023	0.130 ^{efgh} ±0.019	0.107 ^{ghijklmn} ±0.020	0.076 ^{pqrstu} ±0.019	0.077 ^{pqrstu} ±0.016	-	-
TW4	0.131 ^{efg} ±0.029	0.147 ^{cde} ±0.047	0.119 ^{fghijk} ±0.029	0.094 ^{lmnopq} ±0.024	0.073 ^{qrstu} ±0.016	-	-
LW5	0.201 ^a ±0.021	0.168 ^{bc} ±0.028	0.159 ^{bcd} ±0.019	0.127 ^{efghi} ±0.021	0.106 ^{hijklmno} ±0.012	0.066 ^{rstuv} ±0.013	0.060 ^{stuv} ±0.006
LW6	0.201 ^a ±0.043	0.158 ^{bcd} ±0.033	0.139 ^{def} ±0.027	0.121 ^{fghij} ±0.019	0.106 ^{hijklmno} ±0.021	0.058 ^{tuv} ±0.010	0.055 ^{uv} ±0.009
LW7	0.199 ^a ±0.031	0.158 ^{bcd} ±0.035	0.099 ^{ijklmnop} ±0.026	0.088 ^{mnoqr} ±0.019	0.088 ^{mnoqr} ±0.021	0.058 ^{tuv} ±0.007	0.045 ^v ±0.009
LW8	0.181 ^{ab} ±0.071	0.169 ^{bc} ±0.051	0.114 ^{fghijkl} ±0.015	0.104 ^{ijklmno} ±0.020	0.096 ^{klmnopq} ±0.014	-	-

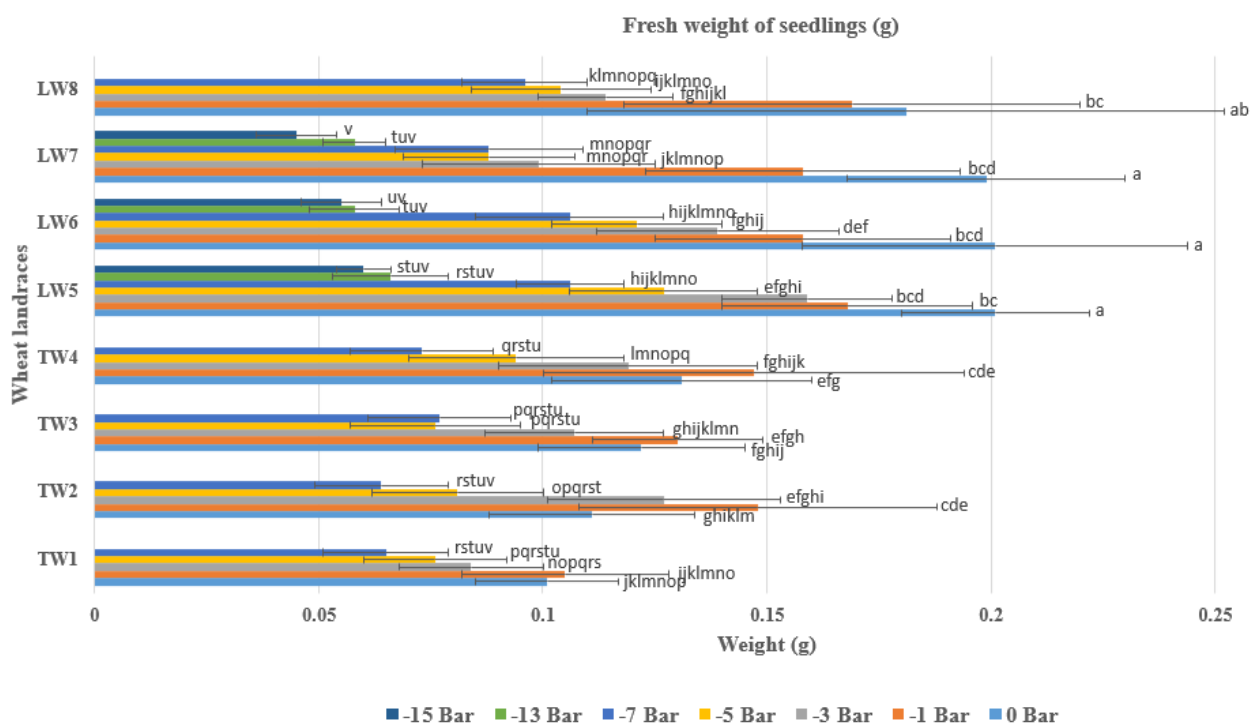


Figure 3.5. Impact of osmotic potentials on fresh weight of seedlings

3.1.6 Dry Weight of Seedlings (g)

The dry weight of seedlings in wheat landrace cultivars under different osmotic potential levels was significantly influenced by the species, osmotic potentials, and their interaction, as demonstrated by the ANOVA analysis ($P \leq 0.05$). The results (**Table 3.6** and **Figure 3.6**) highlight significant variations among the landraces and stress levels. At 0 Bar (control), the highest dry weight was observed in LW8 (0.040 ± 0.008 g), significantly outperforming the other cultivars. LW6 and TW3 followed with dry weights of 0.033 ± 0.005 g and 0.030 ± 0.009 g, respectively, while TW1 and TW2 exhibited lower dry weights at 0.022 ± 0.004 g and 0.019 ± 0.004 g, respectively. As osmotic stress increased, the dry weights of most cultivars showed varying responses. At -1 Bar, LW8 maintained the highest dry weight (0.047 ± 0.011 g), while TW1 and TW4 showed moderate reductions. At -5 Bar, LW8 continued to exhibit the highest dry weight (0.050 ± 0.012 g), whereas TW1 and TW2 displayed lower values (0.026 ± 0.005 g and 0.024 ± 0.006 g, respectively). Under the most severe osmotic stresses (-13 Bar and -15 Bar), most cultivars exhibited no dry weight, except for LW5 and LW6, which maintained minimal dry weights (0.036 ± 0.007 g and 0.042 ± 0.008 g at -13 Bar, respectively). Among the tested wheat landraces, LW6, LW5 and LW7 consistently

demonstrated the highest tolerance to osmotic stress, retaining higher dry weights under various stress levels compared to the other cultivars. These findings emphasize the importance of selecting stress-tolerant cultivars for cultivation in regions prone to salinity and osmotic stress.

Table 3.6. The effects of different levels of osmotic potential, induced by various salt stress concentrations, on the dry weight of seedlings of wheat landraces

Wheat landraces	Dry weight of seedlings (g) at different osmotic potentials						
	0 Bar	-1 Bar	-3 Bar	-5 Bar	-7 Bar	-13 Bar	-15 Bar
TW1	0.022 ^{qrst} ±0.004	0.021 st ±0.006	0.022 ^{rst} ±0.004	0.026 ^{nopqrs} ±0.005	0.026 ^{nopqrs} ±0.005	-	-
TW2	0.019 ^t ±0.004	0.024 ^{opqrst} ±0.007	0.026 ^{nopqrs} ±0.006	0.024 ^{pqrst} ±0.006	0.028 ^{ijklmnopq} ±0.007	-	-
TW3	0.030 ^{hijklmnop} ±0.009	0.036 ^{defghi} ±0.008	0.035 ^{efghij} ±0.006	0.033 ^{fghijklm} ±0.008	0.038 ^{cdef} ±0.009	-	-
TW4	0.021 st ±0.005	0.022 ^{rst} ±0.005	0.024 ^{opqrst} ±0.006	0.029 ^{ijklmnopq} ±0.007	0.025 ^{opqrst} ±0.007	-	-
LW5	0.029 ^{ijklmnop} ±0.004	0.028 ^{klmnopqr} ±0.007	0.032 ^{fghijklmn} ±0.005	0.032 ^{fghijklmn} ±0.005	0.031 ^{ghijklmn} ±0.006	0.036 ^{cdefgh} ±0.007	0.038 ^{cdefg} ±0.002
LW6	0.033 ^{fghijkl} ±0.005	0.031 ^{hijklmno} ±0.008	0.034 ^{efghijk} ±0.007	0.038 ^{cdefg} ±0.009	0.035 ^{defghi} ±0.008	0.042 ^{bc} ±0.008	0.041 ^{cd} ±0.007
LW7	0.022 ^{qrst} ±0.004	0.027 ^{mnpqrs} ±0.005	0.025 ^{opqrst} ±0.006	0.027 ^{lmnopqrs} ±0.006	0.028 ^{lmnopqr} ±0.004	0.032 ^{fghijklmn} ±0.005	0.026 ^{nopqrs} ±0.007
LW8	0.040 ^{cde} ±0.008	0.047 ^{ab} ±0.011	0.047 ^{ab} ±0.006	0.050 ^a ±0.012	0.052 ^a ±0.008	-	-

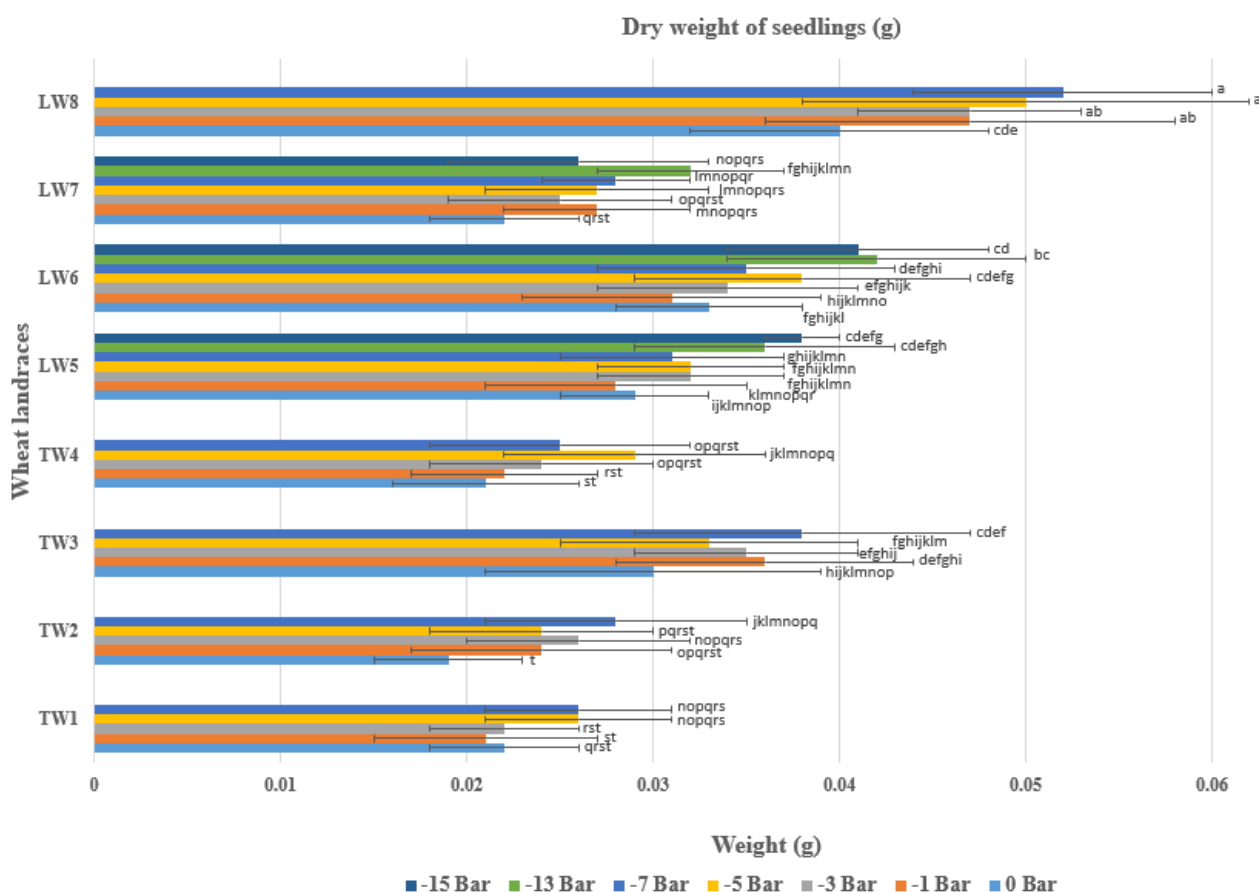


Figure 3.6. Impact of osmotic potentials on dry weight of seedlings

3.1.7 Percentage of Relative Water Content in the Seedlings (%)

The percentage of relative water content in seedlings of wheat landraces under different osmotic potential levels was significantly influenced by the species, osmotic potentials, and their interaction, as demonstrated by the ANOVA analysis ($P \leq 0.05$). The results (**Table 3.7** and **Figure 3.7**) highlight significant variations among the landraces and stress levels. At 0 Bar (control), the highest relative water content was observed in LW7 ($88.73 \pm 1.13\%$), significantly outperforming the other cultivars. LW5 and TW4 followed with relative water content values of $85.42 \pm 1.44\%$ and $83.98 \pm 3.57\%$, respectively, while TW3 and LW8 exhibited lower relative water content at $75.24 \pm 5.49\%$ and $75.73 \pm 8.57\%$, respectively. As osmotic stress increased, the relative water content of all cultivars decreased progressively. At -1 Bar, LW7 and LW5 maintained relatively higher relative water content, while TW3 and LW8 showed moderate reductions. At -5 Bar, a sharp decline in relative water content was observed across all cultivars, with values

ranging between $74.51 \pm 2.24\%$ (LW5) and $52.10 \pm 5.90\%$ (LW8). Under the most severe osmotic stresses (-13 Bar and -15 Bar),

Most genotypes exhibited almost negligible relative water content. However, LW5 and LW7 displayed some tolerance under these conditions, maintaining minimal relative water content ($45.25 \pm 2.16\%$ and $43.79 \pm 7.42\%$ at -13 Bar, respectively). Among the tested wheat landraces, LW7, LW5 and LW6 consistently demonstrated the highest tolerance to osmotic stress, retaining higher relative water content under various stress levels compared to the other genotypes. These findings emphasize the importance of selecting stress-tolerant genotypes for cultivation in regions prone to salinity and osmotic stress.

Table 3.7. The effects of different levels of osmotic potential, induced by various salt stress concentrations, on the percentage of relative water content in the seedlings of wheat landraces

Wheat landraces	Percentage of relative water content in the seedlings (%) at different osmotic potentials						
	0 Bar	-1 Bar	-3 Bar	-5 Bar	-7 Bar	-13 Bar	-15 Bar
TW1	77.97 ^{fghi} ±2.57	79.90 ^{defg} ±3.15	74.09 ^{ijkl} ±4.39	65.05 ^o ±4.67	60.03 ^p ±4.97	-	-
TW2	83.15 ^{bcd} ±2.14	83.59 ^{bcd} ±2.14	79.47 ^{efg} ±2.92	70.88 ^{klmn} ±3.56	55.89 ^q ±4.97	-	-
TW3	75.24 ^{hij} ±5.49	72.91 ^{ijklm} ±4.31	67.53 ^{no} ±2.12	56.11 ^{pq} ±4.78	50.07 ^r ±4.38	-	-
TW4	83.98 ^{bcd} ±3.57	84.60 ^{bc} ±4.15	79.35 ^{efgh} ±2.06	69.32 ^{mno} ±2.86	66.15 ^o ±6.60	-	-
LW5	85.42 ^{ab} ±1.44	83.46 ^{bcd} ±2.52	79.95 ^{defg} ±1.06	74.51 ^{ijkl} ±2.24	70.76 ^{lmn} ±3.43	45.25 ^s ±2.16	36.88 ^t ±3.92
LW6	82.80 ^{bcd} ±3.79	80.54 ^{cdef} ±4.40	75.12 ^{ijk} ±3.63	68.83 ^{mno} ±5.28	67.12 ^{no} ±3.25	27.99 ^u ±3.51	25.23 ^u ±3.27
LW7	88.73 ^a ±1.13	82.91 ^{bcd} ±2.11	74.97 ^{ijkl} ±2.76	68.91 ^{mno} ±6.05	67.71 ^{no} ±5.52	43.79 ^s ±7.42	42.17 ^s ±6.72
LW8	75.73 ^{ghij} ±8.57	69.10 ^{mno} ±13.62	58.40 ^{pq} ±5.24	52.10 ^r ±5.90	45.91 ^s ±6.83	-	-

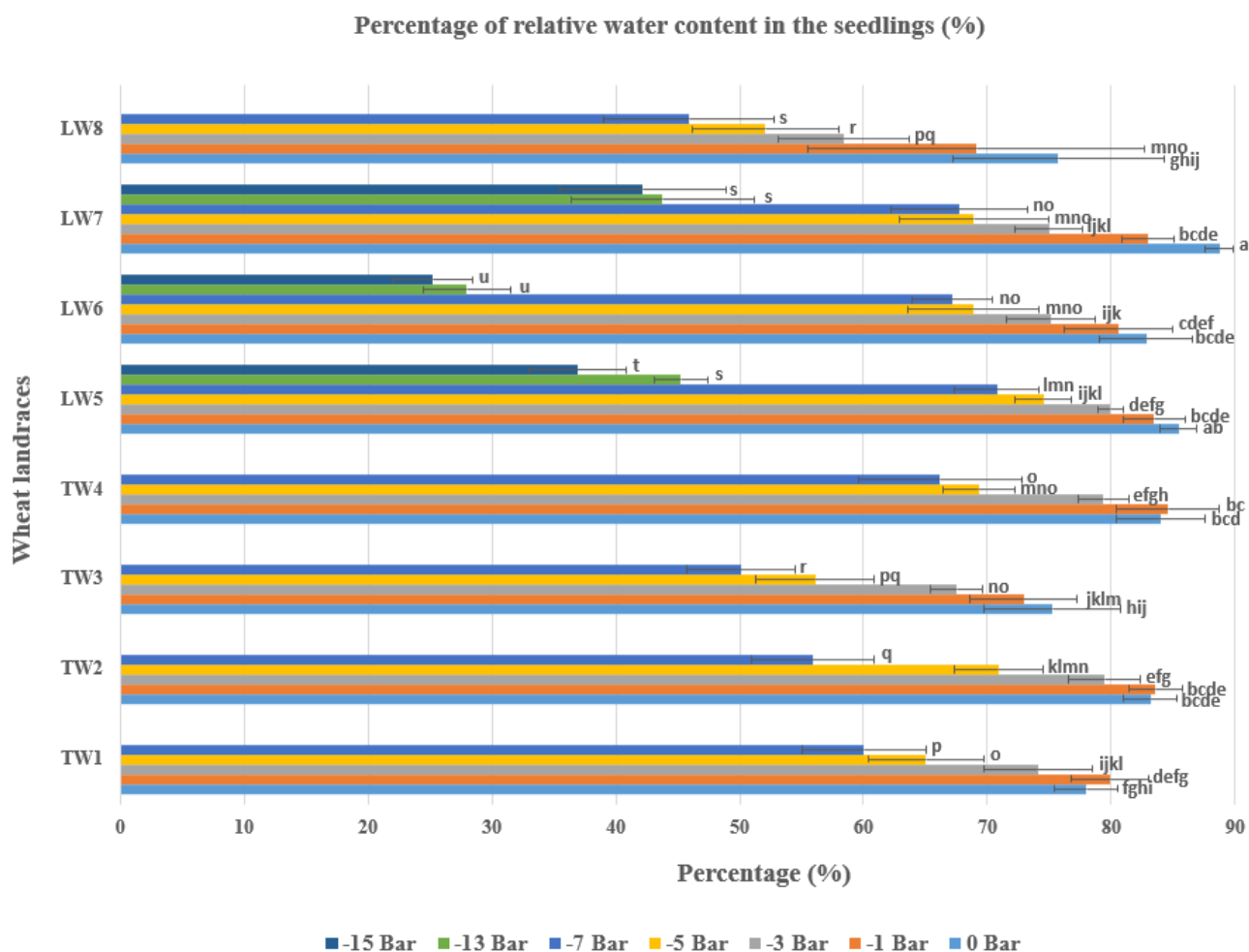


Figure 3.7. Impact of osmotic potentials on percentage of relative water content in the seedlings (%)

3.2 Physiological Analysis

3.2.1 Determination of Chlorophyll a, b and a+b Content in the Seedling Extract

3.2.1.1 Chlorophyll a (mg/g)

The chlorophyll a content in seedlings of wheat landrace cultivars under different osmotic potential levels was significantly influenced by the species, osmotic potentials, and their interaction, as demonstrated by the ANOVA analysis ($P \leq 0.05$). The results (**Table 3.8** and **Figure 3.8**) highlight significant variations among the landraces and stress levels.

At 0 Bar (control), the highest chlorophyll a content was observed in LW8 (29.301 ± 0.096 mg/g), significantly outperforming the other genotypes. LW5 and TW4 followed with chlorophyll a content of 28.324 ± 0.146 mg/g and $22.398 \pm$

1.206 mg/g, respectively, while TW3 exhibited the lowest chlorophyll a content at 5.342 ± 0.012 mg/g. As osmotic stress increased, the chlorophyll a content of all cultivars decreased progressively. At -1 Bar, LW5 and LW8 maintained relatively higher chlorophyll a level, while TW3 and TW1 showed moderate reductions. At -5 Bar, a sharp decline in chlorophyll a content was observed across most landraces, with values ranging from 21.322 ± 0.127 mg/g (LW6) and 2.997 ± 0.130 mg/g (TW4). Under the most severe osmotic stresses (-13 and -15 Bar), most genotypes exhibited almost negligible chlorophyll a content. However, LW5 displayed some tolerance under these conditions, maintaining minimal chlorophyll a content (8.399 ± 0.340 mg/g at -7 Bar).

Among the tested wheat landraces, LW6, LW7 and LW5 consistently demonstrated the highest tolerance to osmotic stress, retaining higher chlorophyll a content under various stress levels compared to the other genotypes. In contrast, TW3 and TW1 were the most sensitive, with chlorophyll a content declining sharply under increasing osmotic stress. These findings emphasize the importance of selecting stress-tolerant genotypes for cultivation in regions prone to salinity and osmotic stress.

Table 3.8. The effects of different levels of osmotic potential, induced by various salt stress concentrations, on the chlorophyll a of wheat landraces

Wheat landraces	Chlorophyll a (mg/g) at different osmotic potentials						
	0 Bar	-1 Bar	-3 Bar	-5 Bar	-7 Bar	-13 Bar	-15 Bar
TW1	13.205 ^k ±0.265	11.718 ^l ±2.533	8.862 ^{mno} ±0.269	4.724 ^q ±0.022	3.127 ^r ±0.008	-	-
TW2	13.194 ^k ±1.668	14.622 ^{ij} ±0.534	9.484 ^m ±0.167	7.184 ^p ±0.179	3.250 ^r ±0.084	-	-
TW3	5.342 ^q ±0.012	4.310 ^q ±0.057	7.912 ^{op} ±0.182	9.894 ^m ±0.270	-	-	-
TW4	22.398 ^{cd} ±1.206	31.771 ^a ±0.437	9.229 ^{mn} ±0.194	2.997 ^r ±0.130	-	-	-
LW5	28.324 ^b ±0.146	28.672 ^b ±0.198	17.591 ^h ±0.514	15.446 ⁱ ±0.222	8.399 ^{no} ±0.340	-	-
LW6	14.353 ^j ±0.115	14.052 ^{jk} ±0.157	15.381 ⁱ ±0.096	21.322 ^e ±0.127	23.171 ^c ±0.283	-	-
LW7	22.072 ^{de} ±0.244	20.035 ^f ±0.103	21.083 ^e ±0.388	19.038 ^g ±0.146	14.205 ^{jk} ±0.103	-	-
LW8	29.301 ^b ±0.096	11.806 ^l ±0.088	4.650 ^a ±0.078	-	-	-	-

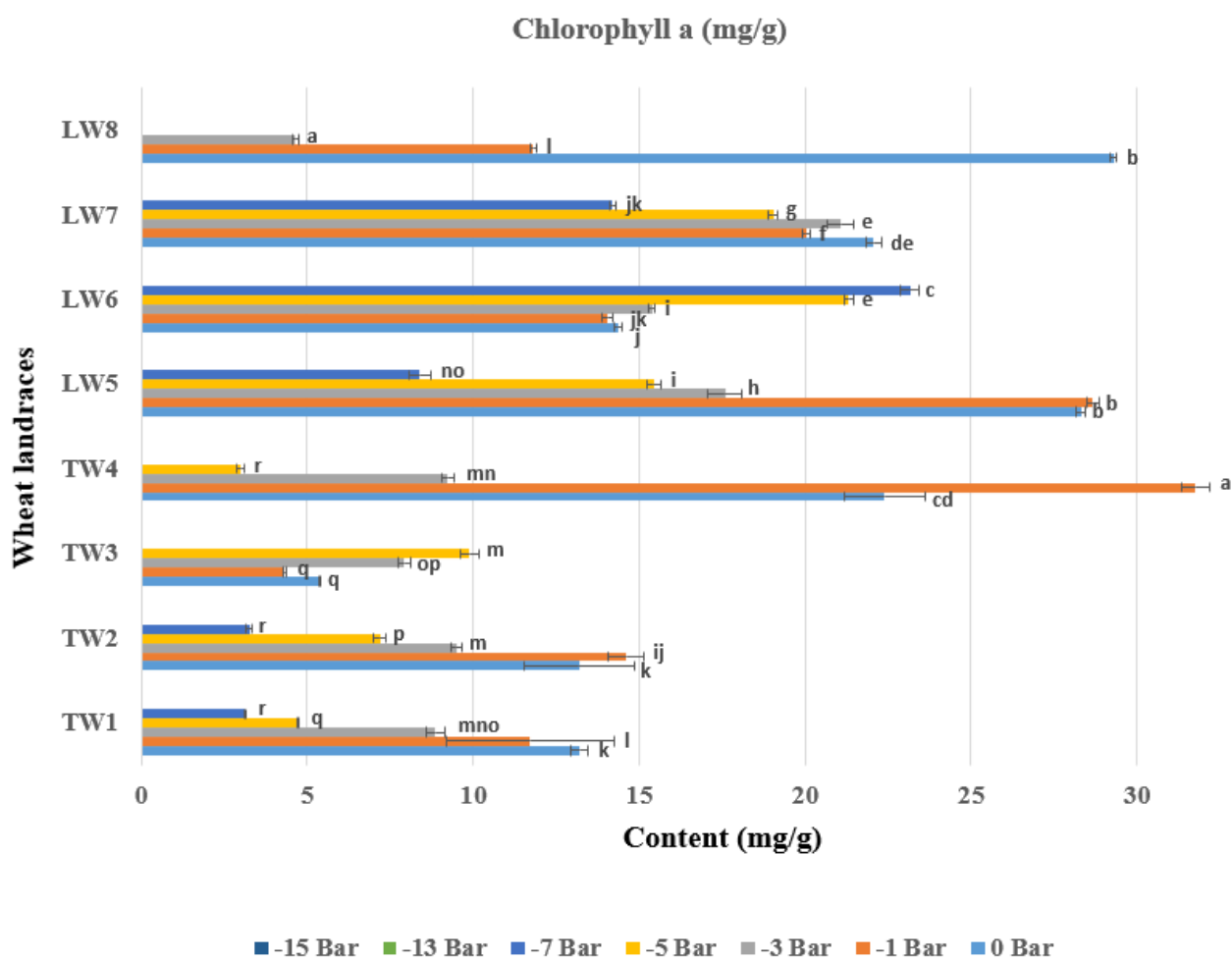


Figure 3.8. Chlorophyll a (mg/g) in somatic potential terms

3.2.1.2 Chlorophyll b (mg/g)

The chlorophyll b content in seedlings of wheat landrace cultivars under different osmotic potential levels was significantly influenced by the species, osmotic potentials, and their interaction, as demonstrated by the ANOVA analysis ($P \leq 0.05$). The results (**Table 3.9 and Figure 3.9**) highlight significant variations among the landraces and stress levels. At 0 Bar (control), the highest chlorophyll b content was observed in TW4 (47.529 ± 5.225 mg/g), significantly outperforming the others. LW7 and LW5 followed with chlorophyll b contents of 18.970 ± 0.506 mg/g and 17.803 ± 0.333 mg/g, respectively, while LW8 exhibited the lowest chlorophyll b content at 4.737 ± 0.298 mg/g. As osmotic stress increased, the chlorophyll b content of most cultivars decreased progressively. At -1 Bar, TW4 maintained the highest chlorophyll b levels (72.180 ± 1.292 mg/g), while LW8 and TW3 showed moderate reductions. At -5 Bar, a sharp decline in chlorophyll b

content was observed across most cultivars, with values ranging from 16.972 ± 0.198 mg/g (LW6) and 4.726 ± 0.327 mg/g (TW4). Under the most severe osmotic stresses (-13 Bar and -15 Bar), most of them exhibited almost negligible chlorophyll b content. However, LW6 displayed some tolerance under these conditions, maintaining minimal chlorophyll b content (17.582 ± 0.136 mg/g at -7 Bar). Among the tested wheat landraces, LW6, LW7 and LW5 consistently demonstrated the highest tolerance to osmotic stress, retaining higher chlorophyll b content under various stress levels compared to the other cultivars. In contrast, LW8 and TW3 were the most sensitive, with chlorophyll b content declining sharply under increasing osmotic stress. These findings emphasize the importance of selecting stress-tolerant landraces for cultivation in regions prone to salinity and osmotic stress.

Table 3.9. The effects of different levels of osmotic potential, induced by various salt stress concentrations, on the chlorophyll b of wheat landraces

Wheat landraces	Chlorophyll b (mg/g) at different osmotic potentials						
	0 Bar	-1 Bar	-3 Bar	-5 Bar	-7 Bar	-13 Bar	-15 Bar
TW1	$10.578^{hij} \pm 0.221$	$14.113^{efg} \pm 9.077$	$7.199^{jklm} \pm 0.373$	$3.698^{nop} \pm 0.078$	$2.579^p \pm 0.088$	-	-
TW2	$9.793^{hij} \pm 0.769$	$11.756^{ghi} \pm 0.691$	$7.726^{jklm} \pm 0.319$	$6.201^{klmn} \pm 0.116$	$3.362^{nop} \pm 0.077$	-	-
TW3	$4.918^{mnop} \pm 0.087$	$2.777^{op} \pm 0.120$	$9.982^{hij} \pm 0.079$	$5.982^{lmno} \pm 0.294$	-	-	-
TW4	$47.529^b \pm 5.225$	$72.180^a \pm 1.292$	$9.547^{hij} \pm 0.675$	$4.726^{mnop} \pm 0.327$	-	-	-
LW5	$17.803^{cd} \pm 0.333$	$15.805^{de} \pm 0.265$	$15.919^{de} \pm 0.309$	$8.420^{ijkl} \pm 0.274$	$9.314^{hijk} \pm 0.194$	-	-
LW6	$12.559^{fgh} \pm 0.100$	$12.482^{fgh} \pm 0.209$	$15.585^{def} \pm 0.047$	$16.972^{cde} \pm 0.198$	$17.582^{cd} \pm 0.136$	-	-
LW7	$18.970^{cd} \pm 0.506$	$16.751^{cde} \pm 0.180$	$19.788^c \pm 0.175$	$17.381^{cde} \pm 0.159$	$12.146^{gh} \pm 0.149$	-	-
LW8	$4.737^{mnop} \pm 0.298$	$10.330^{hij} \pm 0.085$	$4.371^{mnop} \pm 0.121$	-	-	-	-

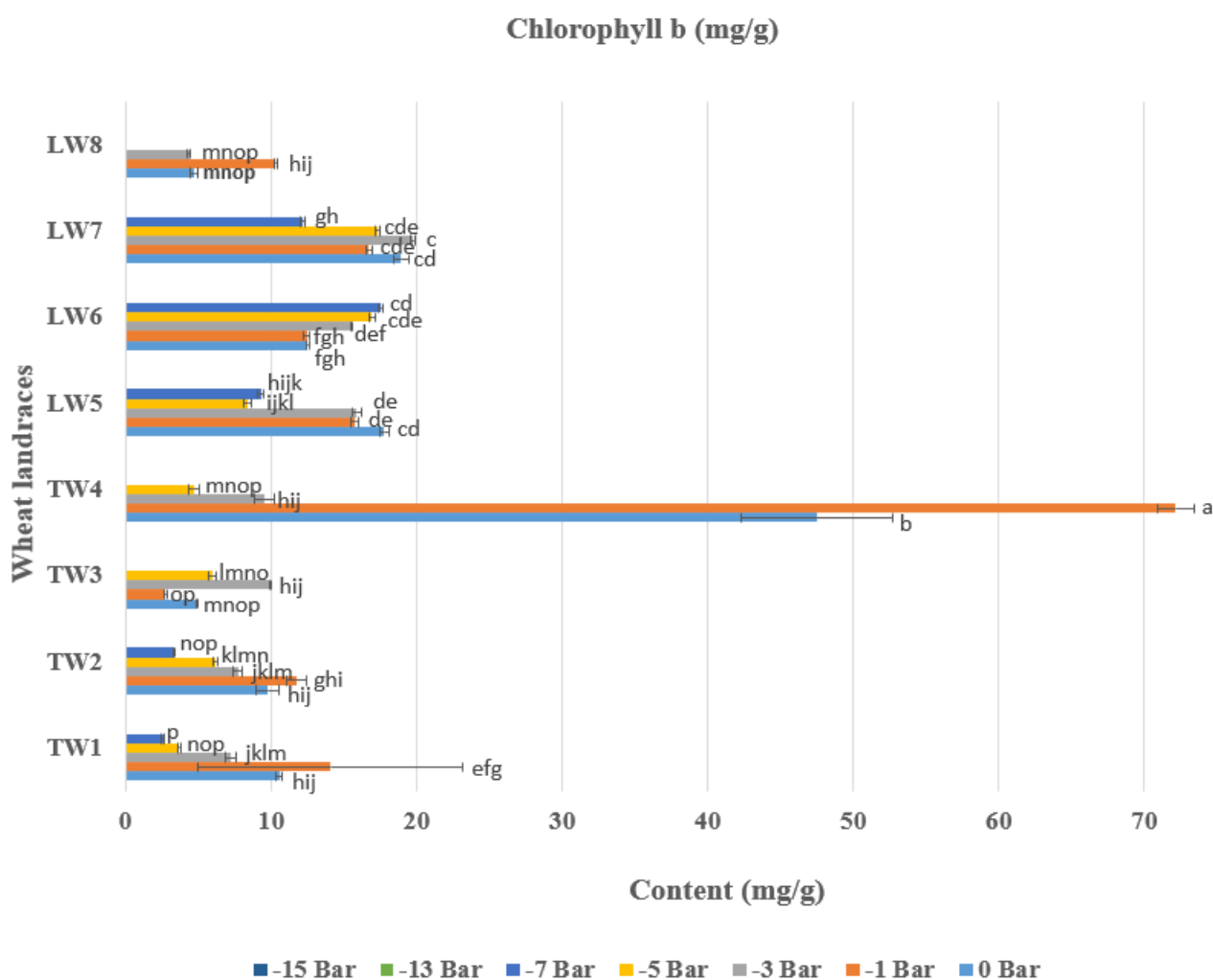


Figure 3.9. Chlorophyll b (mg/g) in wheat seedlings under different osmotic potential levels

3.2.1.3 Chlorophyll a + b (mg/g)

The total chlorophyll content (chlorophyll a + b) in seedlings of wheat landrace cultivars under different osmotic potential levels was significantly influenced by the species, osmotic potentials, and their interaction, as demonstrated by the ANOVA analysis ($P \leq 0.05$). The results (**Table 3.10** and **Figure 3.10**) highlight significant variations among the landraces and stress levels.

At 0 Bar (control), the highest total chlorophyll content was observed in TW4 (69.926 ± 4.034 mg/g), significantly outperforming the other cultivars. LW5 and LW7 followed with total chlorophyll contents of 46.127 ± 0.187 mg/g and 41.042 ± 0.279 mg/g, respectively, while TW3 exhibited the lowest total chlorophyll content at 10.261 ± 0.086 mg/g. As osmotic stress increased, the total chlorophyll content of most cultivars decreased progressively. At -1 Bar, TW4

maintained the highest total chlorophyll levels (103.951 ± 0.871 mg/g), while TW3 and TW1 showed moderate reductions. At -5 Bar, a sharp decline in total chlorophyll content was observed across most cultivars, with values ranging from 38.294 ± 0.079 mg/g (LW6) and 7.723 ± 0.205 mg/g (TW4). Under the most severe osmotic stresses (-13 Bar and -15 Bar), most cultivars exhibited almost negligible total chlorophyll content. However, LW6 displayed some tolerance under these conditions, maintaining minimal total chlorophyll content (40.753 ± 0.386 mg/g at -7 Bar).

Among the tested wheat landraces, LW6, LW7 and LW5 consistently demonstrated the highest tolerance to osmotic stress, retaining higher total chlorophyll content under various stress levels compared to the other cultivars. In contrast, TW3 and TW1 were the most sensitive, with total chlorophyll content declining sharply under increasing osmotic stress. These findings emphasize the importance of selecting stress-tolerant cultivars for cultivation in regions prone to salinity and osmotic stress.

Table 3.10. The effects of different levels of osmotic potential, induced by various salt stress concentrations, on the chlorophyll a + b of wheat landraces

Wheat landraces	Chlorophyll a + b (mg/g) at different osmotic potentials						
	0 Bar	-1 Bar	-3 Bar	-5 Bar	-7 Bar	-13 Bar	-15 Bar
TW1	23.783 ^{ij} ±0.196	25.830 ^{hi} ±6.544	16.061 ^l ±0.193	8.422 ^{nop} ±0.073	5.707 ^q ±0.090	-	-
TW2	22.988 ^j ±0.905	26.378 ^h ±0.222	17.211 ^{kl} ±0.458	13.385 ^m ±0.076	6.612 ^{pq} ±0.009	-	-
TW3	10.261 ⁿ ±0.086	7.087 ^{opq} ±0.082	17.894 ^{kl} ±0.117	15.875 ^l ±0.247	-	-	-
TW4	69.926 ^b ±4.034	103.951 ^a ±0.871	18.776 ^k ±0.481	7.723 ^{opq} ±0.205	-	-	-
LW5	46.127 ^c ±0.187	44.477 ^c ±0.069	33.511 ^f ±0.240	23.866 ^{ij} ±0.138	17.712 ^{kl} ±0.151	-	-
LW6	26.912 ^h ±0.125	26.534 ^h ±0.207	30.966 ^g ±0.144	38.294 ^e ±0.079	40.753 ^d ±0.386	-	-
LW7	41.042 ^d ±0.279	36.786 ^e ±0.158	40.871 ^d ±0.305	36.419 ^e ±0.074	26.350 ^h ±0.092	-	-
LW8	34.037 ^f ±0.270	22.136 ^j ±0.169	9.021 ^{no} ±0.107	-	-	-	-

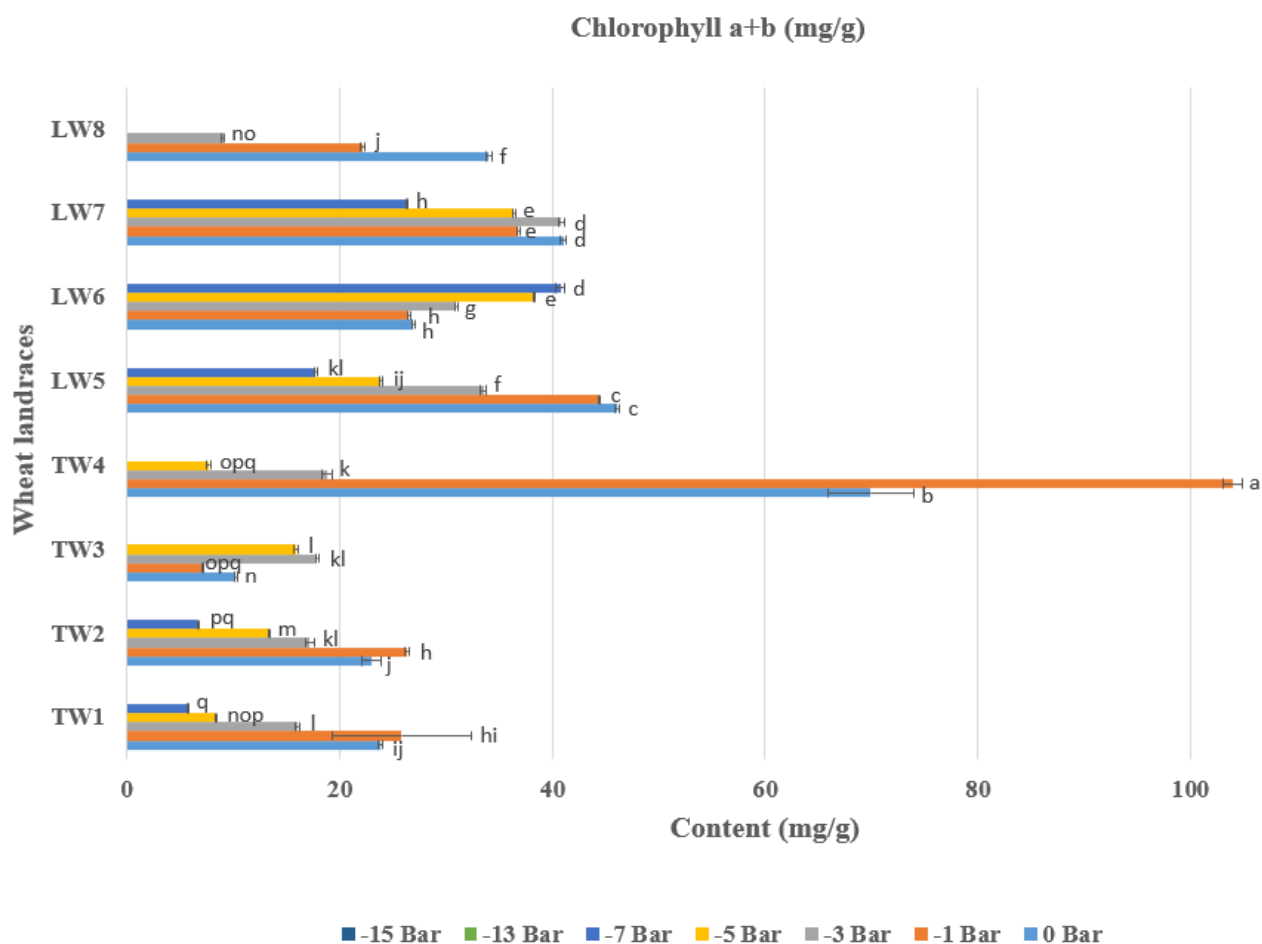


Figure 3.10. Chlorophyll a + b in wheat seedlings under different osmotic potential levels

3.2.2 Determination of the Soluble Sugar Content (mg/g)

The soluble sugar content in seedlings of wheat landrace cultivars under different osmotic potential levels was significantly influenced by the species, osmotic potentials, and their interaction, as demonstrated by the ANOVA analysis ($P \leq 0.05$). The results (**Table 3.11** and **Figure 3.11**) highlight significant variations among the landraces and stress levels. At 0 Bar (control), the highest soluble sugar content was observed in LW5 (1093.506 ± 2.634 mg/g), significantly outperforming the others. TW1 and TW4 followed with soluble sugar contents of 984.310 ± 31.178 mg/g and 648.104 ± 49.670 mg/g, respectively, while LW7 exhibited the lowest soluble sugar content at 72.931 ± 1.035 mg/g. As osmotic stress increased, the soluble sugar content of most cultivars showed varying responses. At -1 Bar, TW1 maintained the highest soluble sugar levels (1176.839 ± 81.934 mg/g), while LW7 and LW8 showed moderate reductions.

At -5 Bar, a sharp decline in soluble sugar content was observed across most cultivars, with values ranging from 1352.126 ± 115.045 mg/g (TW1) and 124.770 ± 0.718 mg/g (LW7). Under the most severe osmotic stresses (-13 Bar and -15 Bar), most cultivars exhibited almost negligible soluble sugar content. However, LW5 displayed some tolerance under these conditions, maintaining minimal soluble sugar content (100.977 ± 4.339 mg/g at -13 Bar). Among the tested wheat landraces, LW7, LW5 and LW6 consistently demonstrated the highest tolerance to osmotic stress, retaining higher soluble sugar content under various stress levels compared to the other cultivars. In contrast, LW7 and LW8 were the most sensitive, with soluble sugar content declining sharply under increasing osmotic stress. These findings emphasize the importance of selecting stress-tolerant cultivars for cultivation in regions prone to salinity and osmotic stress.

Table 3.11. The effects of different levels of osmotic potential, induced by various salt stress concentrations, on the sugar content in the seedling extract of wheat landraces

Wheat landraces	Sugar content (mg/g) at different osmotic potentials						
	0 Bar	-1 Bar	-3 Bar	-5 Bar	-7 Bar	-13 Bar	-15 Bar
TW1	984.310^d ± 31.178	1176.839^b ± 81.934	943.506^d ± 47.542	1352.126^a ± 115.045	780.287^f ± 54.803	-	-
TW2	465.920^i ± 5.267	784.885^f ± 73.763	591.782^h ± 38.949	360.747^{jk} ± 13.392	386.609^j ± 5.267	-	-
TW3	280.172^{lmn} ± 0.690	310.517^{klm} ± 0.690	244.655^{nopq} ± 13.099	385.000^j ± 13.567	868.793^e ± 26.300	-	-
TW4	648.104^g ± 49.670	248.104^{nop} ± 2.603	403.621^j ± 1.035	319.483^{kl} ± 13.180	355.690^{jk} ± 42.732	-	-
LW5	1093.506^c ± 2.634	780.862^f ± 5.172	471.667^i ± 45.300	263.621^{mno} ± 1.724	1147.529^b ± 27.388	100.977^{vw} ± 4.339	74.540^w ± 12.473
LW6	770.517^f ± 18.001	373.966^j ± 18.165	188.333^{qrst} ± 10.103	232.586^{nopqr} ± 1.724	173.966^{stu} ± 10.488	117.644^{uvw} ± 12.110	96.954^{vw} ± 27.388
LW7	72.931^w ± 1.035	87.529^w ± 1.899	89.483^w ± 1.243	124.770^{uvw} ± 0.718	166.609^{stu} ± 1.054	165.690^{stu} ± 0.690	201.667^{pqrst} ± 0.868
LW8	214.770^{opqrs} ± 0.527	194.310^{pqrst} ± 0.345	149.943^{tuv} ± 1.630	207.759^{opqrst} ± 1.503	186.609^{rst} ± 1.211	-	-

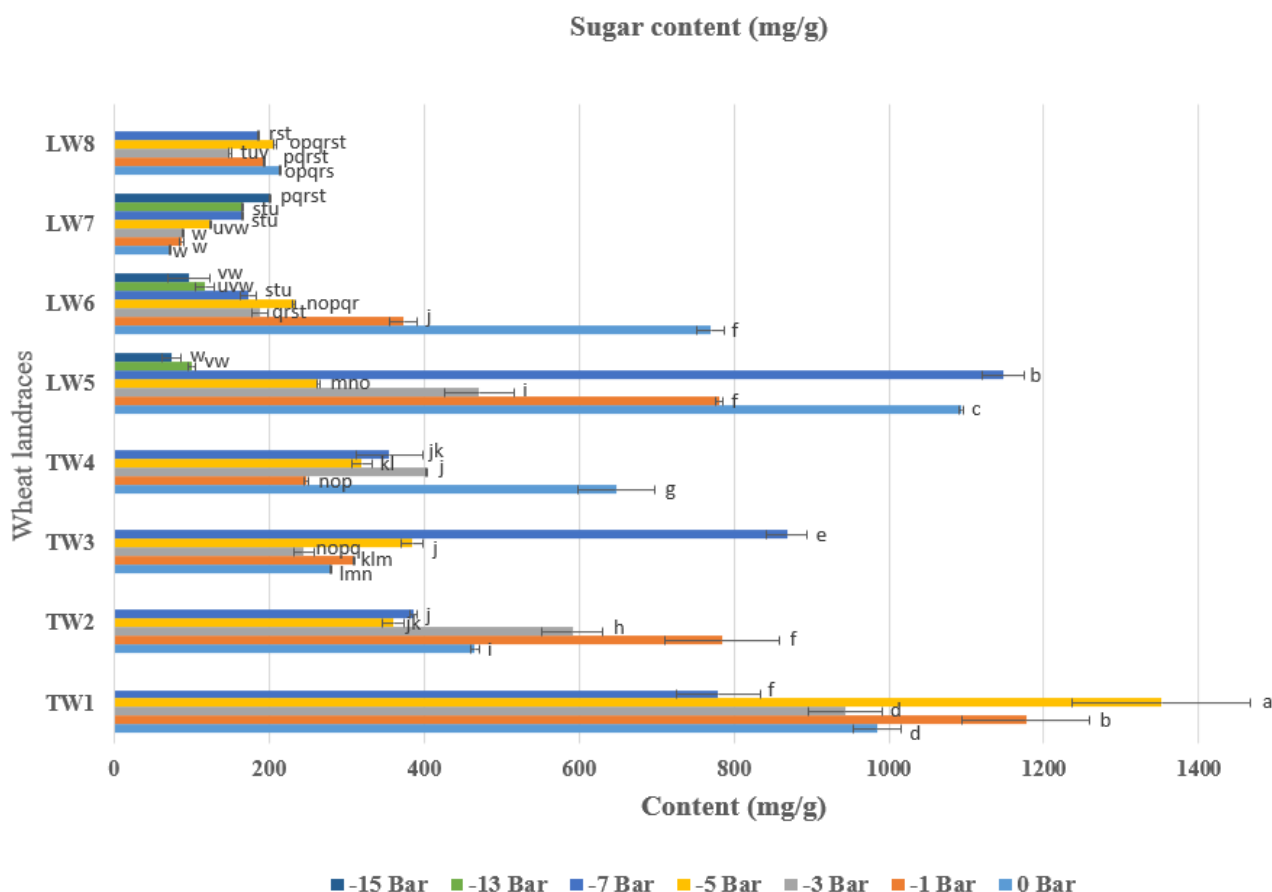


Figure 3.11. Sugar content in wheat seedlings under different osmotic potential levels

3.2.3 Determination of the Soluble Protein Content (mg/g)

Soluble protein content in seedlings of wheat landraces under different osmotic potential levels was significantly influenced by the species, osmotic potentials, and their interaction, as demonstrated by the ANOVA analysis ($P \leq 0.05$). The results (**Table 3.12** and **Figure 3.12**) highlight significant variations among the landraces and stress levels.

At 0 Bar (control), the highest soluble protein content was observed in TW1 (208.909 ± 5.123 mg/g), significantly outperforming the other cultivars. LW8 and LW7 followed with soluble protein contents of 127.242 ± 4.553 mg/g and 130.576 ± 0.694 mg/g, respectively, while TW4 exhibited the lowest soluble protein content at 29.515 ± 0.946 mg/g. As osmotic stress increased, the soluble protein content of most cultivars showed varying responses. At -1 Bar, TW1 maintained relatively high soluble protein levels (163.000 ± 11.418 mg/g), while TW4 and TW3 showed moderate reductions.

At -5 Bar, a sharp increase in soluble protein content was observed in TW1 (222.697 ± 13.130 mg/g), while others like LW6 and TW4 displayed lower values (61.333 ± 2.148 mg/g and 38.909 ± 13.384 mg/g, respectively). Under the most severe osmotic stresses (-13 Bar and -15 Bar), most genotypes exhibited almost negligible soluble protein content. However, LW5 and LW7 displayed some tolerance under these conditions, maintaining minimal soluble protein content (61.333 ± 0.262 mg/g and 124.061 ± 0.694 mg/g at -15 Bar, respectively).

Among the tested wheat landraces, LW7, LW5 and LW6 consistently demonstrated the highest tolerance to osmotic stress, retaining higher soluble protein content under various stress levels compared to the other cultivars. In contrast, TW4 and TW3 were the most sensitive, with soluble protein content declining sharply under increasing osmotic stress. These findings emphasize the importance of selecting stress-tolerant cultivars for cultivation in regions prone to salinity and osmotic stress.

Table 3.12. The effects of different levels of osmotic potential, induced by various salt stress concentrations, on the soluble protein content content in the seedling extract of wheat landraces

Wheat landraces	Soluble protein content (mg/g) at different osmotic potentials						
	0 Bar	-1 Bar	-3 Bar	-5 Bar	-7 Bar	-13 Bar	-15 Bar
TW1	208.909 ^b ±5.123	163.000 ^e ±11.418	166.788 ^{de} ±10.769	222.697 ^a ±13.130	165.424 ^{de} ±4.731	-	-
TW2	126.636 ^{lm} ±4.384	171.636 ^d ±0.455	114.061 ^{op} ±2.777	139.818 ^{hi} ±0.455	142.091 ^{gh} ±1.818	-	-
TW3	46.182 ^y ±0.909	67.242 ^u ±2.922	51.939 ^{xy} ±0.262	134.364 ^{ijk} ±0.909	148.455 ^{fg} ±1.364	-	-
TW4	29.515 ^{aabb} ±0.946	23.152 ^{bb} ±0.694	31.485 ^{aa} ±1.144	38.909 ^z ±13.384	90.879 ^s ±0.946	-	-
LW5	78.000 ^t ±2.531	84.970 st ±1.144	98.000 ^r ±1.981	101.788 ^{qr} ±1.892	58.758 ^{vwx} ±0.694	61.333 ^{uv} ±0.262	114.818 ^{op} ±0.455
LW6	108.455 ^{pq} ±1.639	53.000 ^{wxy} ±0.909	121.030 ^{mno} ±5.007	61.333 ^{uv} ±2.148	59.364 ^{vw} ±1.203	84.818 st ±0.455	35.879 ^{zaa} ±0.694
LW7	130.576 ^{jkl} ±0.694	105.879 ^q ±5.756	117.091 ^{no} ±1.981	116.636 ^{no} ±1.203	187.849 ^c ±1.837	135.424 ^{hij} ±1.144	124.061 ^{lmn} ±0.694
LW8	127.242 ^{klm} ±4.553	117.697 ^{no} ±1.389	135.727 ^{hij} ±0.455	153.303 ^f ±1.144	203.303 ^b ±0.694	-	-

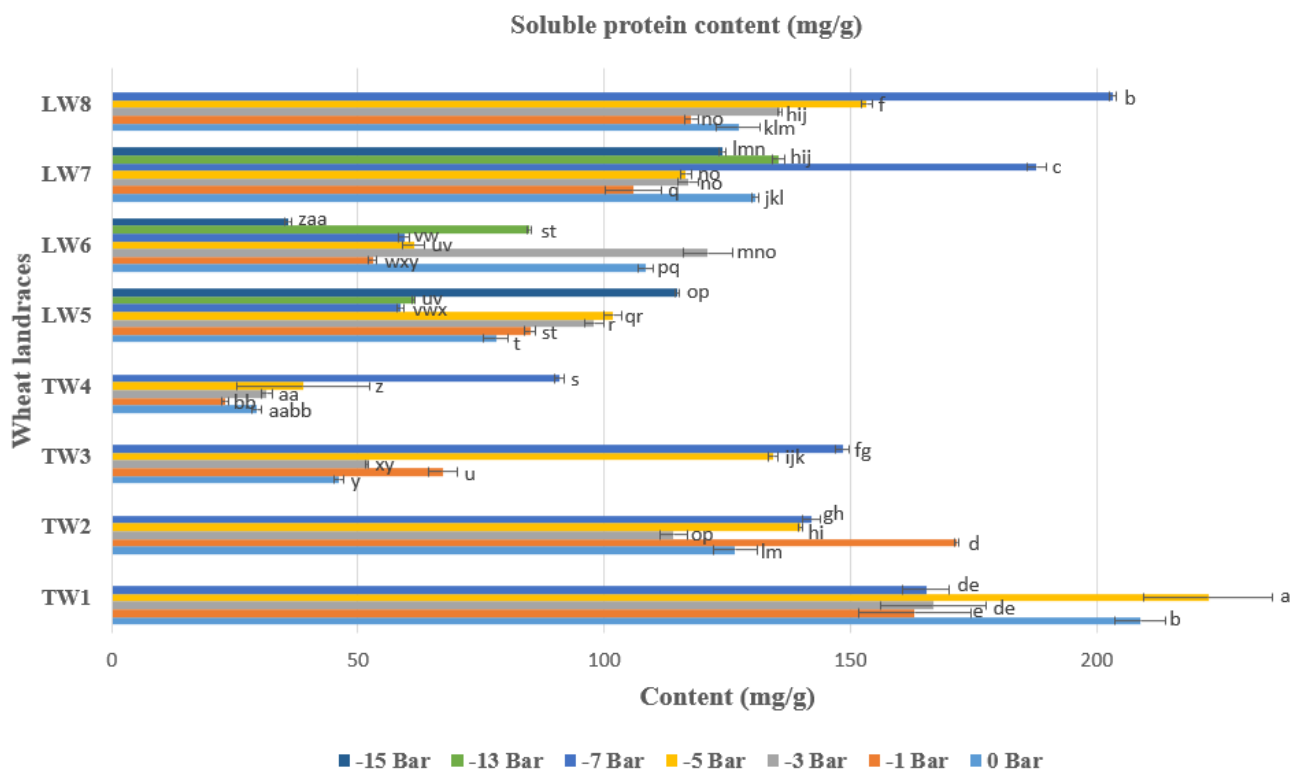


Figure 3.12. Soluble protein content in wheat seedlings under different osmotic potential levels

3.2.4 Determination of the Total Free Amino Acid Content (mg/g)

Amino acid content in seedlings of wheat landraces under different osmotic potential levels was significantly influenced by the species, osmotic potentials, and their interaction, as demonstrated by the ANOVA analysis ($P \leq 0.05$). The results (Table 3.13 and Figure 3.13) highlight significant variations among the landraces and stress levels.

At 0 Bar (control), the highest total free amino acid content was observed in TW1 (327.885 ± 0.722 mg/g), significantly outperforming the other cultivars. LW8 and TW4 followed with total free amino acid contents of 180.386 ± 1.183 mg/g and 187.677 ± 1.183 mg/g, respectively, while LW5 exhibited the lowest total free amino acid content at 113.510 ± 3.083 mg/g. As osmotic stress increased, the total free amino acid content of most cultivars showed varying responses. At -1 Bar, TW1 maintained relatively high total free amino acid levels (376.219 ± 1.654 mg/g), while LW5 and LW6 showed moderate reductions. At -5 Bar, a sharp increase in total free amino acid content was observed in TW1 (515.385 ± 1.573 mg/g), while other cultivars like LW5 and LW6 displayed lower values ($77.156 \pm$

1.362 mg/g and 159.240 ± 0.786 mg/g, respectively). Under the most severe osmotic stresses (-13 Bar and -15 Bar), most cultivars exhibited almost negligible total free amino acid content. However, LW5 and LW7 displayed some tolerance under these conditions, maintaining minimal total free amino acid content (49.031 ± 0.938 mg/g and 108.719 ± 0.625 mg/g at -13 Bar, respectively).

Among the tested wheat landraces, LW7, LW6 and LW5 consistently demonstrated the highest tolerance to osmotic stress, retaining higher total free amino acid content under various stress levels compared to the other cultivars. In contrast, LW5 and LW6 were the most sensitive, with total free amino acid content declining sharply under increasing osmotic stress. These findings emphasize the importance of selecting stress-tolerant cultivars for cultivation in regions prone to salinity and osmotic stress.

Table 3.13. The effects of different levels of osmotic potential, induced by various salt stress concentrations, on the total free amino acid content in the seedling extract of wheat landraces

Wheat landraces	Amino acid content (mg/g) at different osmotic potentials						
	0 Bar	-1 Bar	-3 Bar	-5 Bar	-7 Bar	-13 Bar	-15 Bar
TW1	327.885 ^e 0±.722	376.219 ^d ±1.654	400.594 ^c ±3.903	515.385 ^b ±1.573	548.094 ^a ±6.875	-	-
TW2	87.052 ^{dd} ±3.312	157.573 ^{op} ±1.301	155.385 ^{pq} ±1.183	200.281 ^k ±1.952	252.469 ^g ±1.250	-	-
TW3	111.323 ^{yz} 0±.786	204.031 ^j 0±.827	173.198 ⁿ 0±.477	298.719 ^f ±3.078	149.969 ^r 0±.938	-	-
TW4	187.677 ^l ±1.183	70.177 ^{gg} ±2.195	123.094 ^v 0±.313	200.594 ^k ±1.362	152.677 ^{qr} 0±.651	-	-
LW5	113.510 ^{xy} ±3.083	115.802 ^x ±1.005	111.427 ^{yz} 0±.786	77.156 ^{ee} ±1.362	134.865 ^t ±2.836	49.031 ^{hh} 0±.938	28.302 ^{ji} ±1.263
LW6	133.198 ^t 0±.477	129.344 ^u ±1.127	130.281 ^u ±1.740	159.240 ^o 0±.786	90.385 ^{cc} 0±.786	73.719 ^{ff} 0±.313	40.906 ⁱⁱ 0±.313
LW7	105.906 ^{aa} ±1.127	90.177 ^{cc} 0±.477	95.594 ^{bb} ±1.362	174.552 ⁿ 0±.786	208.823 ⁱ ±1.183	108.719 ^{zaa} 0±.625	90.490 ^{cc} 0±.722
LW8	180.386 ^m ±1.183	234.552 ^h 0±.786	119.552 ^w 0±.477	136.219 ^t ±1.362	139.656 ^s 0±.625	-	-

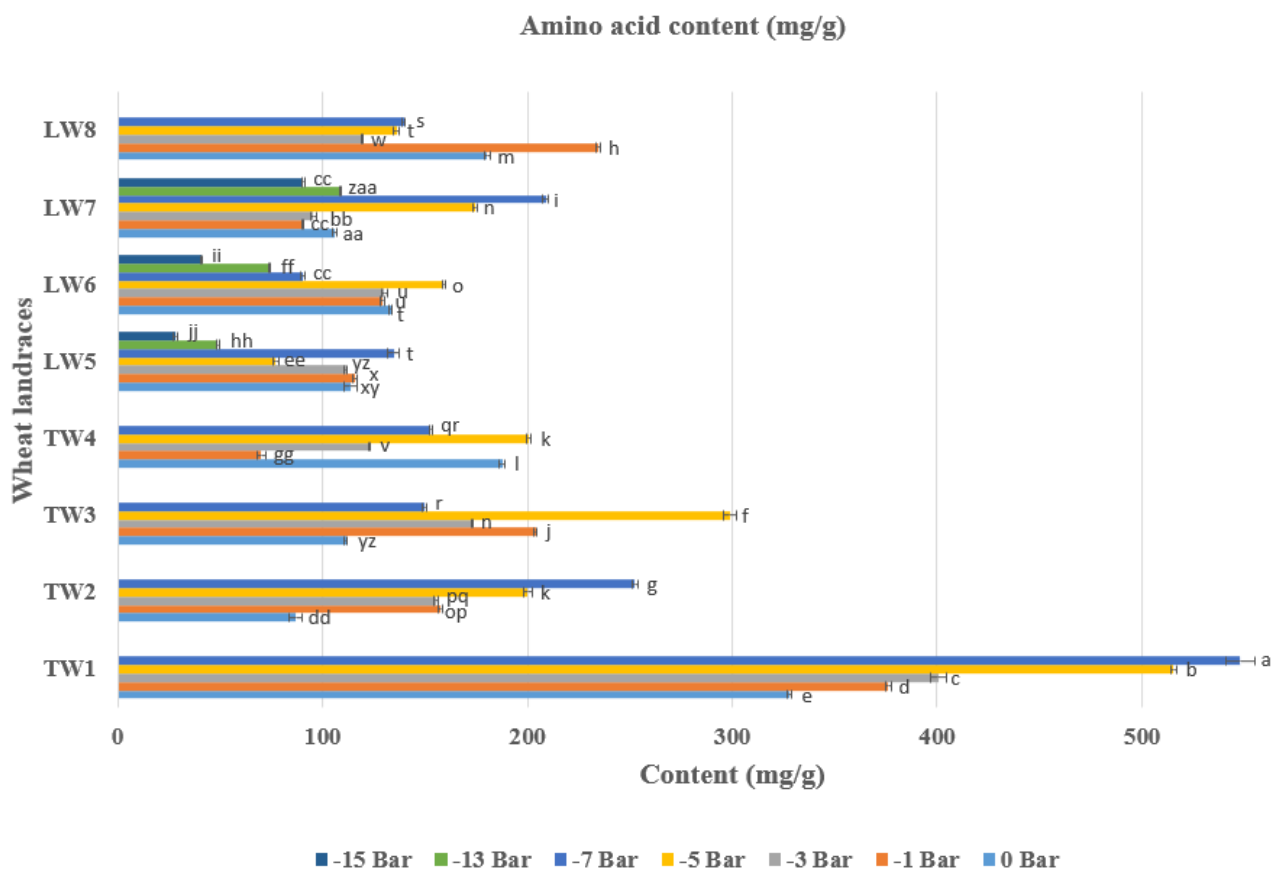


Figure 3.13. Total amino acid content in wheat seedlings under different osmotic potential levels

3.2.5 Determination of Proline Content (mg/g)

The proline content in seedlings of wheat landrace cultivars under different osmotic potential levels was significantly influenced by the species, osmotic potentials, and their interaction, as demonstrated by the ANOVA analysis ($P \leq 0.05$). The results (**Table 3.14** and **Figure 3.14**) highlight significant variations among the landraces and stress levels.

At 0 Bar (control), the highest proline content was observed in TW3 (85.322 ± 5.872 mg/g), significantly outperforming the other cultivars. TW2 and LW5 followed with proline contents of 72.656 ± 7.820 mg/g and 78.211 ± 1.072 mg/g, respectively, while TW1 and TW4 exhibited the lowest proline content at 39.767 ± 1.764 mg/g and 39.878 ± 2.694 mg/g, respectively. As osmotic stress increased, the proline content of most cultivars showed varying responses. At -1 Bar, TW1 maintained the highest proline levels (240.767 ± 7.173 mg/g), while TW2 and TW4 showed moderate increases. At -5 Bar, a sharp increase in proline content was observed in LW5 (254.100 ± 3.512 mg/g), while other cultivars like TW1 and TW2

displayed lower values (85.767 ± 7.024 mg/g and 77.433 ± 4.372 mg/g, respectively). Under the most severe osmotic stresses (-13 Bar and -15 Bar), most cultivars exhibited almost negligible proline content. However, LW6 and LW7 displayed some tolerance under these conditions, maintaining minimal proline content (170.989 ± 4.880 mg/g and 224.767 ± 2.028 mg/g at -13 Bar, respectively).

Among the tested wheat landraces, LW7, LW6 and LW5 consistently demonstrated the highest tolerance to osmotic stress, retaining higher proline content under various stress levels compared to the other cultivars. In contrast, TW1 and TW4 were the most sensitive, with proline content declining sharply under increasing osmotic stress. These findings emphasize the importance of selecting stress-tolerant cultivars for cultivation in regions prone to salinity and osmotic stress.

Table 3.14. The effects of different levels of osmotic potential, induced by various salt stress concentrations, on the determination of proline content in seedling extract of wheat landraces

Wheat landraces	Proline content (mg/g) at different osmotic potentials						
	0 Bar	-1 Bar	-3 Bar	-5 Bar	-7 Bar	-13 Bar	-15 Bar
TW1	39.767 ^y ±1.764	240.767 ^c ±7.173	75.322 ^{uvw} ±2.219	85.767 ^{rstu} ±7.024	68.767 ^w ±2.082	-	-
TW2	72.656 ^{vw} ±7.820	53.878 ^x ±1.836	109.656 ^{pq} ±2.411	77.433 ^{tuvw} ±4.372	119.322 ^{nop} ±4.623	-	-
TW3	85.322 ^{rstu} ±5.872	85.100 ^{rstu} ±4.096	107.544 ^q ±3.168	88.100 ^{rst} ±2.333	121.878 ^{no} ±5.481	-	-
TW4	39.878 ^y ±2.694	165.100 ^k ±8.570	182.211 ^j ±12.725	139.433 ^l ±2.333	124.544 ^{mn} ±3.238	-	-
LW5	78.211 ^{stuvw} ±1.072	86.433 ^{rstu} ±1.856	245.100 ^{bc} ±3.180	254.100 ^b ±3.512	89.767 ^{rs} ±19.221	68.544 ^w ±5.189	81.767 ^{rstuv} ±8.963
LW6	112.656 ^{opq} ±3.949	83.433 ^{rstuv} ±4.667	116.100 ^{nopq} ±3.528	134.100 ^{lm} ±1.856	214.989 ^{de} ±9.628	170.989 ^k ±4.880	181.433 ^j ±4.842
LW7	105.767 ^q ±4.933	115.100 ^{nopq} ±13.077	185.322 ^{ij} ±6.703	194.322 ^{hi} ±3.672	211.767 ^{ef} ±0.882	224.767 ^d ±2.028	202.211 ^{gh} ±6.703
LW8	52.544 ^x ±3.097	92.433 ^r ±9.074	111.322 ^{opq} ±1.072	208.010 ^{ef} ±7.211	282.544 ^a ±6.678	-	-

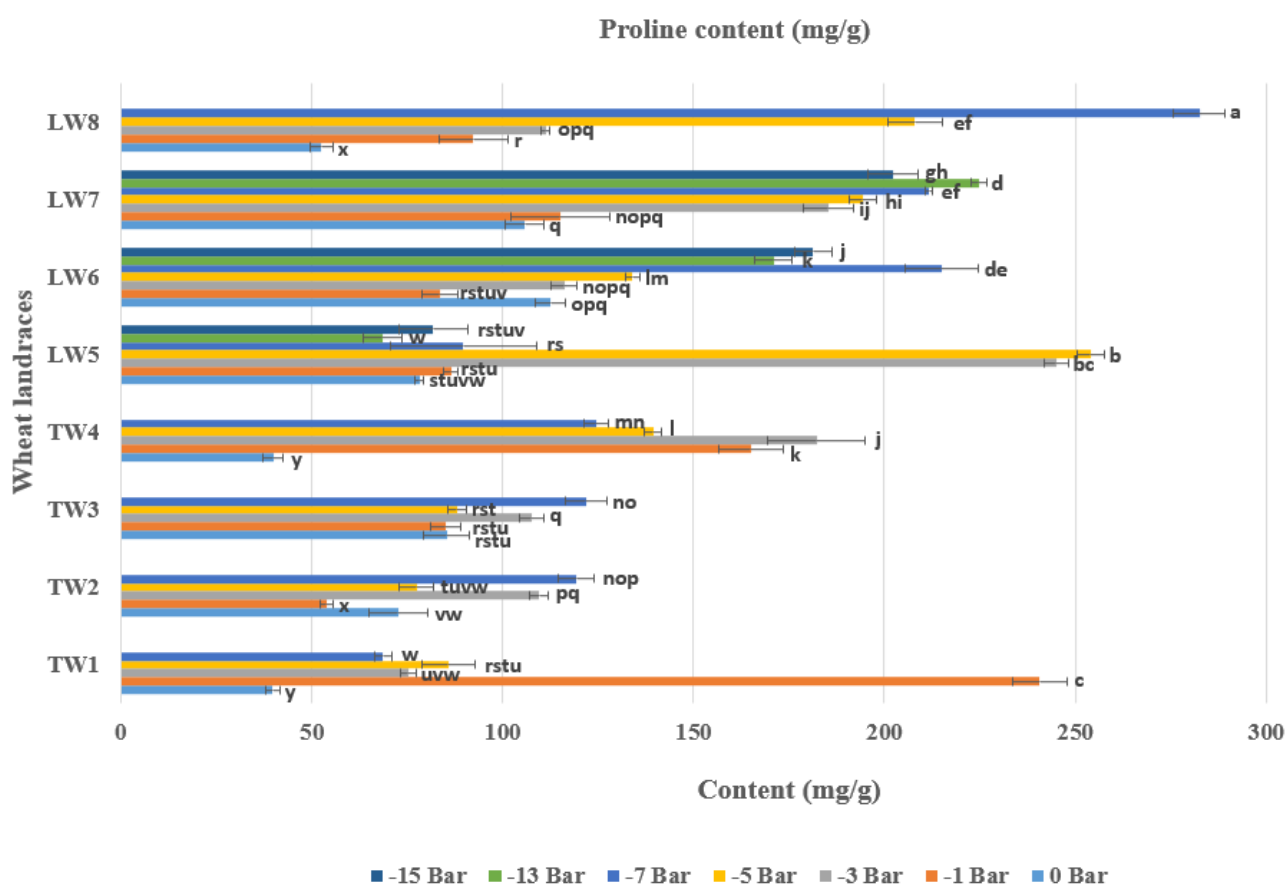


Figure 3.14. Proline content in in wheat seedlings under different osmotic potential levels

3.3 Molecular Analysis

3.3.1 DNA yield (µl) and quality

Enough DNA was extracted using CTAB method of genomic DNA isolation from the eight genotypes and all samples of isolated DNA, 5 µl were run on a 0.8 % agarose gel (Doyle, 1990) (Figure 3.15).

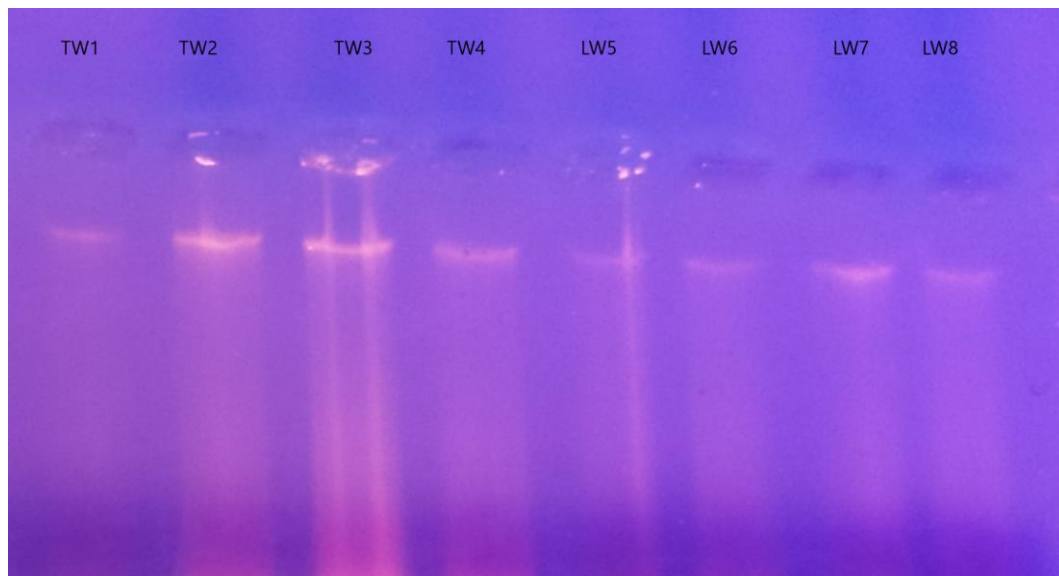


Figure 3.15. DNA isolated from eight wheat landraces was run on a 0.8% agarose gel electrophoresis

3.3.2 PCR Amplification

Only three genes were amplified with PCR, SOS1, RAS1 and TAS14. Seven samples of PCR products were obtained with SOS1 primers (Figure 3.16), four samples with RAS1 primers (Figure 3.17) and three samples with TAS14 primers (Figure 3.18).

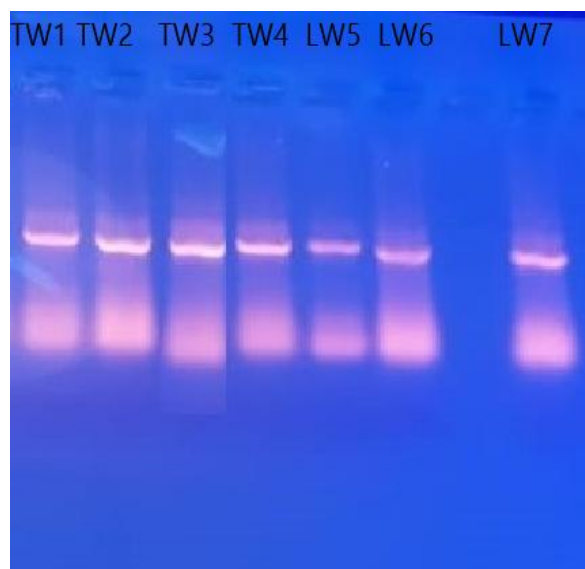


Figure 3.16. PCR product amplification from seven genotypes with SOS1 primers (TW1=710 bp, TW2=592 bp, TW3=1103 bp, TW4=690 bp, LW5=793 bp, LW6=1690 bp, and LW7=708 bp).

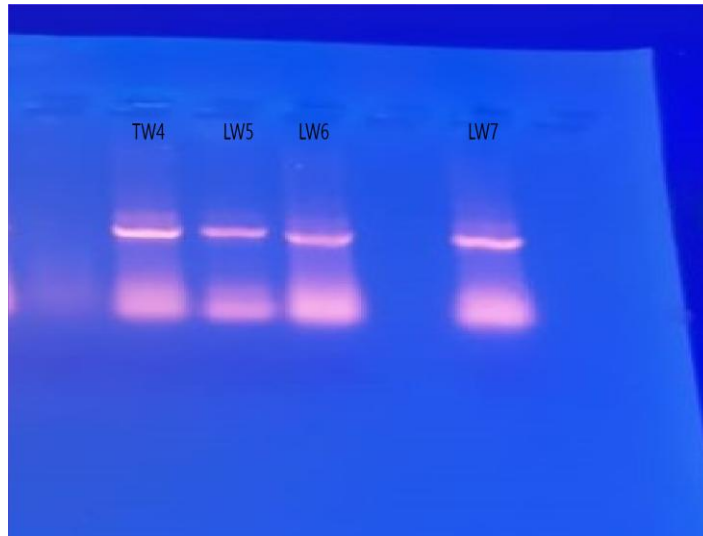


Figure 3.17. PCR product amplification from four genotypes with RAS1 primers (TW4=1575 bp, LW5=1645 bp, LW6=1155 bp, and LW7=1033 bp).

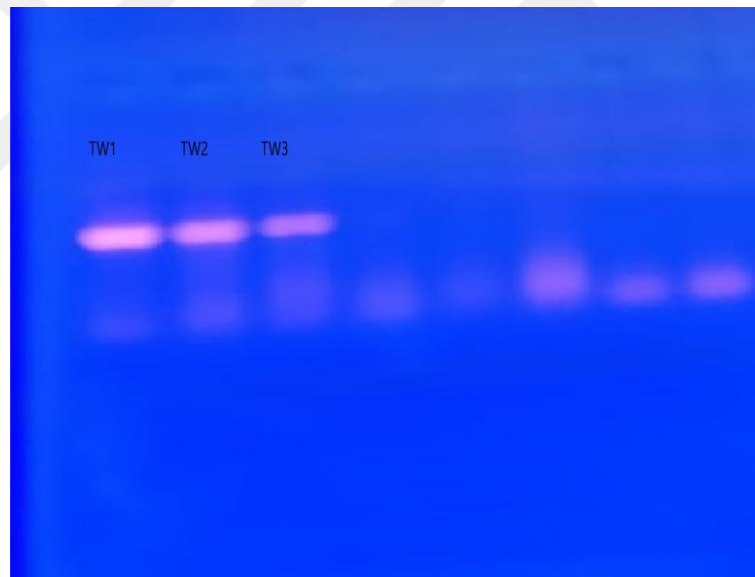


Figure 3.18. PCR product amplification from three genotypes with TAS14 primers (TW1=588 bp, TW2=324 bp, and TW3=568 bp).

3.3.3 Genetic Relationship of Wheat Landraces

The genetic relationship among the genotypes was evaluated based on DNA sequence to confirm the genetic bases of salt tolerance, identify the most tolerant genotypes for the breeding program and determine whether the DNA profile can explain salt tolerance responses.

3.3.3.1 SOS1 gene

SOS1 is essential for the homeostasis of both Na⁺ and K⁺. SOS1 gene expression is concentrated in cells surrounding the xylem, suggesting that SOS1 may function in loading Na⁺ into the xylem for long-distance transport (Shi et al., 2000). The preferential expression of SOS1 at the symplast/xylem boundary would also help explain the K⁺ transport defect of SOS1 mutant plants. It is well known that K⁺ and Na⁺ transport are closely linked at the xylem/symplast interface (Lacan & Durand, 1996). A dendrogram showing the relationship between different sequences related to the SOS1 gene in various wheat genotypes was constructed using the MEGA11 sequence alignment program.

The phylogenetic tree was based on DNA sequences of PCR products obtained from eight genotypes of wheat under study. DNA sequences of SOS1 gene in different genotypes evaluated revealed high variation between sequences. The sequences varied from 592 bp in TW2 to 1690 bp in TW1.

The inclusion of *Hordeum secalinum* in the analysis highlights its genetic relationship with the seven genotypes based on the SOS1 gene sequence. In the phylogenetic tree, *H. secalinum* is placed alongside the wheat genotypes, showing its relative proximity or distance based on sequence similarity. The 65% similarity indicates that the *H. secalinum* SOS1 sequences are relatively distantly related to wheat genotypes. This low similarity suggests that while *H. secalinum* and wheat share a common evolutionary ancestor, they diverged significantly.

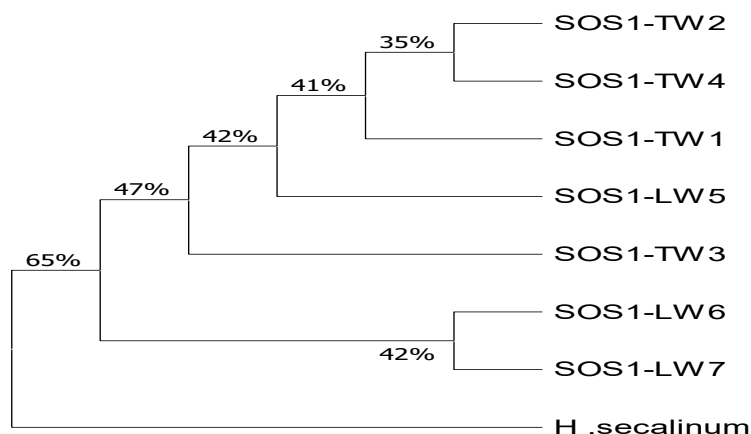


Figure 3.19. Maximum parsimony tree based on SOS1 gene PCR sequences among seven wheat landrace genotypes

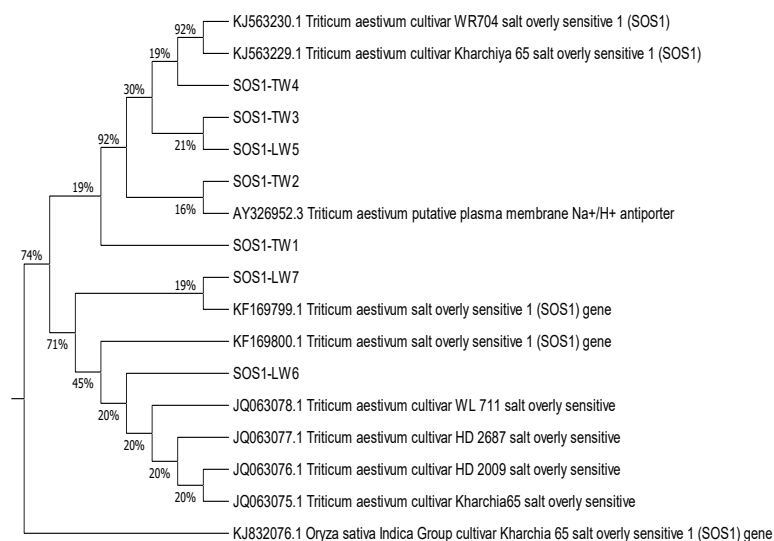


Figure 3.20. Phylogenetic tree constructed using the maximum parsimony method based on SOS1 gene sequences of seven wheat genotypes and reference accessions from NCBI GenBank

The dendrogram shows the relationship among different sequences of SOS1 gene in wheat genotypes and one outgroup, *H. secalinum*. The outgroup (*H. secalinum*) is more distantly related to the other sequences. Figure 3.19 shows two clusters, in the first, *H. secalinum*, while the second cluster includes the sequences of wheat genotypes, and this cluster is divided into different subclusters.

A sequence of TW4 is more related to TW2 and the sequences of LW6 and LW7 are more similar. LW5 and TW3 are more similar to the sequences of SOS1 gene. Another alignment study illustrated in Figure 3.20 included different sequences for SOS1 gene from GenBank to confirm the existence of SOS1 gene controlling salt tolerance in these genotypes.

Rice (*KJ832076.1*) is positioned at the farthest branch in the dendrogram, serving as an outgroup. This supports its evolutionary distinction from the wheat SOS1 gene sequences, consistent with the fact that rice and wheat belong to different genera. The SOS1 sequences in the tested genotypes show varying degrees of similarity with the reference sequences. Similarities or differences help identify specific genotypes' evolutionary adaptations and potential functional divergences in response to environmental stresses.

These results assisted in wheat breeding program for salt tolerance and helped to correct the selection of good parents. SOS1 is essential for regulating Na⁺ efflux at the cellular level and enhancing Na⁺ transportation in the organelles and tissues. salt overly sensitive (SOS) engaged Na⁺ /H⁺ antiporters (Yousefirad *et al.*, 2018; Wu *et al.*, 2019). Amino acid sequence alignment of TW2 showed similarity with the GPD gene and illustrated conserved domain hits.

Zhou *et al.*, (2022) reported the function of the SOS pathway and its association with the natural variation of salt tolerance in maize. Taken together, this study demonstrated that the maize SOS pathway confers a conservative salt-tolerant role, and the components of SOS pathway confer the natural variations of Na⁺ regulation and salt tolerance in maize, therefore introducing important gene targets for breeding salt-tolerant crops. Ali *et al.*, (2023) found that overexpression of SOS1 reduces Na⁺ accumulation in transgenic Arabidopsis, resulting in enhanced salt tolerance.

3.3.3.2 RAS1 gene

Positional cloning of the Response to ABA and Salt 1 (RAS1) quantitative trait locus (QTL) identified a previously uncharacterized gene that is induced by ABA and salt stress. Genetic and molecular analyses demonstrate that RAS1 acts as a negative regulator of salt tolerance and plays a key role in modulating ABA sensitivity (Ren *et al.*, 2010).

The response to ABA and Salt 1 (RAS1) genes acts as a protection mechanism against high salinity and water deficit in several plant species. Identifying gene sequences involved in plant tolerance to abiotic stresses is vital for optimizing molecular tools targeted at genetic selection and the improvement of salt-tolerant varieties.

In this study, we obtained four genotypes represented by bands with RAS1 primers after the PCR reaction. The sequence analysis using MEGA11 demonstrated that the dendrogram contains two clusters: one with LW6 and TW4, and the other with LW5 and LW7 (Figure 3.21). The maximum parsimony tree for the RAS1 gene shows relationships between four genotypes and a related sequence (*Hordeum jubatum*). *H. jubatum* was included to assess its genetic relationship with the wheat genotypes concerning the RAS1 gene, which is associated with stress responses and abscisic acid signaling. The tree places *H. jubatum* in a comparative framework, highlighting how its RAS1 sequences relate to wheat.

The dendrogram shows the relationship among four sequences of the RAS1 gene in wheat genotypes and one outgroup, *H. jubatum*. The outgroup (*H. jubatum*) is more distantly related to the other sequences. The genotypes (LW6, TW4, LW5, LW7) are clustered based on genetic similarity. The four genotypes were analyzed with different accessions of wheat in NCBI to study the relationship between them. The results indicate that LW6 and TW4 represented more homologous sequences and were related to RAS1 gene sequences in NCBI (Figure 3.22).

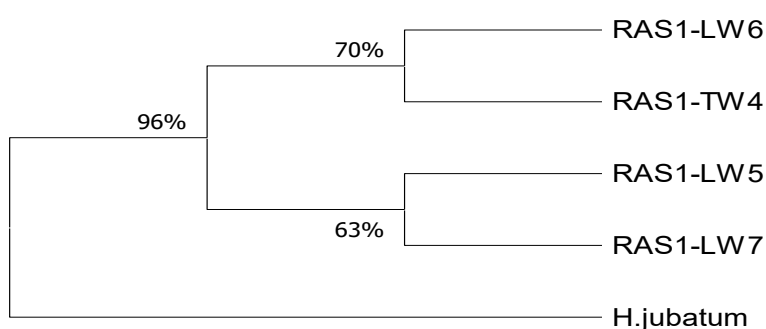


Figure 3.21. Maximum parsimony tree based on PCR-amplified RAS1 gene sequences from seven genotypes

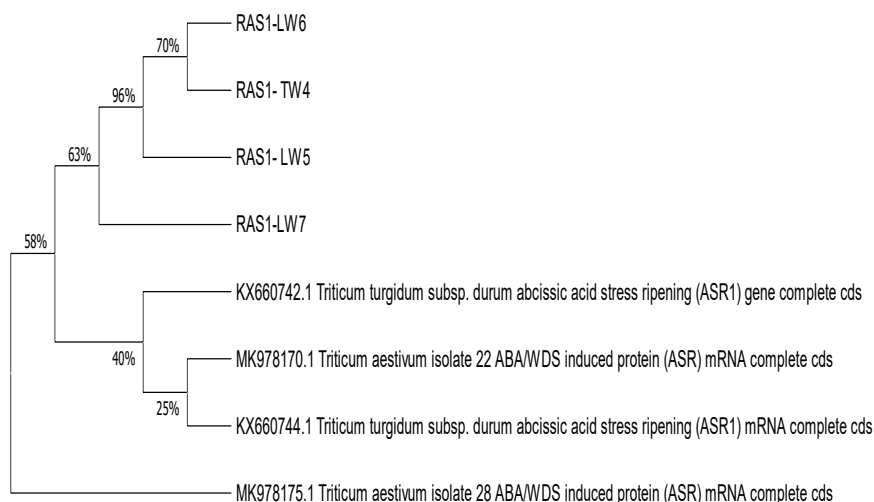


Figure 3.22. Phylogenetic tree constructed using the maximum parsimony method based on RAS1 gene sequences of four wheat genotypes and selected accessions from NCBI GenBank

3.3.3.3 TAS14 gene

TAS14 belongs to the Dehydrins (DHN) protein family, which was first isolated and characterized in tomato (Godoy et al. 1990). Osmotic stress and ABA treatment could induce the expression of TAS14 in tomato seedlings and adult plants (Godoy et al. 1990). Overexpression of TAS14 conferred transgenic plants long-term drought tolerance without affecting plant growth (Muñoz-Mayor et al. 2012). The functions and regulatory mechanisms of DHN proteins in tobacco response to drought stress are largely unknown (Hu et al., 2024).

In this study, 3 salt stress-related genes were screened from TW1, TW2 and TW3 genotypes of wheat. Only three amplicons for three genotypes were amplified with TAS14 primers.

3.3.3.4 Relationship between SOS1, RAS1 and TAS14 Sequences

To study the relationship between sequences obtained from different primers of SOS1, RAS1, and TAS14, we used MEGA11 to construct the phylogenetic trees and the result is represented in the dendrogram (Figure 3.23).

The maximum parsimony tree of 14 sequences, based on PCR products, separated the sequences into two major groups, TAS14-TW3, RAS1-LW5 in the first cluster and the rest of the sequences in the second cluster. SOS1 (LW5, LW6, LW7, TW3 and TW1) and TAS14-TW2, RAS1-LW7 sequences in the sub-cluster and the rest of the sequences in another sub-cluster, which is divided into different sub sub cluster subcluster, TAS14-TW1 in the sub-cluster, RAS1 (TW4 and TW6) and SOS1-TW2 in the subcluster.

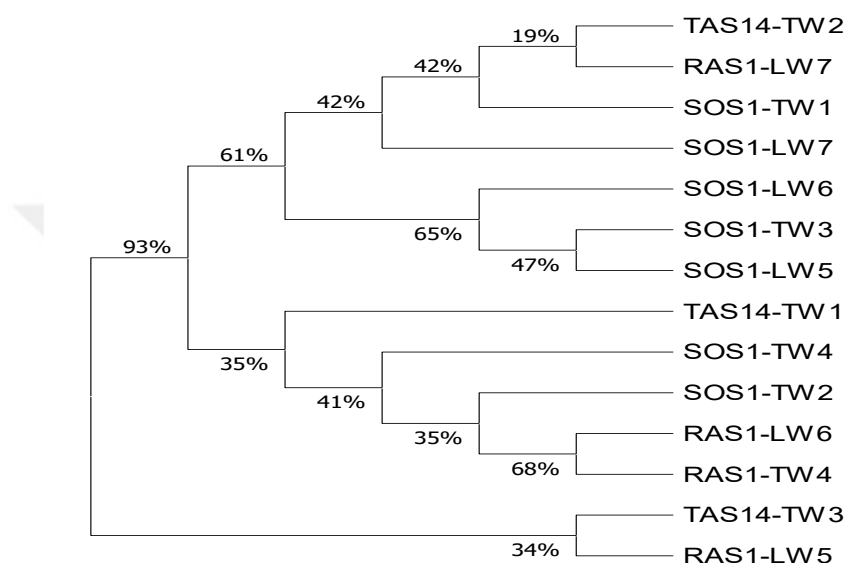


Figure 3.23. Phylogenetic tree constructed using the maximum parsimony method based on DNA sequences of the SOS1, RAS1, and TAS14 genes

3.3.4 Phylogenetic Study Based on Amino Acid Sequences

DNA sequences were translated to amino acid sequences by using “Expasy” online software (<https://web.expasy.org/translate/>). In this section, the relationship between amino acid sequences was evaluated, and phylogenetic trees were constructed using the neighbor-joining method. Evolutionary analyses were conducted in MEGA11 (Tamoura et al., 2021).

3.3.4.1 SOS1 Gene

The results of phylogenetic analysis indicate two clusters in the dendrogram, the first includes LW5, TW4 and LW7, while the second cluster is divided into two subclusters, one containing TW2 and the other containing TW1, TW3 and TW6 (Figure 3.24).

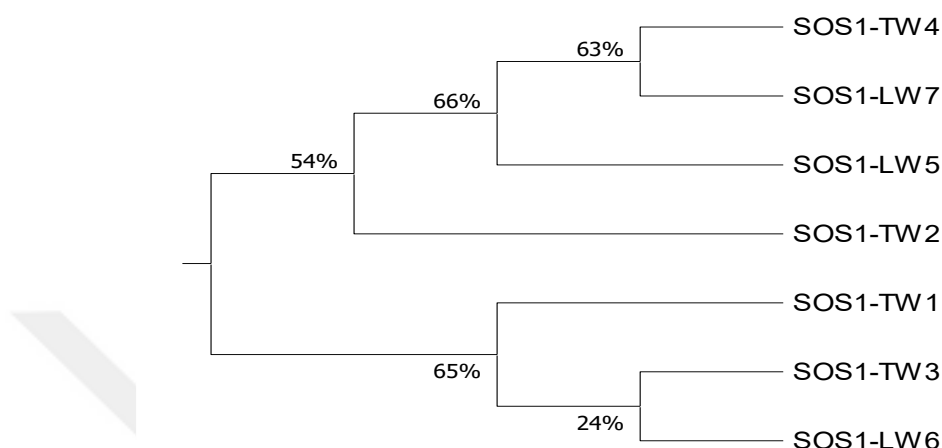


Figure 3.24. Maximum parsimony tree based on amino acid sequences of the SOS1 gene from seven genotypes

No conserved domain was found in any of the different SOS1 sequences.

3.3.4.2 RAS1 Gene

In the phylogenetic tree based on amino acid sequence, RAS1-LW7 appears alone, while in the second subcluster, LW5 and TW4 and LW6 are grouped (Figure 3.25).

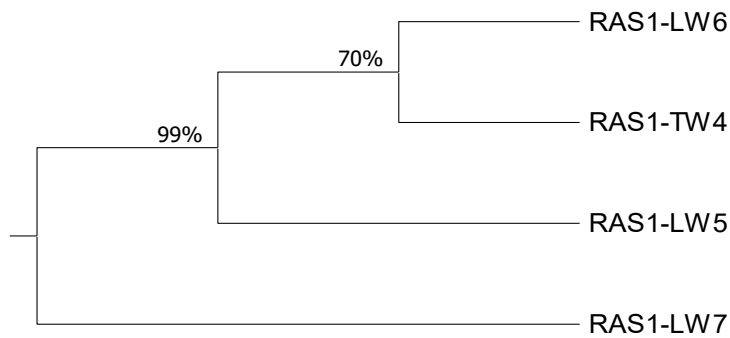


Figure 3.25. Phylogenetic tree constructed using the maximum parsimony method based on RAS1 gene-derived amino acid sequences from seven genotypes

All four sequences demonstrated conserved domains as follows:



Figure 3.26. Conserved domains of the RAS1_LW6 amino acid sequence gene

RAS1-LW6 has two conserved domains. The first is NADH dehydrogenase subunit 2, Provisional MTH00135, is a member of the superfamily cl39059 (Figure 3.26). The second conserved domain is a proton-conducting membrane transporter. This is a family of membrane transporters that includes some 7 of the potentially 14-16 TM regions. In many instances, the family forms part of complex I, which catalyzes the transfer of two electrons from NADH to ubiquinone in a reaction that is associated with proton translocation across the membrane. In this context, the combination predominantly includes subunits 2, 4, 5, 14, L, M and N. In many bacterial species, these proteins are probable stand-alone transporters not coupled with oxidoreduction. The family as a whole represents homologs across the phyla (Lu et al., 2020; Wang et al., 2023).

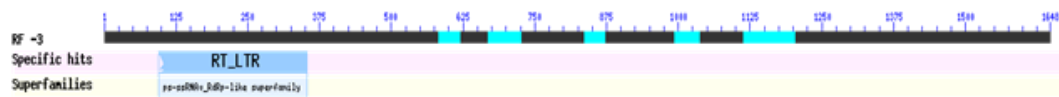


Figure 3.27. Conserved domain of the RAS1_LW5 amino acid sequence gene.

Reverse transcriptases (RTs) from retrotransposons and retroviruses have long terminal repeats (LTRs) in their DNA copies but not in their RNA template (Figure 3.27). RT catalyzes DNA replication from an RNA template and is responsible for the replication of retroelements. An RT gene is usually indicative of a mobile element such as a retrotransposon or retrovirus. RTs are found in a variety of mobile elements, including retrotransposons, retroviruses, group II introns, bacterial msDNAs, hepadnaviruses, and Caulimoviruses (Lu *et al.*, 2020; Wang *et al.*, 2023).

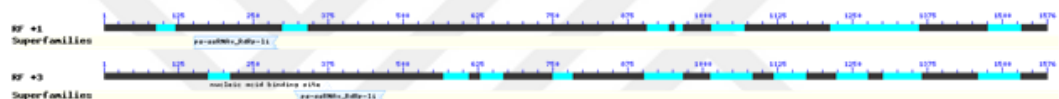


Figure 3.28. Conserved domains of the RAS1_TW4 amino acid sequence gene.

Reverse transcriptases (RTs) from retrotransposons and retroviruses have long terminal repeats (LTRs) in their DNA copies but not in their RNA template (Figure 3.28). RT catalyzes DNA replication from an RNA template and is responsible for the replication of retroelements. An RT gene is usually indicative of a mobile element such as a retrotransposon or retrovirus. RTs are found in a variety of mobile elements, including retrotransposons, retroviruses, group II introns, bacterial msDNAs, hepadnaviruses, and Caulimoviruses (Lu *et al.*, 2020; Wang *et al.*, 2023).



Figure 3.29. Conserved domain of the RAS1_LW7 amino acid sequence gene

This is a family of plant proteins induced by water deficit stress (WDS), abscisic acid (ABA) and ripening (Figure 3.29). ABA/WDS-induced protein; are a family of plant proteins triggered by water deficit stress (WDS), or abscisic acid (ABA) and ripening (Lu *et al.*, 2020; Wang *et al.*, 2023).



4. DISCUSSION

4.1 Morphological Insights into Salt Stress Tolerance in Wheat

Landraces

The morphological traits of wheat landraces under salt stress exhibited considerable variability, highlighting differences in their ability to withstand osmotic stress. These findings underscore the potential for selecting and breeding salt-tolerant wheat varieties, particularly among landraces that demonstrated superior morphological responses. The results confirmed that LW7, LW5, and LW6 exhibited the highest resilience across multiple morphological parameters, positioning them as strong candidates for agricultural applications in saline-prone environments (Munns & Tester, 2008; Ashraf et al., 2013).

Germination percentage, a crucial determinant of early seedling establishment, varied significantly among the tested landraces. The superior germination rates observed in TW2, LW5, and LW7 indicate their inherent ability to withstand high salt concentrations. The ability to germinate under osmotic stress is indicative of robust cellular mechanisms that enable the seeds to maintain water uptake and metabolic function despite the challenging conditions (Zörb et al., 2019). This suggests that these landraces possess adaptive traits that enhance their viability in saline soils, making them suitable for further breeding and cultivation in affected regions (Munns et al., 2006).

Root length was another key parameter influenced by salt stress. LW7 consistently demonstrated the highest root elongation under all stress levels, suggesting its superior capacity for water uptake and nutrient absorption in saline environments. In contrast, LW6 and LW5 exhibited a sharp decline in root growth as salinity levels increased. This reduction in root length could be attributed to ion toxicity and osmotic stress, which impair cell division and elongation (Flowers et al., 2010). The superior performance of LW7 suggests that it may have mechanisms that enable it to sustain root growth under stress, such as enhanced ion transport regulation and osmotic adjustment (Hosseini et al., 2015).

Root number is another critical factor that determines a plant's ability to explore soil resources. The findings revealed that LW7, LW5, and LW6 maintained higher root numbers under various osmotic stress levels compared to the other genotypes. An increased number of roots in salt-tolerant genotypes can contribute

to better water and nutrient uptake, providing an advantage in saline environments. The ability of these genotypes to sustain root proliferation under stress suggests that they may possess efficient root architecture modifications or enhanced hormonal signaling pathways that regulate root development in adverse conditions (Yamaguchi & Blumwald, 2005).

Coleoptile length, which plays a vital role in seedling emergence and early growth, was significantly influenced by salinity stress. The results indicated that LW7, LW5, and LW6 consistently maintained longer coleoptiles under increasing salinity levels. This suggests that these landraces possess traits that help mitigate the negative effects of osmotic stress on shoot elongation (Julkowska & Testerink, 2015). The maintenance of coleoptile length under stress conditions implies that these genotypes may have mechanisms that enhance cell expansion and division, allowing for continued growth even in saline environments. This trait is particularly beneficial for seedling establishment in saline soils, where early growth is crucial for plant survival (Munns et al., 2020).

Fresh weight of seedlings was also analyzed as an indicator of overall seedling vigor under salt stress. LW5, LW6, and LW7 demonstrated the highest fresh biomass retention across varying osmotic stress levels, suggesting their ability to sustain metabolic processes despite salinity-induced water deficits (Rahman et al., 2016). The retention of fresh weight in these genotypes indicates superior osmotic adjustment mechanisms that enable them to maintain cellular hydration and structural integrity. This adaptation is crucial for ensuring proper physiological functions and minimizing the adverse effects of dehydration caused by salt stress (Deinlein et al., 2014).

Similarly, dry weight analysis provided insights into biomass accumulation and stress tolerance. LW6, LW5, and LW7 exhibited the highest dry weights under increasing salinity levels, reinforcing their resilience to salt stress. Dry weight retention is indicative of a plant's ability to sustain growth and biomass accumulation despite osmotic and ionic stress (Shabala, 2013). The ability of these genotypes to maintain dry matter production under saline conditions suggests that they possess efficient photosynthetic performance, nutrient allocation, and metabolic adjustments that contribute to sustained growth under stress (Parida & Das, 2005).

The percentage of relative water content in seedlings further highlighted the adaptability of the tested landraces to salinity stress. LW7, LW5, and LW6 exhibited higher relative water content values under varying osmotic stress conditions, indicating their superior capacity to retain cellular water and maintain turgor pressure. relative water content is a key indicator of plant water status, and higher values suggest effective osmotic regulation mechanisms that prevent excessive water loss (Hasegawa et al., 2000). The ability of these genotypes to retain water under salt stress may be attributed to their capacity for osmoprotectant accumulation, enhanced stomatal regulation, or efficient ion compartmentalization strategies (Acosta-Motos et al., 2017).

Overall, the morphological analyses demonstrated that LW7, LW5, and LW6 exhibited superior tolerance to osmotic stress across multiple parameters, including germination percentage, root growth, coleoptile length, seedling biomass, and water retention. Their consistent performance under salt stress suggests that they possess key physiological and genetic adaptations that enable them to survive and thrive in saline environments. These findings emphasize the importance of selecting and utilizing salt-tolerant landraces for breeding programs aimed at improving wheat production in saline-prone regions (Roy et al., 2014). Future research should focus on further exploring the underlying genetic and physiological mechanisms that contribute to salinity tolerance in these genotypes, as well as conducting field trials to validate laboratory results in real agricultural conditions (Munns & Gilliam, 2015).

4.2 Physiological Insights into Salt Tolerance Mechanisms in Wheat Landraces

The physiological responses of wheat landraces to osmotic stress induced by salinity revealed significant differences among genotypes, emphasizing the importance of adaptive mechanisms in mitigating salt-induced damage. The findings indicate that LW6, LW7, and LW5 consistently demonstrated superior physiological tolerance across multiple biochemical markers, highlighting their resilience under saline conditions. These landraces exhibited higher chlorophyll content, soluble sugars, proteins, free amino acids, and proline levels, indicating that their survival and growth were supported by enhanced osmotic adjustment mechanisms (Acosta-Motos et al., 2017; Ashraf et al., 2013).

Chlorophyll content, a key determinant of photosynthetic efficiency, was notably impacted by salt stress. However, LW6, LW7, and LW5 exhibited a relatively higher retention of chlorophyll a, chlorophyll b, and total chlorophyll content under osmotic stress conditions compared to other genotypes. The ability to sustain chlorophyll levels despite increasing salinity suggests that these landraces possess protective mechanisms to prevent chlorophyll degradation, maintain photosynthetic efficiency, and ensure continued energy production for growth and development (Munns & Tester, 2008). Chlorophyll degradation under salt stress is typically associated with oxidative damage and reduced pigment stability, but the high chlorophyll retention observed in these landraces indicates their ability to counteract oxidative stress through enhanced antioxidant activity and efficient compartmentalization (Deinlein et al., 2014).

Soluble sugars are essential for osmotic adjustment, helping maintain cellular hydration and stabilizing metabolic processes. The results demonstrated that LW7, LW5, and LW6 retained higher soluble sugar content under salinity stress, suggesting their capacity to regulate osmotic balance effectively. Sugars act as osmoprotectants, reducing the effects of ion toxicity by balancing intracellular osmotic pressure, scavenging reactive oxygen species (ROS), and stabilizing proteins and membranes (Parida & Das, 2005). The increased accumulation of soluble sugars in these genotypes suggests that they employ carbohydrate metabolism as a defense mechanism against osmotic stress, contributing to their enhanced tolerance to salinity (Hasegawa et al., 2000).

Protein accumulation under salt stress is indicative of cellular adjustments to maintain metabolic functions. The present study found that LW7, LW5, and LW6 had significantly higher soluble protein content under varying osmotic stress levels. This suggests that these genotypes may possess stress-responsive proteins, such as heat shock proteins, late embryogenesis abundant (LEA) proteins, and ion transporters, which help in protecting cellular structures and maintaining enzymatic activity under salt stress (Ashraf et al., 2013). The retention of protein content under stress conditions is crucial for sustaining cellular metabolism and enzymatic reactions necessary for plant survival in saline environments (Munns et al., 2006).

Free amino acids also play a fundamental role in osmotic regulation and stress adaptation. The results demonstrated that LW7, LW6, and LW5 exhibited higher total free amino acid content under osmotic stress conditions, further supporting their enhanced tolerance mechanisms. Amino acids such as proline, glycine betaine, and glutamate act as osmoprotectants by stabilizing proteins, scavenging ROS, and maintaining membrane integrity under salt stress (Yamaguchi & Blumwald, 2005). The increased accumulation of free amino acids in these genotypes suggests that they employ nitrogen metabolism as a means of mitigating the detrimental effects of salinity, enhancing their ability to sustain metabolic functions in stressful environments (Rahman et al., 2016).

Proline accumulation is a well-documented response to salinity stress, acting as an osmoprotectant that aids in cellular water retention and macromolecule protection from oxidative damage. The results confirmed that LW7, LW6, and LW5 exhibited the highest proline accumulation under varying osmotic stress levels, reinforcing their ability to tolerate high salinity conditions. Proline functions as an osmolyte, a stabilizer of subcellular structures, and a signaling molecule that regulates stress-responsive pathways (Munns et al., 2020). The elevated proline levels in these genotypes suggest an enhanced capacity to counter salt-induced osmotic stress through increased biosynthesis and reduced degradation, reinforcing their resilience (Shabala, 2013).

Overall, the physiological analyses suggest that LW6, LW7, and LW5 possess multiple adaptive mechanisms that contribute to their superior tolerance to osmotic stress. The retention of chlorophyll content and the accumulation of soluble sugars, proteins, free amino acids, and proline highlight their ability to mitigate the adverse effects of salinity through osmotic adjustment, antioxidant defence, and stress-responsive metabolic pathways. These findings support the potential of these landraces as valuable genetic resources for breeding programs aimed at enhancing wheat production in saline-prone regions (Roy et al., 2014). Future studies should focus on elucidating the molecular regulatory mechanisms underlying these physiological adaptations and conducting field trials to validate laboratory findings under real-world agricultural conditions (Munns & Gilliham, 2015).

4.3 Molecular Insights into Salt Stress Tolerance in Wheat: Analysis of SOS1, RAS1, and TAS14 Genes

4.3.1 SOS1 Gene Analysis

The role of the SOS1 gene in Na⁺ ion efflux and salt tolerance was confirmed. The phylogenetic tree based on SOS1 sequences revealed significant variability among genotypes, consistent with previous findings on natural genetic diversity in salt-tolerance mechanisms (Shi et al., 2000; Yousefirad et al., 2018). Alignment with GenBank accessions confirmed the presence and functional integrity of SOS1 in these genotypes. Furthermore, the identification of conserved domains in amino acid sequences supports SOS1's evolutionary conservation and critical role in ionic homeostasis, as suggested by Ali et al. (2023).

The phylogenetic tree constructed from SOS1 gene sequences (Figures 5 and 6) grouped the genotypes into distinct clusters, highlighting genetic diversity in the salt overly sensitive (SOS) pathway. The separate clustering of LW6 and LW7 from the other genotypes suggests a unique sequence that may influence their functional properties. Conserved domain analysis further confirmed SOS1's role in regulating Na⁺/H⁺ antiport activity, a key mechanism critical for maintaining ionic homeostasis under salt stress (Shi et al., 2000; Ali et al., 2023).

The alignment with GenBank accessions reinforced the evolutionary conservation of SOS1, consistent with its pivotal role in plant salt tolerance. Similar findings were reported by Wu et al. (2019), who demonstrated the importance of SOS1-mediated Na⁺ leakage in conferring salt tolerance across diverse plant species. Phylogenetic and domain analyses highlight SOS1 as a valuable molecular marker for breeding salt-tolerant wheat.

4.3.2 RAS1 Gene Analysis

ABA signaling is crucial for regulating stress adaptation mechanisms, including stomatal closure, osmotic adjustment, and stress-responsive gene expression (Cutler et al., 2010). The involvement of the RAS1 gene in ABA sensitivity and its negative regulation of salt stress was confirmed. Phylogenetic analysis grouped the genotypes into distinct clusters, highlighting genetic diversity in response mechanisms.

The dendrogram for RAS1 gene sequences (Figures 7 and 8) revealed two main clusters, separating LW6 and TW4 from LW5 and LW7. This distinction suggests differential regulation of the RAS1 pathway among genotypes, which could influence their response to salt stress. Conserved domain analysis of RAS1 highlighted key features, including proton-conducting membrane transporters and reverse transcriptase, which are indicative of its role in modulating ABA sensitivity and salt stress responses (Ren et al., 2010; Lu et al., 2020).

The conserved domains identified within the RAS1 sequences corroborate its functional integrity and evolutionary relevance, paralleling findings in other crops such as maize (Ren et al., 2010; Zhou et al., 2022). These findings suggest that genotypes clustering together may share functional similarities in their stress responses, offering targets for breeding programs aimed at improving salt tolerance.

4.3.3 TAS14 Gene Analysis

The TAS14 results demonstrated that TW2 and TW3 were more closely related than TW1, indicating potential functional similarities in their dehydrin proteins. Dehydrins play a critical role in osmotic stress tolerance by stabilizing cellular structures during dehydration (Godoy et al., 1990). Amplification of TAS14 was observed in three genotypes, and sequence alignment with *Solanum* species suggests shared evolutionary pathways in stress adaptation. These findings align with previous studies on the functional roles of dehydrins in abiotic stress resistance, particularly under salinity- or drought-induced osmotic stress (Muñoz-Mayor et al., 2012; Hu et al., 2024).

The evolutionary conservation of TAS14 across plant taxa further underscores its importance in stress adaptation mechanisms. This is supported by recent studies highlighting the multifunctionality of dehydrins in stabilizing membranes and proteins under adverse environmental conditions (Wang et al., 2023).

4.3.4 Integrated Analysis of SOS1, RAS1, and TAS14

The combined dendrogram (Figure 11) integrating SOS1, RAS1, and TAS14 sequences illustrates the interconnected roles of these genes in mediating salt tolerance. The clustering patterns indicate that certain genotypes, such as TW4 and LW6, exhibit overlapping functional characteristics, possibly due to shared

regulatory pathways. The conserved domains identified across these genes suggest that their functions are deeply embedded in evolutionary adaptations to abiotic stresses. The relationship between conserved domains and phylogenetic clustering provides a framework for understanding genotype-specific responses to salt stress. These insights are instrumental in selecting genotypes with optimal combinations of SOS1, RAS1, and TAS14 functionality for breeding programs aimed at enhancing wheat's resilience to saline conditions.



5. CONCLUSION

This study comprehensively examined the morphological, physiological, and molecular responses of wheat landraces to osmotic stress, particularly regarding salinity tolerance. The findings reveal substantial genetic variability in the salinity tolerance mechanisms of the tested wheat genotypes, highlighting their potential for breeding programs aimed at improving wheat resilience to saline environments. The results emphasize the necessity of integrating multiple analytical approaches to gain a holistic understanding of wheat responses to abiotic stress conditions.

Wheat landraces are not wheat varieties. The agronomic characteristics (Nearly all) of them have not been determined and are not officially recognized. In addition, the agronomic traits of wheat landraces are not stable and they have survived through breeding processes, spontaneous growth in nature and have survived all living and non-living abiotic stress (such as salinity, heavy metal, etc.) conditions they have encountered during this process. In addition, their (Wheat landraces) seeds' nutritional profiles are rich in nutrients (especially P and Fe), but their yield levels are very low (90-100 kg/da). In contrast, modern wheats have high yields (200-350 kg/da) but low nutrient profiles and is not resistant to abiotic-biotic stresses.

Morphological analyses demonstrated that wheat genotypes exhibit distinct responses to increasing osmotic stress. Certain wheat landraces; LW5, LW7, and LW6, maintained high germination rates, root lengths, root numbers, and overall seedling vigor, even under severe salinity stress. Their ability to sustain robust root development underscores the critical role of root architecture in salinity tolerance. These findings align with previous research, which highlights early seedling establishment as a key determinant of salt stress resilience. The selection of such high-performing genotypes can significantly contribute to breeding programs focused on improving wheat productivity in saline soils.

Physiological analyses further confirmed variations in osmotic stress responses among wheat landraces. LW7, LW5, and LW6 exhibited higher chlorophyll a, chlorophyll b, and total chlorophyll content under stress conditions, indicating superior photosynthetic efficiency and resilience against oxidative damage. The accumulation of soluble sugars, proteins, and amino acids in these genotypes suggests that osmotic adjustment plays a key role in conferring salt

tolerance. Notably, increased proline and total free amino acid content reinforce their capacity to mitigate osmotic stress by stabilizing cellular structures, scavenging ROS, and maintaining metabolic homeostasis. These biochemical traits make them strong candidates for breeding salt-tolerant wheat varieties.

On the molecular level, the study identified significant genetic variability in the expression of stress-responsive genes SOS1, RAS1, and TAS14, which are critical for maintaining ionic balance, regulating osmotic stress responses, and facilitating ABA-mediated stress signaling. Phylogenetic and conserved domain analyses confirmed the evolutionary significance of these genes in mediating salt stress adaptation. The distinct clustering of LW6 and TW4 suggests unique regulatory mechanisms that could be exploited in breeding programs. The observed genetic diversity in salt tolerance genes supports the use of molecular markers in wheat breeding, enabling the development of genotypes specifically tailored for high-salinity environments.

The combined dendrogram, integrating SOS1, RAS1, and TAS14, highlights the interconnected roles of these genes in salinity tolerance. The clustering patterns indicate that genotypes like TW4 and LW6 share functional characteristics, likely due to overlapping regulatory pathways. These findings provide crucial insights into the molecular basis of salt tolerance and suggest that genomic selection strategies, including marker-assisted selection (MAS), could enhance wheat productivity under abiotic stress conditions.

In summary, this study provides an in-depth analysis of the mechanisms underlying wheat's response to salinity stress at morphological, physiological, and molecular levels. The identification of superior genotypes with enhanced tolerance traits presents valuable opportunities for breeding programs aimed at developing wheat cultivars capable of thriving in salinity-prone environments. Moving forward, further research should explore the broader regulatory networks governing wheat's response to salinity, particularly by characterizing additional stress-related genes that interact with SOS1, RAS1, and TAS14. Additionally, transcriptomic and proteomic approaches should be integrated to provide a more comprehensive understanding of gene expression patterns and protein modifications under stress conditions, ultimately guiding the development of genetically enhanced wheat varieties.

To ensure sustainable wheat production in saline environments, future related agronomic strategies should combine advanced molecular tools, precision breeding techniques, and sustainable agronomic practices. Moreover, field validation of laboratory findings is essential, as environmental variables such as soil composition, microbial activity, and fluctuating climatic conditions can significantly influence plant responses to salinity. Conducting large-scale field trials would bridge the gap between controlled experimental conditions and real-world agricultural settings, thereby improving the practical applicability of research findings. Furthermore, there is a critical need to explore additional candidate genes involved in ion transport, osmotic balance, and oxidative stress defense to gain deeper insights into the genetic basis of salinity tolerance in the genotype. Expanding the genetic and biochemical analyses beyond SOS1, RAS1, and TAS14 could uncover novel regulatory pathways that contribute to salt stress adaptation. Such integrative approaches will be instrumental in developing high-yielding, salt-tolerant wheats, ensuring food security, and promoting sustainable agriculture in regions affected by soil salinity. Future studies should focus on identifying additional salinity tolerance mechanisms in wheat beyond SOS1, RAS1, and TAS14. Investigating other candidate genes with putative roles in ionic transport, osmotic balance, and oxidative stress defense could provide deeper insights into the genetic basis of salinity tolerance. Additionally, field validation studies are crucial to complement laboratory findings, as environmental factors—such as soil composition, microbial interactions, and climate variability—can significantly influence plant responses to salinity stress. Fully integrated studies bridging laboratory and field research will enhance the practical applicability of findings, facilitating the development of resilient wheat varieties suited for saline-prone soils.

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APPENDICES

APPENDIX 1: Morphology

Estimation of Germination Percentage (%)

Tests of Between-Subjects Effects

Dependent Variable: (Germination Percentage - %)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1514181.667 ^a	55	27530.576	107.051	.000
Intercept	8973171.667	1	8973171.667	34891.768	.000
OsmoticPotentialsCode	1514181.667	55	27530.576	107.051	.000
Error	417646.667	1624	257.172		
Total	10905000.000	1680			
Corrected Total	1931828.333	1679			

a. R Squared = .784 (Adjusted R Squared = .776)

Root Length (cm)

Tests of Between-Subjects Effects

Dependent Variable: (Root Length - cm)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7557.903 ^a	45	167.953	75.475	.000
Intercept	12793.606	1	12793.606	5749.176	.000
OsmoticPotentialsCode	7557.903	45	167.953	75.475	.000
Error	1117.097	502	2.225		
Total	21604.160	548			
Corrected Total	8675.001	547			

a. R Squared = .871 (Adjusted R Squared = .860)

Root Numbers (n)

Tests of Between-Subjects Effects

Dependent Variable: (Root Numbers - n)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	758.960 ^a	45	16.866	34.407	.000
Intercept	9764.356	1	9764.356	19919.993	.000
OsmoticPotentialsCode	758.960	45	16.866	34.407	.000
Error	246.070	502	.490		
Total	10810.000	548			
Corrected Total	1005.029	547			

a. R Squared = .755 (Adjusted R Squared = .733)

Coleoptile Length (cm)

Tests of Between-Subjects Effects

Dependent Variable: (Coleoptile Length – cm)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6013.478 ^a	43	139.848	87.567	.000
Intercept	11957.361	1	11957.361	7487.183	.000
OsmoticPotentialsCode	6013.478	43	139.848	87.567	.000
Error	707.491	443	1.597		
Total	22455.940	487			
Corrected Total	6720.968	486			

a. R Squared = .895 (Adjusted R Squared = .885)

Fresh Weight of Seedlings (g)

Tests of Between-Subjects Effects

Dependent Variable: (Fresh Weight of Seedlings - g)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.929 ^a	45	.021	30.973	.000
Intercept	6.934	1	6.934	10404.701	.000
Osmotic Potentials Code	.929	45	.021	30.973	.000
Error	.337	505	.001		
Total	8.211	551			
Corrected Total	1.265	550			

a. R Squared = .734 (Adjusted R Squared = .710)

Dry Weight of Seedlings (g)

Tests of Between-Subjects Effects

Dependent Variable: (Dry Weight of Seedlings - g)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.035 ^a	45	.001	17.833	.000
Intercept	.534	1	.534	12143.927	.000
OsmoticPotentialsCode	.035	45	.001	17.833	.000
Error	.022	505	4.399E-5		
Total	.592	551			
Corrected Total	.058	550			

a. R Squared = .614 (Adjusted R Squared = .579)

Percentage of Relative Water Content in the Seedlings (%)

Tests of Between-Subjects Effects

Dependent Variable: (Relative Water Content - %)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	134769.533 ^a	45	2994.879	134.860	.000
Intercept	2517506.090	1	2517506.090	113363.671	.000
OsmoticPotentialsCode	134769.533	45	2994.879	134.860	.000
Error	11214.709	505	22.207		
Total	2664919.232	551			
Corrected Total	145984.242	550			

a. R Squared = .923 (Adjusted R Squared = .916)

APPENDIX 2: Physiological

Determination of Chlorophyll a, b, and a+b content in the seedling extract

Chlorophyll a (mg/g)

Tests of Between-Subjects Effects

Dependent Variable: Chlorophyll a (mg/g)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6843.488 ^a	35	195.528	567.206	.000
Intercept	21856.022	1	21856.022	63401.985	.000
OsmoticPotentialsCode	6843.488	35	195.528	567.206	.000
Error	24.820	72	.345		
Total	28724.330	108			
Corrected Total	6868.308	107			

a. R Squared = .996 (Adjusted R Squared = .995)

Chlorophyll b (mg/g)

Tests of Between-Subjects Effects

Dependent Variable: Chlorophyll b (mg/g)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	17388.251 ^a	35	496.807	156.332	.000
Intercept	19324.637	1	19324.637	6080.939	.000
OsmoticPotentialsCode	17388.251	35	496.807	156.332	.000
Error	228.809	72	3.178		
Total	36941.697	108			
Corrected Total	17617.060	107			

a. R Squared = .987 (Adjusted R Squared = .981)

Chlorophyll a + b (mg/g)

Tests of Between-Subjects Effects

Dependent Variable: Chlorophyll a+b (mg/g)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	38491.041 ^a	35	1099.744	637.643	.000
Intercept	82283.442	1	82283.442	47708.815	.000
OsmoticPotentialsCode	38491.041	35	1099.744	637.643	.000
Error	124.178	72	1.725		
Total	120898.661	108			
Corrected Total	38615.219	107			

a. R Squared = .997 (Adjusted R Squared = .995)

Determination of the Soluble Sugar Content in the Seedling Extract (mg/g wet weight)

Tests of Between-Subjects Effects

Dependent Variable: (Sugar Content - mg/g wet weight)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	16179167.737 ^a	45	359537.061	378.505	.000
Intercept	25155983.809	1	25155983.809	26483.154	.000
OsmoticPotentialsCode	16179167.737	45	359537.061	378.505	.000
Error	87389.536	92	949.886		
Total	41422541.082	138			
Corrected Total	16266557.273	137			

a. R Squared = .995 (Adjusted R Squared = .992)

Determination of the Soluble Protein Content in the Seedling Extract (mg/g wet weight)

Tests of Between-Subjects Effects

Dependent Variable: (Soluble Protein Content - mg/g wet weight)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	344702.288 ^a	45	7660.051	433.438	.000
Intercept	1669480.015	1	1669480.015	94466.206	.000
OsmoticPotentialsCode	344702.288	45	7660.051	433.438	.000
Error	1625.895	92	17.673		
Total	2015808.198	138			
Corrected Total	346328.183	137			

a. R Squared = .995 (Adjusted R Squared = .993)

Determination of the Total Free Amino Acid Content in the Seedling Extract (mg/g wet weight)

Tests of Between-Subjects Effects

Dependent Variable: (Amino Acid Content - mg/g wet weight)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1682008.478 ^a	45	37377.966	11588.318	.000
Intercept	3931474.404	1	3931474.404	1218877.822	.000
OsmoticPotentialsCode	1682008.478	45	37377.966	11588.318	.000
Error	296.745	92	3.225		
Total	5613779.627	138			
Corrected Total	1682305.222	137			

a. R Squared = 1.000 (Adjusted R Squared = 1.000)

Determination of Proline Content in Seedling Powder (mg/g dry weight)

Tests of Between-Subjects Effects

Dependent Variable: (Proline Content - mg/g dry weight)

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	543638.745 ^a	45	12080.861	314.058	.000
Intercept	2337090.561	1	2337090.561	60755.745	.000
Osmotic Potentials Code	543638.745	45	12080.861	314.058	.000
Error	3538.963	92	38.467		
Total	2884268.269	138			
Corrected Total	547177.708	137			

Df: Degrees of Freedom, F: F-statistic, Sig.: Significance, a. R Squared = .994 (Adjusted R Squared = .990)

APPENDIX 3: DNA Sequences

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APPENDIX 4: Outgroup Sequences

>AF516445.1 *Hordeum secalinum* clone HSEC030 5S ribosomal RNA gene, complete sequence

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>Z49841.1 *H. jubatum* chloroplast rbcL gene for ribulosebiphosphate carboxylase

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GTGCAATGCATGCAGTTATTGATAGACAGAAAAATCATGGTATGCATTTC
CGTGATTAGCTAAAGCATTGCGTATGTCTGGGGGAGATCATATCCACTC
CGGTACAGTAGTAGGTAAGTTAGAAGGGGAACGCGAAATGACTTTAGG

TTTTGTTGATTTATTGCGCGATGATTTTATTGAAAAAGATCGTGCTCGCG
GTATCTTTTTCCTCAGGACTGGGTATCCATGCCAGGTGTTATACCGGTA
GCTTCAGGTGGTATTCATGTTTGGCATATGCCAGCTCTGACCGAAATCTT
TGGGGACGATTCTGTATTACAATTTGGTGGAGGAACTTTAGGACATCCT
TGGGGGAATGCACCTGGTGCAGCAGCTAATCGAGTGGCTTTAGCAGCC
TGTGTACAAGCTCGTAACGAAGGGCGTGATCTTGCTCGCGAAGGTAAT
GAAATTATCCGAGCAGCTTGCAAATGGAGTCCTGAACTAGCCGCAGCTT
GTGAAGTATGGAAGGCGATCAAATTCGAGTTCGAGCCGGTAGATACTAT
CGATTAA



APPENDIX 5: DNA Sequences Alignment

Alignment of SOS1 sequences:

```
SOS1-TW3  -----TCGGGGGAAGGGCGCGGCGAATGAAAAGAAGA--GAAGATCAGG
SOS1-LW6  -----GCGATGAGAACGACCAGTCGAGAGACGAAGAGCATGATGATAAGA
SOS1-LW7  ---GGAGTAAATGGATGGTTGAACTGGTTGATTGATGA-GAGAACCCTCCACCTTCGG
SOS1-LW5  GCGTGGGGATAATCCTTTGATGA-CTGATTCATT-GATGA-GTGAGCCTTCCACCTTCGG
SOS1-TW1  -----
SOS1-TW2  -----
SOS1-TW4  -----

SOS1-TW3  CTTGAATGAAGAACTCTCCCTCTGATACCTTGTGGAACCGAACCAGAAGAGCTCAA-CA
SOS1-LW6  TTTGATTGTATAACTCTATTATCTGATCCCTTGTTGAACCCAACCAGAAGAACATAGGCA
SOS1-LW7  TGTCCGTGTAGGCCTGAATCAGCAAAATCCTTGAGATGTCCTACCAGGAGAGAACGAACC
SOS1-LW5  TGTCTTGTAGGCCTGACTAAGCAAAATCCTTGAATCCCCCTACCACTCGAACGTACCAT
SOS1-TW1  -----AAGTACCGCAATCAAAAG--CCTCACCT
SOS1-TW2  -----AGTAGTGGGTTTGAGAATTCCTTTGCT
SOS1-TW4  -----AAGAATTAAGAAGGGCCTTACG

SOS1-TW3  GCGGACCCCTTGATGATCCAGAAGCGAACCAGAAGAACTTTGATCACAGCTCCAGCTGTGC
SOS1-LW6  TCTTATTGGAGGGATTCTGAAGACAGAGAACCGAACTTTGATCACACCTCCAGCTGTGC
SOS1-LW7  TCTTGA-AGCAAAATCCTTGATGCTAACCTGCAAGAGCTGATACGAGACGCTAGCGGGCG
SOS1-LW5  TCTTGC-ATACATATCCTTG-----TGCAAGGGCTAGCA-GAGAAGCTACCAGACT
SOS1-TW1  TCGTTCGGGCAGGCTCG-CGGTGTACATC-GAGACCAACCGGC-ACGCGATTGTCAGGAA
SOS1-TW2  TTGTTTTGTCTAGTCTCGTCGGTTGACACCCGTGACGAAGAGGG-AGGCG-TCAGCAGAGG
SOS1-TW4  TTTTG--AATTAGCCCCACGGTGTA---CCGCGAGACCCGCGGGAAGTTGTTGCCCCGAG

SOS1-TW3  TGCTGA-GTCACACACTGATATGAGCTTATTTGGCCTGCAG--TCTCAAGTTCTCTATA
SOS1-LW6  TGCTGCCGTCAAACACTGATTTGAACTTATTTGGCCTGCAG--TCTTCAGGTTCTCTATA
SOS1-LW7  GACTTCTGATT-CCCATTTCTTGTTCAGGCCCGCTGGATCTCCGGAAACTCTCCG
SOS1-LW5  GGCTTCTCATTT-CGCTAAGCTTGCAATTCAGGCCCTCTG-ATCTCCGGAAACTCTCCC
SOS1-TW1  GGATTCGCAGAG----CG---TTCCTGTTTCCGCCCTG-AGCAGCTCCGAGTTGCTCGCA
SOS1-TW2  GAGCAGAGATGA----CAACCTTCTTGCTCCGCCCTTCAAGTGAGCCTTGTCTTCTCG
SOS1-TW4  GGACCGCAGTGC-GACTGGTCATGGATGGT--GGCCTGCTG---CTTGGGAGAGATGGAT
```

SOS1-TW3 TTTTCATCAATCGAGAAGTGTCTGTTTTCTAAAGCAAAAACAGTGATTTATTTTGGTCTT

SOS1-LW6 TTTTCATCAATCAAGCAGTGGCTGTTTTCTGAAGCAAAAAGCAGTGATTTATTTTCTTTTA

SOS1-LW7 GCCACCTGAGCAAACCGACATGCTCTTTGCTCTGTGTTTCGCGATGGTCCAAAAGCTGCAG

SOS1-LW5 -CCACCTGAGCAAACCTACATGCGCTTTGCGCAGTGCTCGCGGAAGGCCAAGTCATG-AA

SOS1-TW1 AAGGATTTCTCATTTTAACCCATGCATTCGATG-GCTAGGCGCGGGGCTAGATCCAGGAT

SOS1-TW2 GTGGTGGTGAAGACACCAGTCGACTCTACGACGTAATATGCGCCGGTCTCGCTCCATGGT

SOS1-TW4 TTAAGATTAATGGCGCATTTCGATGCTTTACTCG---TCTGCGA-GATCCTTGATATGCAG

SOS1-TW3 AAAG-GGTTGGATGATTTGACATTGTGTTGTGTGCAGCCCAGGTTTGGCACCGGCGCTGG

SOS1-LW6 AAAGTAATTGAATGCTGAAGTGCTCTCTTGTTTACAGGCATGAATCAGATAGACTACTG-

SOS1-LW7 AAAACAACGATCCACTTCTTTGCAG----ATCGG-ATTCTATATCTGGCCTCCTC-----

SOS1-LW5 GAGCCAACCACTTTCATGGTGG---CTGGGTAAAAGAGATTTGCCATTTCGCGGAAG

SOS1-TW1 TGGTACACTGGACTACGTTGC-TGATTAAGCGTACGGCCTAG-TCCGTGATAGTCAAGAT

SOS1-TW2 ATGTTGGCGGGGTCCTTCTCCATGTGGAAGCGGATGGTCTTG-CCGTTGACGGTCAGGTT

SOS1-TW4 TTGT--ACTGGTTGCTGGACGTCGTACAAGTTTGCCCGTGAGAACCGCGATTGCCGGG--

SOS1-TW3 TTTATACAGTGCCCTTCTGAATGGTGGCAACTACTATCAAGGGTTGCGCTCGT-TGCGGG

SOS1-LW6 -TTATACTGCTCCCAGTTATCAGGTGGAGGCTAG-AGCAAGGGTCATCTTATT-TGAAAT

SOS1-LW7 -ATTTCAAATATTATGCCCTTGCCCTAGCCTCGACCTGATAGCTGTTATCTC-CATAGC

SOS1-LW5 TATTTCAAGAGTTATTCTAAATGG---AGGCAAATACTAGCAACTTTTGGATCACGTAGT

SOS1-TW1 GGTGCCGTCACCTGGATCGCACCTGGAAGTGGACGTGTGAGCTGGCATAACCATTTGCA

SOS1-TW2 GTTGCCGTCGACCTTGATCTCACCTTGAACTGGCCGTGTGAGCTGTCATACT-TGAGCA

SOS1-TW4 -TCTCGGTCGAATCACACGGGAGAGGACTGTCATATTAGGGGCAAGTTCCTAGCTGCC

SOS1-TW3 ACTTAGCCCA---ACATCTCACGACACGAGCTGACG-ACAACCATGCTCCACCTGTCACT

SOS1-LW6 GAGGAGTCCAGATATAGAATCCGATCTGCAAAGAAGTCATCGTTGCTTTCTCCAGCGCT

SOS1-LW7 G-GTGGCTAT---CTGATTTCATGAGGGTAAGGCA----CATTTACTTCTTCGTCAAG---

SOS1-LW5 TCGAAGATGT---CCGGTACTTATGGGCAAACCGACTGCGTGCACGCTCCAGATGAGACG

SOS1-TW1 TGTCTGCCCA--CAGGCACGCTGACCTGGGAACCAAGGAACAAATGGCCACCAAG-CG

SOS1-TW2 TGTATGCCTA--CATGGACGTTGGCCTGCAATCCACTAGGAACAAATGGCCTCCAAC-CG

SOS1-TW4 CCGAGGCCGA-----GTGAGGTGGGGACCTTGACGAAAAGTTGGCCAACATTTAT

SOS1-TW3 CTGCCCC-----CGAAGGGGACGTCCTATGACTAGGATTGTCAGAGGATGG

SOS1-LW6 CGGACCGTCACGAACGCAGAGCAAAGAGCATGACCGATTGCTCAGGTGGCCGGAAGATT

SOS1-LW7 -TACATT-----AAAGAACAAGCCCTTTCTGATACCAAACCCCACTACTCG

SOS1-LW5 TTGCCCC-----CACGAGGGACGTCTCATGAAAAGGAGTCGGAGAGGAGGG

SOS1-TW1 CTGGGAC-----CATATGGAACGCGCCGCGGATTG---TCGACAAGACTGG

SOS1-TW2 CCGCGAT-----CATATGGAACGCGCAGCGGATTG---TCTGGAAGACTGT

SOS1-TW4 CTAAAAG-----ATCAAGAACATGCCGCACAGTGT-TTGCTTTAGCACGG

SOS1-TW3 CTTGA--CCTGGTAAGGTTTCGTCCCCTTGCTTGGAATTAAACCACATGCT---CCACCGG

SOS1-LW6 CCGGAGATCCCGAAAGGCTCGAAATGCTAGCTTAGCTGAAATGCCAAGGCAGGCTGCGAG

SOS1-LW7 CATGA--TCTGTTGA---CCTGACTGCATCACTGCCCCCTCTATCTTTGCT--ATCCC--

SOS1-LW5 CTTGA--GCGGGTAAGGTTCTGAACAAATTGAGGGCAATTTTGACAAAGGCC--AAACAAG

SOS1-TW1 GGTGT-ATCTGGGGA--TCCCTACGGAGTAGAGGGGCTTGACGAATGGGTCTTTTACCGG

SOS1-TW2 CTTGT-ATCCGGGGA--TACCTACGGCGTAGTGGGGCTCGATGAAGGGGTCTTTTACGGC

SOS1-TW4 CCTAT--CCTACCAG----CGCCACGGATGATTGGGCCGATCGCAGGAGTTGGTTCGAGG

SOS1-TW3 ATTGTGCGGGGCCCCGTGCTTTCCTTTTCAGTTTAACTCTTG-GGACCGTACTCCGCAGGC

SOS1-LW6 CTTCTCTGGTGGAGGCTTTTTACTCAGCATTAGGGTCTAGTGGTCCATGCACCCCTGGC

SOS1-LW7 -TCGCAGTAGGTGTCGCGGGCA---ACTTAAAAACGGTCACTTACACAACACCTTTTGT

SOS1-LW5 ATTAAGACGGGGGTCGTGCTTAGACACCTGCTGAACTTTCGGGAAAGGACACCGATGGC

SOS1-TW1 GAGAATGCCGACGTCGTTGTGCTCGACCCTGCGACATGTG-CGATCTTTGA--TATGGCT

SOS1-TW2 GACAATGTCGACGTCGTTGTGCTCGACCCTGTGAGACTCGGTTAGCTTTGA--TATGGCA

SOS1-TW4 GCGACGGGCGTGGTGGTTAGTATCGTTTCTGCGCCACTCTTTAATCTATTC--CACATCA

SOS1-TW3 GGAGA-TTTTGATGTGTTAGCT-GCAGCACTG---AAGGGGCTCACA-CCCCCTAAAACA

SOS1-LW6 CTTTTCTTTTGCCACGTCAGATCGCGGCACTGTCCGCGTAATTGACG-CCTCTAAGATGA

SOS1-LW7 GACCCTGCTGGG-----AGAC-GGGAGGCGG---AGGAGACCCA---GTACTATTCGGA

SOS1-LW5 CATTGAGTTTTGTTTTTCAGAT-GGTGAACTG---AAGGCGCTCTCACGCACAAAAACGA

SOS1-TW1 CAGTTTTCGGGCTCAATCATTATTG-GAATTTTGGAAGA-GAAGCGGTAAATGCAGAATC

SOS1-TW2 GAATTAACGGGCTCAATCAAACCTACGCATTGCGGAAGACGATACGGCCAATGCAGAA--

SOS1-TW4 CGGGTTTTGGCGCAGTGTGCATGAATTTCTTAGTACGTAACCTCTGAAAGTATCCGCA

SOS1-TW3 TATTATCGTCGGTTGCGGCG-TGGACTGCCAGGGATCTGAG----CCAGTA-CGCTCCC

SOS1-LW6 CGACTTTCGAAAGGTGGGACCCTGAACTGTTTCGAGGGTAGCCATTTTCAGTAATAATTTA

SOS1-LW7 CTCCTT--GGCTGATTGACTG--GTATTCCTGAAGTTT-----CTAGGATTGGTTTA

SOS1-LW5 CTTTCTCCGTCTGGTGGGGAA-TGAACTGCCAGGTAA-----CTGTGATAGGCTAG

SOS1-TW1 CTCTGTCCGGAGTTTCATGCGACAGGGGCCAAAGAAGTGGCATCCGTCGTCACGGCTGTA

SOS1-TW2 -----

SOS1-TW4 CTCCCACCATAATTCACCGCA--GCGCCAGACAAGAATGTCCTCCAGCGCGA-CGTCCAA

SOS1-TW3 CACGCTTTCGCTCCTCAG----CGTCAGTTACAGACCAGAGAG----TCGGCTTCA-CC

SOS1-LW6 GAAGACTAAACTCCCTGGGAAACCACCTGATAAAAGACACAGAAAAGTCCGGCTTTGGTC

SOS1-LW7 CCAG----GATTGGT-----

SOS1-LW5 CTCGTC--AGGTGGACGC---TCCCCGCGGAAGGTACGAACGGATAGTCCCCTTCGCCC

SOS1-TW1 AAGGGTTACAGGCTCTAGACGTGCTGGGTGGAGTACCGGTGAACGGTTATTCGTCCTGG

SOS1-TW2 -----

SOS1-TW4 AAGGGTTGCAGGCTCAAGATGAGTGTGTTCTATTTCCAGTGAACCGATTGGTCTCCATTC

SOS1-TW3 GCTGGTGTTCCTCCAC----ATCTCTACGCAT--TTCACCG----CTACACGTGGAATTC

SOS1-LW6 GGGGGGGGTCCCCACCGGGACTCCTACCCAAAATTCAACGGGTTTCACGTGGGGAGTGC

SOS1-LW7 -----

SOS1-LW5 CTGGGTGTTCACAGGA-----

SOS1-TW1 CGCACC-----

SOS1-TW2 -----

SOS1-TW4 TACCG-----

SOS1-TW3 CACTCT-CCTCTTCTGCGCTCA--AGTTCCC--AGTTTCCAATGACCTCCCCG-----G

SOS1-LW6 CAGTGGACCGGTTCTGGGAATATGGGTCTCGGAGTTATCCAAGAGGGCCGTGCGCGGGG

SOS1-LW7 -----

SOS1-LW5 -----

SOS1-TW1 -----

SOS1-TW2 -----

SOS1-TW4 -----

SOS1-TW3 TTTGAGCCGGGG---CTTTCA-CATCAGACTA-----AGAAACGGCTGCGA---GCCC

SOS1-LW6 GTTGGGGGGGGGGGGGTTTTAGTTTTGGAATGTAAAAGAAAAGCGCCTGGGGAGTGCCC

SOS1-LW7 -----

SOS1-LW5 -----

SOS1-TW1 -----

SOS1-TW2 -----

SOS1-TW4 -----

SOS1-TW3 TTA----CGCCAATAATTCGGACACGCTGCCA-CTACGTATTAC-AGCGGCTGCTGGCA

SOS1-LW6 TTGGCCAGCGCAACGAGGTAGGGGGTAGGATTGGCTATGGGTAACGAGCAGTAATTGGCA

SOS1-LW7 -----

SOS1-LW5 -----

SOS1-TW1 -----

SOS1-TW2 -----

SOS1-TW4 -----

SOS1-TW3 CGTATAGCGAGCT-----TCTGTAGTACCGTCAGTACGCCCTATCG---ACGGAAGT

SOS1-LW6 GGGG-GGTGGGCTGGGAAACGGGTGAGTTGTGAGGGGGGGGTTGATGGTGGATGTGGAGG

SOS1-LW7 -----

SOS1-LW5 -----

SOS1-TW1 -----

SOS1-TW2 -----

SOS1-TW4 -----

SOS1-TW3 TCTCCTACAACAAGCTTACGATCGAAACTCATCGTCAGCGGGTTG---CTCGTCGACTTC

SOS1-LW6 GTCCGCGTGAGAGGGGAACGCACGATAGTCGTGAAGGGGGTGTGGGGTTCGTTACCGAT

SOS1-LW7 -----

SOS1-LW5 -----

SOS1-TW1 -----

SOS1-TW2 -----

SOS1-TW4 -----

SOS1-TW3 T-GCATTGGTAAGATTCCTATGTGATC-----

SOS1-LW6 TAGAACCCAGAAGGACGGAGAGTGAAGGGTGAAAGGGAAGTGTGCGCCGGGGTGCAGGGGC

SOS1-LW7 -----

SOS1-LW5 -----

SOS1-TW1 -----

SOS1-TW2 -----

SOS1-TW4 -----

SOS1-TW3 -----
 SOS1-LW6 GGGGGGGCTTGTGGTCCGGGGTGTCTGAATTGTATTGGCCGCAGTGAAGGGGGAGAGGAAA
 SOS1-LW7 -----
 SOS1-LW5 -----
 SOS1-TW1 -----
 SOS1-TW2 -----
 SOS1-TW4 -----

SOS1-TW3 -----
 SOS1-LW6 GATTCTTGAGAGCAGGTGGCGGTCTGACTGCGAATAGGCGAGAGAAGTGCGTGGACACGG
 SOS1-LW7 -----
 SOS1-LW5 -----
 SOS1-TW1 -----
 SOS1-TW2 -----
 SOS1-TW4 -----

SOS1-TW3 -----
 SOS1-LW6 CGGCGACTCGAGGAAACGCGCGGTGTGGGTGGGAGGAGGAAGGAAAAGCCTGGATGGTGT
 SOS1-LW7 -----
 SOS1-LW5 -----
 SOS1-TW1 -----
 SOS1-TW2 -----
 SOS1-TW4 -----

SOS1-TW3 -----
 SOS1-LW6 CGACACATGCAATGGGTGAGGGTAGCGAAACGAGTGAGGGAGGAACTCGGTTGAAAACC
 SOS1-LW7 -----
 SOS1-LW5 -----
 SOS1-TW1 -----
 SOS1-TW2 -----
 SOS1-TW4 -----

SOS1-TW3 -----

SOS1-LW6 TGGCGGGAAGGGTAAGGGGGGAACGAGGGGGGGGTATAGGGAGGATGGTGGGGGGGGGG

SOS1-LW7 -----

SOS1-LW5 -----

SOS1-TW1 -----

SOS1-TW2 -----

SOS1-TW4 -----

SOS1-TW3 -----

SOS1-LW6 TCCGGATTCGAGAGGTTGAACGAGGGGGTGGGGGCAGACAAAAAAAAAAGGATATGGGACA

SOS1-LW7 -----

SOS1-LW5 -----

SOS1-TW1 -----

SOS1-TW2 -----

SOS1-TW4 -----

SOS1-TW3 -----

SOS1-LW6 GGAGGAATGAGGGGAGAGGAAGGAACATCGGAGGAGAGGGGGAGGGTGGGATGGCAA

SOS1-LW7 -----

SOS1-LW5 -----

SOS1-TW1 -----

SOS1-TW2 -----

SOS1-TW4 -----

SOS1-TW3 -----

SOS1-LW6 CAACCCAACAAAACAAAAAAACCCAACAAAG

SOS1-LW7 -----

SOS1-LW5 -----

SOS1-TW1 -----

SOS1-TW2 -----

SOS1-TW4 -----

Alignment of RAS1 sequences:

RAS1-LW6	ACATGCACTTTAGCAGATTTGTAAGA----TTTAGCTAACATA---GTGTTATGGGTTT-
RAS1-TW4	CCAGGAACTGAAGCAAGTTTAAAAAAGATTTTGAAAAAGGCATGTGCTTCATGCGTCCT
RAS1-LW5	----AATGGTTTGCAATTCAAAAAAGCTTCTCTTTCCAAGCTT--AATTTTTGAAACA-
RAS1-LW7	-----
RAS1-LW6	-AATACTTCACAATGAATCTTTTA-CTTTGAGCCCGTTAGGATGA-CCAATTATCTAGT
RAS1-TW4	AAGCACTTCATAATGATCCCTTTAACTTTGAGCTCGTTAGGATAGTCCTATTATCTAGT
RAS1-LW5	GATTGGTTTGCTACTTTACTCATGGTTTGGCATTTCACACAAAAATAATCCAAACTTAGT
RAS1-LW7	-----ATGGTAAAAATACCCCCCTACGCGGC
RAS1-LW6	GCCCCAAAATA---CCCAA-CATAACGTACG-TATGT-TGATTGTAG-TAGCATTAATAA
RAS1-TW4	GCCGAAAAGAGGATCGCTA-CATCACGTATGATATGTCTGATTGTAGACAGCATTAATAA
RAS1-LW5	TTTAAATGCTGTTTTCCATTCATCTCCCAAT-TTCATACGAATTTGA-TGGCATCCACTA
RAS1-LW7	TACGGCAGGTCCGCTGCCGGCGACGACTACG-----AGAGGATAGCTAGGCTAAAGCC
RAS1-LW6	TATTACTATTCGTTATCGTCATCCTATTCTAGGCTAGATGATA-TGCTTGATGAATTGA
RAS1-TW4	TATTACTATTCGTTATCGTCATCCTATTCTAGGCTAGATGATA-TGCATGATGAATTGA
RAS1-LW5	CGCAA--ATCAACTTTGGAGAA--TATTGTAGAGCCACTCAATT-CATCAAGCATATCAT
RAS1-LW7	TACAAATTAGGAAGAGGGGAAGGCTATTGGAGTCCTCGTTTTTTGTCTTGGTGGAGGAA
RAS1-LW6	GTGGCTCTACAAAATGCTCCAAAGTTGATTTGCGTAGTGGATACCATCAAATTCGTATGA
RAS1-TW4	GCGGCTCTACAATATTCTCCAAAGTTGATTTGCGTAGTGGATACCATCAAATTCGTATGA
RAS1-LW5	CTAGCCTAGGAATAGGAT--GACGATAA--CGAATAGTA-ATATTATTAA-TGCCTCTAC
RAS1-LW7	GGAATACAGCCCCGTCTCCAGGGACAGGCCCTTGGCAGGAACCTCCGG-TCCGGAGGC
RAS1-LW6	AATTGGGA-G-ATGAAT-GGAAAACAGCATTT---AAAATAAGGTTGCATT---ATATG
RAS1-TW4	AATTGGGACG-ATGAAAAGGAAAACACCATT---AAAATAAGTTTGGATT---ATATG
RAS1-LW5	AATCAACACACATACGTGATGTACCATCCTTTTTTCGGCACTAAAATAATAGG---AACAG
RAS1-LW7	GACAACTACG-ACACCGGTTACAACAAGCAGTCCAGCAACGAGGAGTACGGCCGCAACAA
RAS1-LW6	AGTGGTTAGTCATGCCTTTAGGGGTAACTAATGCACCTAGTACTTTCAGTAGATTAATGG
RAS1-TW4	AGTGGTTAGTCATGCCTTTAGGGGTAACTAATGCACCTAGTACTTTCATGAGATTAATGA
RAS1-LW5	CACAAGGACTAAGGGACTCGCGGCTGTACACCTTTGTGCAACCTCTTGAC-TTAACGC

RAS1-LW7 GCCCCGCGACGACGACTACGACCGCAGCGCCGGCGGGTACAAGAAGTCCGGCGACGATGA

RAS1-LW6 ACTGA-GTTTTACGAGCGTGCGTTGGACGATTTGTGTCAGTTTACTTTGAGCCCGTTT--

RAS1-TW4 ACGAA-GTTTTACGTGCTTTCATTGGACGATTTGTGGTAGTTTACTTTGACCGCTTTTGG

RAS1-LW5 ACGCCCGACTTACAGGC---CAGTGGATTGTCAACAACCTATCATTTTTTTTCATTTCAA

RAS1-LW7 GTACAGCGGTGGCGGGTAC-AAGAAGGCGGGTGGCGAC-GACGAGTACAGCGGTGGCGGG

RAS1-LW6 ----GGATGCTATTGCCCTAGCAATAAAAATTGGC-----CTGGCCCCCCTTCACATGTG

RAS1-TW4 AAAAGAAGGCCGCTGCCCCGCTCCTTGGGTCTGGG-----TGGGTGCCTTTCGC-CGTG

RAS1-LW5 CCACAAAAGCTATTGCCCTAGCAATAAAAATTGGC-----CTGGCCCCCCTTCACATGTG

RAS1-LW7 TACAAGAAGTCGGGGGGCGACGACCGGGGGTACAGCGACGACTACGACCGCAGCGCCGGCG

RAS1-LW6 ACTCCCAGAA-GTCCTCCAAGGCCTGACCCTGCAAACGGGCTTAATCCTCCTCACATGAC

RAS1-TW4 GCTCCCTGTG-GTCCGTTGGGGCCTTCCCCGAAAACGGGGTTA--TCGTCTCGACCGAC

RAS1-LW5 ACTCCCAGAAAGTCTCCTCCGAGGCCTGACCCTGCAAACGGGCTTAGTCTCTCCGCATGAC

RAS1-LW7 GGTACAAGAA-GTCCGGCGACGATGAGTACAGCGG--GGAATAAAGGGCGCCAGAAAGAA

RAS1-LW6 AAAAACTAGCCCCAAAG-GACCGCCTTCTTTCAATTTTCTAGCAAGACC--AGCTAAACC

RAS1-TW4 AAAAACTAGTTCCACA-GCCCTCCCTCTA-CACAATTCTAACCACGCC--ACCTGACCC

RAS1-LW5 AAAAACTAGCCCCAGGAGCCCGCCTGCCAGCAAGTTTCTAAGCACGCCTAGGCTGACCC

RAS1-LW7 ATTTAAAATTTTCCACACCCCCCCTCCGGGGAAAAACCATATCTCC--GAGGAAATC

RAS1-LW6 TTCTAATAAATGGGGGAATCCTTTCTACAATCCTGGGGGGT--GGGGGGGATTAAACC

RAS1-TW4 TCCCAGTA--TTGGGGGCAGTCCTTCTACT-TCCTTGGCGGT--GGGGGGGCATTAACCC

RAS1-LW5 TGCCAAGAAATGGGGGGGAGGCTTTTACGATCCTTGGGGGTGGGGGGGGGTTAACCG

RAS1-LW7 TGTTTTAA-TAAAGGGGAGG-TGAGTACA---GCGGCGGC---GGGTACAAGAAGTCC

RAS1-LW6 AACTTCTGCTGCGC--AAAGCCTAG-CTTTT-CATTCAATGCCC---CTATTGGG--GGA

RAS1-TW4 AACTCCCCTTCGGC--AAATCATAGGCATT-CATCAGACGCCC---ACATGGGGCTGAG

RAS1-LW5 GGCTCTAGCTACGCGAAAATCTAAGGCTTTTTCATCGGGTCGCCTGGACATTGGATGGGG

RAS1-LW7 GGCGGCGACGACAC--AGAGTACGGGT-----CGTCCAGGG-----ACGAGACCGAG

RAS1-LW6 AAATCCG-----GAAATAAATT--GCACACCAGACTCTCCC--TTTTTAACCGTG-GAA-

RAS1-TW4 TAATCCTCCGCCAATAAAAATC--GCACCCCAACCTCTCCCCTTTTTTACCCCG-GGGG

RAS1-LW5 AAGTCTGG-GTAGATGAAAGTTTTGCAACCCGACCGTGGCCCTTTTTTGGAGGGGTGGGA
 RAS1-LW7 AAGTACAG----GAAGGAGGAGAAGGAGCACAAGCACAAGGAGCACCTCGGCGAGATAGG

 RAS1-LW6 ----TGCCTAAATCA---ACTTACCA-----TTATCTCTTTTTTCAAATT----ACTA
 RAS1-TW4 AATTTACCTAAACAGGAACCTACCCCTATATTTTTCTTAATTCTGAAATTTTCCACTC
 RAS1-LW5 GAGTTGGGTGAAGCCAAGAGCTACCGC-TAGATTTTCTTTATCTCTGAAATT--CAGCT
 RAS1-LW7 ---CACCTCGCCT-----CCGGCGC-----CTTCGCCATGTACGAGAAG---CACCA

 RAS1-LW6 GAGTTTC-----AAAAAATTTAGAATC-ATCTTTCAG---TGGCCCAGGGCCAACTTAA
 RAS1-TW4 AGGCTTTTCGAAAGAAAAAATTAGCACG-AAACCCTTAT---TCTTCCGG-CCGTTTGGG
 RAS1-LW5 GGCCTTCGAAGGAGGATTATCGAGGACGTAATCGTTGGGGTTGGGGCGGAGGGGTCCCAA
 RAS1-LW7 GGT-----GAAGAAGGACCCGGAGAACGCGCACCGACACAAGATCGAGGAGGAGGTGG

 RAS1-LW6 CGGCTATAGATACATTC-----TCTATTGGTCGCA-----GCGGGC
 RAS1-TW4 GCCCCGAGGGGGCCCC-----GCCAACGCCC-CT-----TTAAAA
 RAS1-LW5 AGCCTTAAAGCGGCCCCAGCTAAATGCCATTGGCCGTTTGGTGAGCATAGAGGCGGGGGC
 RAS1-LW7 CCGCGGTGGCGGCCGTG-----GGCAGCGGCGGTT-----ACGCGT

 RAS1-LW6 TTTCGTCTCTCTAGGTTTAGGCAAAAGATTAATGGCCAGAACTAGAAAA---CATCCAC
 RAS1-TW4 CCCCCCCCCCTAACCTAAAAATACCTATTCTCGCCCTTATTTTGGGGG---CCACCGA
 RAS1-LW5 CTTTTCCGGCCCCCTTTTGAAGGGGTTTTGGGGGGAAGGGGGAAGGGGGAATTTGGCCCA
 RAS1-LW7 TCCACGAGCACCACGAGAAGAAGGAGTACAAGGAGTCGTCGGCCCTCACAACGGAGGAGG

 RAS1-LW6 AGCTTCCTCAGAGTTATCTCTCTCCAC-CCTCTTGCGCCTTTTTTCTAGGCTAGGGGAT
 RAS1-TW4 AAAAGGGGCGGGGGGGCCCTTTTTCTACCCCCCCCCCTCCCTTCTCGGGGGGGGGT
 RAS1-LW5 AAGAAGGGTGTGGGAAAAAAAAAACCTAAAGAGGGAGATTGCGCTTGAAGATGTAT
 RAS1-LW7 GAGTGTCTCGGGCCAAGTAAGAACAAAACATAAATAATATTATTATCTATTTAAGAAA

 RAS1-LW6 TACGGCGTCGCTAGCCCAACTATCTCTTCCGCGACTTGGCAGCT-TAGCTTAC----GA
 RAS1-TW4 TTCTTCACTGGGGGCGCGCAAAAAAGGGGGGTAAAAATCTATGGCCCCCCCCGAAA
 RAS1-LW5 TTCCCTCCCG-TGGCCCCCCCCCTTGATGGCCGCTTTTTTTTTTTTGTATAGA
 RAS1-LW7 TGAGATGACAAAAACATAATATTATACTGACTGAATATATAATTCATGT-----GA

 RAS1-LW6 CAGTCCCGTGATCTCACCTCATTCGTCTTATCCAGCTGCTATAATGAGCAGGCGAGA-

RAS1-TW4 GAAACCCCTTTTGTGGAGAAAAACCCAAAAAATTACCTCTAAAAAGAGGGGAGGGTT-
 RAS1-LW5 GGGGAAGAGGGGGAGGGTATTTAAGGGGCCGCCCCGCCGCGAGACCGCCAAAAGAATA
 RAS1-LW7 AAGATAATTAATAT-GAAATATAAGTGAT-----

 RAS1-LW6 -CATA--GCGGACGACGGACAGACTGA-----
 RAS1-TW4 -TTTTCCGCGCAAAAAGAATATATATATTTCTCCCCCCCCCTCCTCCCCCCCCCCC
 RAS1-LW5 TTTTCTCCTCGTTGCCAGGGCGGGAGGGGGTTGTGGGCCGCCACGCGCTTATATAGATCG
 RAS1-LW7 -----

 RAS1-LW6 -----
 RAS1-TW4 CCCCCCTCTTTAGGCGCCCGCCGCTGTTTTTTTTTTATTATCATAATCAGGGGCGT
 RAS1-LW5 TTTACGAGATGCCGATGGTGGCGCCCCCTGTTGAATGTTATTCGACATGCCGCCCTC
 RAS1-LW7 -----

 RAS1-LW6 -----
 RAS1-TW4 GAGAGGGAGCGGAGGAGGTTTTAGGGGGGCGCCCTCCCCGCCCCGAGGAGGCGCCCC
 RAS1-LW5 CAAATGGTTGTCGCGGGCCCTTTTTTAATCGCCCCGCCGCGGCGGTGGGGGTTTTTG
 RAS1-LW7 -----

 RAS1-LW6 -----
 RAS1-TW4 CAAAAAAATACTTTTCCCCCTTTTCCCCCGGGGCGAGGTGTGGGTGGGGCCGACCAC
 RAS1-LW5 GAATAAAATTTGTAAAAAGCATTTGGCGCGGAGGGAGGAGGGGACGGTCGGTCGCACAC
 RAS1-LW7 -----

 RAS1-LW6 -----
 RAS1-TW4 CTTTTAAAAGCCTTTGAACGCCGAGCGAGGGCCCCC-----CCTTAGAAATTTTC
 RAS1-LW5 CGTTGTGGAACAGCGAAGAAGTGGGGGAGCGCGATGTGGGGGGGTGCCGAGGGAGTGA
 RAS1-LW7 -----

 RAS1-LW6 -----
 RAS1-TW4 CTCCCACCCCCCTTTTCTCCCTCCCTCTTTTACTCCCCCCCCCGGCGGGTGG
 RAS1-LW5 AAGCCAAACGCGACGATTGCGTGGGCGCGACGCTGTGGGCAGGCGAGGGACGGGTAAAA
 RAS1-LW7 -----

RAS1-LW6 -----
RAS1-TW4 GGGCGACTAAAAGAAAATTATAAAAAATTTTGGG-----
RAS1-LW5 TTCCGGGCGGTGGAGAGCGGGCGTAGGGCTTGAGGAGATGAATTGACCATATATATCTGG
RAS1-LW7 -----

RAS1-LW6 ---
RAS1-TW4 ---
RAS1-LW5 TGC
RAS1-LW7 ---

Alignment of TAS14 sequences:

TAS14-TW2 -----ACAGCACTGCG-----
TAS14-TW3 -----ACGGTTTGTATGAGTTTATGAGCCCTGATGTATTGT
TAS14-TW1
GTGGGCATCTGTAGCATCTCGGGATAGGGTCGCTGGGGGTTCTAGCCCCCAAATGCGC
TAS14-TW2 -----
TAS14-TW3
ATGGTGCTTATGATGTCGAGGATTACTTTGTCTATTAATCCTATTCCGCATCAACTGAA
TAS14-TW1 AAG-----
ACTGATGAATATGGAAACCATGTCCAAGAAACAGGAGTCTATCAAGGTA
TAS14-TW2 -CCGTTTCGGAAACAGAGTTTCGGAATAAGCATGTGTTTATGGGCTCTTACACT-----
-
TAS14-TW3
ATCGTTCTTACGTCAATTACCCACCATAGGCGTTTGTATAATATCATTCAATTAAGATGTG
TAS14-TW1
CCGGTACTGTACTGTCCACCTACAATGCACCTCCGTGTAATTTCTATTATGTTTCGACGG
TAS14-TW2 -----TCGCACTAATCTTTTACTGTCTGCAG-----CCTCGTTAGGGCCTCC--
TAS14-TW3 TGTTCCTTCGCCCCCTTATCATAAGTCTGTTA-----CTTATTTTGTGCCAATGC
TAS14-TW1
CGGTATGATGGGGGGCACGGGTACTGGCGGTATGATGGGGGGCACTGGTGGAGAATATGG
TAS14-TW2 -----CATTAATTAAGAAGAGGATAGCTAG-----

TAS14-TW3
 AAAATATACAAAAATATTTTTGCAGATTGATCTAAGACTATTCACCGCTACCAAAT--

TAS14-TW1
 AACTCAAGGTGCTAGTCATAAACCATGGCGTTCCAACCTATCTGTATGTATATATATAGG

TAS14-TW2 --GCTAAAGCCTACAAATTAGG-----AAGAGGGGAAGG-

TAS14-TW3 CAGAAATTGCCCTCGAATTTTATTACGGCATATTATCCACCCATT--
 AAGATACGATCG-

TAS14-TW1
 CCGCCATGCCCCGGCCGCTGTGTTTTGTACTTCCGTAAAATCTTTAGAAAGATCTGATACG

TAS14-TW2 -----CTATTGGAGTCCTCGTTTTTTGTTCTTGGTGGAGGAAGGGAATACAGCCCCG

TAS14-TW3 -----ATAAACAGGCCAAAGATTTTTCCTTTCTAAAGATGGTAAAAATACCCCCC

TAS14-TW1
 TTTTATAAAAAAACCTACCCTGACTTTTTGTTGATTTTCAAGACTGTTTTGACAGAGAAA

TAS14-TW2 TCTCCCAGGGACAGG-----
 CCCTTGGCAGGAACTTCCGGATAATCTAATGAGGCTAGC

TAS14-TW3
 CTACCCAACCAACTATTACATACAATAGCACCGCCTCCCATTAATTAAGAAGAGGATAGC

TAS14-TW1
 TTGAAAAATTAAATACTAGAAAATCCCACTCTACCCACTTTTCGGCAAACGCGAGGATTAC

TAS14-TW2 GG-----CGACATACGATGCTCCCTCTCGTATATGTGTT

TAS14-TW3
 TAGGCTAAAGCCTACAAATTAGGAAGAGGGGAAGGCTATTGGAGTCCTCGTTTTTTGTTC

TAS14-TW1 CGGCCCCGGGCCCATCG-----AGTCTAACCTCGTCCACCGCTCGTGTCCGGTAT

TAS14-TW2 GCGTTGCGGACACCCTAATA-----

TAS14-TW3
 TTGGTGGAGGAAAATATTATACTGACTGAATATATAATTCATGTGAAAGATAATTAATAT

TAS14-TW1 TTATGAAAGCGAGGCCGATGATAGAGG-----
 GCTCATTCGGGTGATTGCCGTATTGGGG

TAS14-TW2 -----

TAS14-TW3 GAAATATAAGTGAT

TAS14-TW1 CCATCTAAAAAG—

APPENDIX 6: Amino Acid Sequences

Amino acid of SOS1 sequences

>SOS1-TW1

STAIKSLTFVPAGSRCTSRPTGTRFAGRIRRAFLFPPAAPSCSQRISHFNPCIR
WLGAGLDPGLVHWTTLIKRTASVIVKMVPSWIPWNWTCELAYHLHV
CPQARPGNHQEQMATKRWDHMERAAADCRQDWGVSGDPYGVVEGLDEW
VFYRENADVVLDPATCAIFDMAQFSGSIIIGILEEKRLMQNPLSGVSCDR
GQRSGIRRHGCKGLQALDVLGWSTGERLFLVLAH

>SOS1-TW2

LHWPYRLPQCVSLIEPVNSAISKLTESHHRVEHNDVDIVAVKDPFIEPHYAVGI
PGYKTVFQTIRCAFMIAAVGGHLFLVDCRPTSMAYMLKYDSSHGQFKGE
IKVDGNNLTVNGKTIRFHMEKDPANIPWSETGAYYVVESTGVFTTTEKDK
AHLKGGAKKVVISAPSADASLFTGVNRRD-QNKAKEFSNPL

>SOS1-TW3

RGEGRGEKEEKIRLERTLPSDTLWNRTTRAQQATLDDPEANQKNFDHSSS
CAAESHTDMSLFGQLQSSSSLYFHQSRSVCFLKQKQFILVLKGWMIHCVVC
SPGLAPALVYTVPFMVATTIKGCARCGTPNISRHETTTLHLHSLCPRRGRP
MTRIVRGWLDLVRVFPLLGIKPHAPDCAGPRAFLSVLLGPYSAGGDFDV
LAAALKGLTPPKTYYSVAAWTARDLSQYAPHAFAPQRQLQTRESASPLVF
LHISTHFTATRGIPLSSALKFPVSNLDPGLSRGFHIRLRNGCEPLRQFGHAA
TTYYSGCWHVRASVSTVSTPYRRNCLLQQAYDRNSSSAGCSSTSALVRFL
CD

>SOS1-TW4

ELRRALRFELAPRCTARPAGSCCPEGPQCDWSWMVACCLGEMDLRLMAH
SMLYSSARSLICSTGCWTSYKFARENRCRVSVESPRERTVILGASSLAAP
RPSEVGTLTKSWPTFIKIKNMPHSVCFSTAYPTSATDDWADRRSWFEGDGR
GGYRFCATLSIPHHGFWRSVHEFSYVTPLKYPHSHHNSPQRQTRMSSSATS
KRVAGSRVCSISSEPIGLHST

>SOS1-LW5

AWGSFDDFIDEAFHLRCPCRPDAKSLNPPTTRTYHSCIHILVQGLAEKLPD
WLLISLSLHFQALSPETLPHLSKPTCALRSARGRPSHEEPTTHFMVAGKRF
AIRGSISEVILNGGKYQLLDHVVRRCVLMGKPTACTLQMRRCPHEGRML
KRSRRGGLERVRFEQIEGNFDKGQTRLRRGSCLDTCTLRERTPMAIEFCFS
DGELKALSRTKTTFSVWWGMNCPGNCDRLARQVDAPRGRYERIVPFAPG
CSHR

>SOS1-LW6

RERPVERRRADLIVLYYLIPCTQPEEHRHLIGGISEDREP NFDHTSSCAA AV
KHFELIWPVFRFSIFSSIKQWLFSEAKAVIYFLLKVIECSALLFTGMNQIDY
CYTAPSYQVEARARVILFEMRSPDIESDLQRSASLLSPALGPSRTQSKEHDR
LLRWPERFRRSRKARNASLAEMPRQAASFSGGGFLLSIGLVVHAPLAFSFA
TSDRGTVRVIDASKMTTFERWDPELFEGSHFQFRRLNSLGNHLIKDTEKSG
FGRGGFPTGTPTQNSTGFTWGVVPDRFW EYGFSELSKRAVRGGWGGGGF
QFWNVKEKRLGSALGQRNEVGGRIGYGRAVIGRGVGVWETGELGGGWW
MWRVRVRGERTIVVKGVLGFTDNPEGRRVKGERELCAGVRGGGACGP
GCRIVLAAVKGERKDSEQVAVLRIGERSAWTRRRLEETRGRVGGRRKEKPG
WCRHMQWVRVAKRVREETRLKTWREGGGNEG GGIGRMVGGGSGFERL
NEGVGADKKKGYGTGGMRGEEGTSEERRGRVGVWQQPNKTKKTQTK

>SOS1-LW7

TNPGKPILETS GIPVNQPRSPNSTGSPPPVVSQQGHKRCCVSDRFLSCPRHL
LRGIAKIGGAVMQSGQQIMRVVGF GIRKGLFFNVLDEEVNVPYPHESDSH
RYGDNSYQVEARARGIIFEMRRPDIESDLQRSGSLFSAAFGPSRTQSKEHV
GLLRWPESFRRSSGPGKQGNGKSEVRPLASRISSCRLASRILLQEVRSLLVG
HLKDFADSGLHGHRWRVLSSNQPVQPSIYS

Amino acid of RAS1 sequences

>RAS1-TW4

RNSKFKKRFWK RHV LHASALHNDPFNFELVRIVLLFCRKEDRYITYDMSD
CRQHYYYSLS SSYSARYAIERLYN ILQSF AWIPSNSYEIGTMKRKTPFKTKF
GLYEWLV MPLGLTNAPSTFMRLMNEVLRAFIGRFVVVYFDRFLKRRPLPR
SLGLGGCLSPWLPVVRWGLP PKTGLSSRPTKTSSHSPSTQFPRHLTLPVL
GAVLLL PWRWGGINPTPLRQIIGISSDAHMGLSNPPPIKIAPQLPFFYPGGI
YLKQEPTYIFLNSEIFHSGFRKKKISTNPYSFRPFGAPRGPPPTPLNPPPLTK
YLF SPLILGATEKGAGGPFFSPPPPSLLGGGVSSLGARQKKGGGLKIYGPPRK
KPFLLEKKPKKLPLKRGE GFFPRKKNIYISSPPPPPPPPPPPLAPAALFFFY
HNQGRERERRRFLGGRPPPPPRRRPQKKYFSPFFPPGRGVGGADHLLKAF
ERRSEGPPPKFFLPPPPFFSPPPLLLPPPGGWGRLKENYKKFW

>RAS1-LW6

HALQICKILTCYGFNTSQIFYFEPVRMTNYSSAQKYPTRTYVDCSSINNITIR
YRHPIRLDDMLDELSGSTKCSKVDLRSGYHQIRMKLGD EWKTAFKTKV
ALYEWLV MPLGVTNAPSTFSRLMDVLRACVGRFVS VYFEPVWMLLPQKL
AWPPFTCDSQKSSKAPCKRASSSHDKNPQRTAFFQFSSKTSTFMGGILSTIL

GGGGGLNQLLLRKALFIQCPYWGKIRKIAHQTLFPWNAINLPLSLFSNYS
FKKFRIIFQWPRANLTAIDTFSIGRSGLSSLVAKDWPETRKHPQLPQSYLSPP
SCAFFSRLGDYGVASPTISSATWQLSLRQSRDLTSFVSYPAAIMSRRDIADD
GQT

>RAS1-LW5

HQIYMVNSSPQALRPLSTARNFNPSLACPQRRRAHAIVAAGFHSRLRHPPTSRS
PHFFAVPQRCATDRPPPPSAPNAFYKFYSKNPHRRAAGRLKRGPAITTIWRG
GMSNNIQQGRRHRLVNDLYKRVAAHNPLPPWQRRKNILLGGLAAGPA
PIPSPLQKKKRRRAIKGGGGPREGNTSSKRKSPSLGFFFFPHPSLGQIFPL
FSPQNPFKRGAGKGPRLYAHQTANGILGPLGFGTPPPQPQLRPRSSFEGQL
NFRDKENLAVALGFTQLSHPSKKGPRSGCKTFIYPDFPIQCPGDPMKKPIFA
LEPGPPPQPPRIVKSLPPISWQQQPRRAKLGRRAPGGFLSCGEDARLQGG
ASEDFLGVTCEGGPGQFLLGQLLWLKKKMIGCQSTGLVGRALSTRGCRQ
RQPRVPSLCCSYFSAEKGWYITYVCLRHYYSLSSSYSARYAIEWLYNIL
QSAWMPSNSYEIGRMENSINVWIIFVNAKPVKQTNLFQKIKLGKRSFFEL
QTI

>RAS1-LW7

HLYFILIIFHMNYIFSQYNIMFLSSHFLNRYNNIYVLFLLGPRHSLLRCEGR
RLLVLLLLLVVVERVTAAAHGRHRGHLDDLVSVRVLRVLLHLVLLVHGE
GAGGEGAYLAEVLLVLVLLLLLPVLLGLVPGRPVLVCVVAAGLLVPAAAVLT
SPLLKTDFLGDMGFSPEGGVWENFKFLSGALYSPLYSSSPDFLYPPALRSSS
LTPGRRPPTSCTRHRCTRRRHPPSCTRHRCTHRRRTSCTRRRCGRSRRRA
CCGRTPRCWTACCNRCRSCRLRTGSSCQGPVPGRRGCIPFLHQEQKTRTPI
AFPSSFVGFSLAILSSSPAADLPPRRGGIFT

Amino acid of TAS14 sequences

>TAS14- TW1

WASVASRDRVAGGSPPKCARLMNMETMSKKQESIKVPVLYCPTYNAPPCN
FYYVRRRYDGGHGYWRYDGGHWWRIWNSRCSTMAFQLICMYIYRPPCP
AAVFCTSVKSLERSDTFHKKTYPDFLLIFKTVLTEKLKNILENPTLPTFGKR
EDYRPGPIESNLVHRSCPVMKARPMIEGSFGLPYWGHLK

>TAS14-TW2

LGVSATQHIYEREHRMSPLASLDYPEVPAKGLSLGDGAVFPSSTKNKKRGL
QPSPLPNLALALSSSLMGGPNEAADSKRLVRSVRAHKHMLIPKLCFRNGA
VL

>TAS14-TW3

ITYISYLSFTIHSVSIIFSSTKNKKRGLQPSPLPNLALALSSSLMGGGAIVCN
SWLGRGGIFTIFRKGKIFGLFYRSYLNQWIIICRNKIRGQFLIWRIVLDQICK
NIFCIFICIGTKVTDLGGRKNTHLNEYTAYGGLTERFQLMRKDTKSSTSA
PYNTSGLINSYKP

A similar SOS1 gene sequence

>SOS1-TW1

AAGTACCGCAATCAAAAGCCTCACCTTCGTTCCGGCAGGCTCGCGGTG
TACATCGAGACCAACCGGCACGCGATTTGCAGGAAGGATTCGCAGAGC
GTTCTGTTCCTCGCCCTGAGCAGCTCCGAGTTGCTCGCAAAGGATTTCT
CATTTTAACCCATGCATTCGATGGCTAGGCGCGGGGCTAGATCCAGGAT
TGGTACACTGGACTACGTTGCTGATTAAGCGTACGGCCTAGTCCGTGAT
AGTCAAGATGGTGCCGTCACCCTGGATCGCACCTGGAAGTGGACGTG
TGAGCTGGCATAACCATTTGCATGTCTGCCCACAGGCACGCTGACCTGGG
AACCACCAGGAACAAATGGCCACCAAGCGCTGGGACCATATGGAACGC
GCCGCGGATTGTCGACAAGACTGGGGTGTATCTGGGGATCCCTACGGA
GTAGAGGGGCTTGACGAATGGGTCTTTTACCGGGAGAATGCCGACGTC
GTTGTGCTCGACCCTGCGACATGTGCGATCTTTGATATGGCTCAGTTTTC
GGGCTCAATCATTATTGGAATTTTGGGAAGAGAAGCGGTTAATGCAGAAT
CCTCTGTCCGGAGTTTCATGCGACAGGGGCCAAAGAAGTGGCATCCGT
CGTCACGGCTGTAAAGGGTTACAGGCTCTAGACGTGCTGGGTTGGAGT
ACCGGTGAACGGTTATTCGTCCTGGCGCACC

>SOS1-TW2

AGTAGTGGGTTTGAGAATTCCTTTGCTTTGTTTTGTCAGTCTCGTCGGTT
GACACCCGTGACGAAGAGGGAGGCGTCAGCAGAGGGAGCAGAGATGA
CAACCTTCTTGCTCCGCCCTTCAAGTGAGCCTTGTCCTTCTCGGTGGT
GGTGAAGACACCAGTCGACTCTACGACGTAATATGCGCCGGTCTCGCTC
CATGGTATGTTGGCGGGGTCCTTCTCCATGTGGAAGCGGATGGTCTTGC
CGTTGACGGTCAGGTTGTTGCCGTCGACCTTGATCTCACCTTGAAGTGC
GCCGTGTGAGCTGTCATACTTGAGCATGTATGCCTACATGGACGTTGGC
CTGCAATCCACTAGGAACAAATGGCCTCCAACCGCCGCGATCATATGGA
ACGCGCAGCGGATTGTCTGGAAGACTGTCTTGTATCCGGGGATACCTAC
GGCGTAGTGGGGCTCGATGAAGGGGTCTTTTACGGCGACAATGTCGAC
GTCGTTGTGCTCGACCCTGTGAGACTCGGTTAGCTTTGATATGGCAGAA
TTAACGGGCTCAATCAAACCTTACGCATTGCGGAAGACGATACGGCCAAT
GCAGAA

>SOS1-TW3

TCGGGGGGAAGGGCGCGGCGAATGAAAAGAAGAGAAGATCAGGCTTG
AATGAAGAACTCTTCCCTCTGATACCTTGTGGAACCGAACCAGAAGAG
CTCAACAGGCGACCCCTTGATGATCCAGAAGCGAACCAGAAGAAGCTTTG
ATCACAGCTCCAGCTGTGCTGCTGAGTCACACACTGATATGAGCTTATT
TGGCCTGCAGTCTTCAAGTTCTCTATATTTTCATCAATCGAGAAGTGTCT
GTTTTCTAAAGCAAAAACAGTGATTTATTTTGGTCTTAAAGGGTTGGAT
GATTTGACATTGTGTTGTGTGTCAGCCCAGGTTTGGCACCGGCGCTGGTT
TATACAGTGCCCTTCTGAATGGTGGCAACTACTATCAAGGGTTGCGCTC
GTTGCGGGACTTAGCCCAACATCTCACGACACGAGCTGACGACAACCA
TGCTCCACCTGTCACTCTGCCCCCGAAGGGGACGTCCTATGACTAGGAT
TGTCAGAGGATGGCTTGACCTGGTAAGGTTTCGTCCCGTTGCTTGGAATT
AAACCACATGCTCCACCGGATTGTGCGGGGCCCCGTGCTTTCCTTTCAG
TTTAACTCTTGGGACCGTACTCCGCAGGCGGAGATTTTGATGTGTTAGC
TGCAGCACTGAAGGGGCTCACACCCCCTAAACATATTATTCGTCCGTT
GCGGCGTGGACTGCCAGGGATCTGAGCCAGTACGCTCCCCACGCTTTC
GCTCCTCAGCGTCAGTTACAGACCAGAGAGTCGGCTTCACCGCTGGTG
TTCCTCCACATCTCTACGCATTTACCGCTACACGTGGAATTCCACTCTC
CTCTTCTGCGCTCAAGTTCCCAGTTTCCAATGACCTCCCCGGTTTGAGC
CGGGGCTTTCACATCAGACTAAGAAACGGCTGCGAGCCCTTACGCCAA
TAATTCGGACACGCTGCCACTACGTATTACAGCGGCTGCTGGCACGTAT
AGCGAGCTTCTGTTAGTACCGTCAGTACGCCCTATCGACGGAAGTGTCT
CCTACAACAAGCTTACGATCGAAACTCATCGTCAGCGGGTTGCTCGTCG
ACTTCTGCATTGGTAAGATTCCTATGTGATC

>SOS1-TW4

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GAGACCCGCGGGAAGTTGTTGCCCGAGGGACCGCAGTGCAGTGGT
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GCATTCGATGCTTTACTCGTCTGCGAGATCCTTGATATGCAGTTGTACTG
GTTGCTGGACGTCGTACAAGTTTGCCCGTGAGAACCGCGATTGCCGGG
TCTCGGTCGAATCACCACGGGAGAGGACTGTCATATTAGGGGCAAGTTC
CCTAGCTGCCCCGAGGCCGAGTGAGGTGGGGACCTTGACGAAAAGTTG
GCCAACATTTATCTAAAAGATCAAGAACATGCCGCACAGTGTTTGCTTT
AGCACGGCCTATCCTACCAGCGCCACGGATGATTGGGCCGATCGCAGG
AGTTGGTTCGAGGGGCGACGGGCGTGGTGGTTAGTATCGTTTCTGCGCCA
CTCTTTAATCTATTCCACATCACGGGTTTTTGGCGCAGTGTGCATGAATTT
TCTTAGTACGTAACCTCTGAAGTATCCGCACTCCCACCATAATTCACC
GCAGCGCCAGACAAGAATGTCTCCAGCGCGACGTCCAAAAGGGTTGC

AGGCTCAAGATGAGTGTGTTCTATTTCCAGTGAACCGATTGGTCTCCAT
TCTACCG

>SOS1-LW5

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ACCTTCGGTGTCTTGTAGGCCTGACTAAGCAAAATCCTTGAATCCCCC
TACCACTCGAACGTACCATTCTTGCATACATATCCTTGTGCAAGGGCTAG
CAGAGAAGCTACCAGACTGGCTTCTCATTTCGCTAAGCTTGCATTTCCA
GGCCCTCTGATCTCCGGAAACTCTCCCCCACCTGAGCAAACCTACATGC
GCTTTGCGCAGTGCTCGCGGAAGGCCAAGTCATGAAGAGCCAACCACC
CACTTCATGGTGGCTGGGTAAAAGAGATTTGCCATTCGCGGAAGTATTT
CAGAGGTTATTCTAAATGGAGGCAAATACTAGCAACTTTTGGATCACGT
AGTTCGAAGATGTCCGGTACTTATGGGCAAACCGACTGCGTGCACGCTC
CAGATGAGACGTTGCCCCCACGAGGGACGTCTCATGAAAAGGAGTCGG
AGAGGAGGGCTTGAGCGGGTAAGGTTTCGAACAAATTGAGGGCAATTTT
GACAAAGGCCAAACAAGATTAAGACGGGGGTCGTGCTTAGACACCTGC
TGA ACTCTTCGGGAAAGGACACCGATGGCCATTGAGTTTTGTTTTTCAG
ATGGTGA ACTGAAGGCGCTCTCACGCACAAAAACGACTTTCTCCGTCT
GGTGGGGAATGA ACTGCCAGGTA ACTGTGATAGGCTAGCTCGTCAGG
TGGACGCTCCCCGCGGAAGGTACGAACGGATAGTCCCCTTCGCCCCCTG
GGTGTTCCACAGGA

>SOS1-LW6

GCGATGAGAACGACCAGTCGAGAGACGAAGAGCATGATGATAAGATTT
GATTGTATAACTCTATTATCTGATCCCTTGTTGAACCCAACCAGAAGAAC
ATAGGCATCTTATTGGAGGGATTTCTGAAGACAGAGAACCGAACTTTGA
TCACACCTCCAGCTGTGCTGCTGCCGTCAAACACTGATTTGAACTTATT
TGGCCTGCAGTCTTCAGGTTCTCTATATTTTCATCAATCAAGCAGTGGCT
GTTTTCTGAAGCAAAAGCAGTGATTTATTTTCTTTTAAAAGTAATTGAAT
GCTGAAGTGCTCTCTTGTTTACAGGCATGAATCAGATAGACTACTGTTAT
ACTGCTCCCAGTTATCAGGTGGAGGCTAGAGCAAGGGTCATCTTATTTG
AAATGAGGAGTCCAGATATAGAATCCGATCTGCAAAGAAGTGCATCGTT
GCTTTCTCCAGCGCTCGGACCGTCACGAACGCAGAGCAAAGAGCATGA
CCGATTGCTCAGGTGGCCGGAAAGATTCCGGAGATCCCGAAAGGCTCG
AAATGCTAGCTTAGCTGAAATGCCAAGGCAGGCTGCGAGCTTCTCTGGT
GGAGGCTTTTTACTCAGCATTTAGGGTCTAGTGGTCCATGCACCCCTGG
CCTTTTCTTTTGCCACGTCAGATCGCGGCACTGTCCGCGTAATTGACGC
CTCTAAGATGACGACTTTCGAAAGGTGGGACCCTGAACTGTTTCGAGGG
TAGCCATTTTCAGTAATAATTTAGAAGACTAAACTCCCTGGGAAACCAC
CTGATAAAAGACACAGAAAAGTCCGGCTTTGGTCGGGGGGGGTTCCCC

ACCGGGACTCCTACCCAAAATTCAACGGGTTTCACGTGGGGAGTGCCA
GTGGACCGGTTCTGGGAATATGGGTTCTCGGAGTTATCCAAGAGGGCCG
TGCGCGGGGGTTGGGGGGGGGGGGGTTTTCAGTTTTGGAATGTAAAAG
AAAAGCGCCTGGGGAGTGCCCTTGGCCAGCGCAACGAGGTAGGGGGT
AGGATTGGCTATGGGTAACGAGCAGTAATTGGCAGGGGGGTGGGCTGG
GAAACGGGTGAGTTGTGAGGGGGGGGGTTGATGGTGGATGTGGAGGGT
CCGCGTGAGAGGGGAACGCACGATAGTCGTGAAGGGGGTGTGGGGT
TCGTTACCGATTAGAACCCAGAAGGACGGAGAGTGAAGGGTGAAAGG
GAACTGTGCGCCGGGGTGCGGGGCGGGGGGGCTTGTGGTCCGGGGTG
TCGAATTGTATTGGCCGCAGTGAAGGGGGAGAGGAAAGATTCTTGAGA
GCAGGTGGCGGTCTGACTGCGAATAGGCGAGAGAAGTGCCTGGACAC
GGCGGCGACTCGAGGAAACGC CGCGGTGTGGGTGGGAGGAGGAAGGA
AAAGCCTGGATGGTGTGACACATGCAATGGGTGAGGGTAGCGAAACG
AGTGAGGGAGGAACTCGGTTGAAAACCTGGCGGGAAGGGTAAGGGG
GGAACGAGGGGGGGGGTATAGGGAGGATGGTGGGGGGGGGGTCCGGA
TTCGAGAGGTTGAACGAGGGGGTGGGGGCAGACAAAAAAAAAAGGATA
TGGGACAGGAGGAATGAGGGGAGAGGAAGGAACATCGGAGGAGAGG
AGGGGGAGGGTGGGATGGCAACAACCCAACAAAACAAAAAAAAACCCA
AACAAAG

>SOS1-LW7

GGAGTAAATGGATGGTTGAACTGGTTGATTTGATGAGAGAACCCTCCAC
CTTCGGTGTCCGTGTAGGCCTGAATCAGCAAAATCCTTGAGATGTCCTA
CCAGGAGAGAACGAACCTCTTGAAAGCAAAATCCTTGATGCTAACCTGC
AAGAGCTGATACGAGACGCTAGCGGGCGGACTTCTGATTTCCCATTTCC
TTGTTTCCCAGGCCCGCTGGATCTCCGGAAACTCTCCGGCCACCTGAGC
AAACCGACATGCTCTTTGCTCTGTGTTTCGCGATGGTCCAAAAGCTGCAG
AAAACAACGATCCACTTCTTTGCAGATCGGATTCTATATCTGGCCTCCTC
ATTTCAAATATTATGCCCCTTGCCCTAGCCTCGACCTGATAGCTGTTATCT
CCATAGCGGTGGCTATCTGATTCATGAGGGTAAGGCACATTTACTTCTTC
GTCAAGTACATTAAAGAACAAGCCCTTTCTGATACCAAACCCCACTACT
CGCATGATCTGTTGACCTGACTGCATCACTGCCCCTCCTATCTTTGCTAT
CCCTCGCAGTAGGTGTGCGGGGCAACTTAAAAAACGGTCACTTACACA
ACACCTTTTGTGACCCTGCTGGGAGACGGGAGGCGGAGGAGACCCAG
TACTATTCGGAATCCTTGGCTGATTGACTGGTATTCCTGAAGTTTCTAGG
ATTGGTTTACCAGGATTGGT

>JQ063077.1 *Triticum aestivum* cultivar HD 2687 salt overly sensitive

ACACACTCATTTTCATACTGAGTGGGGTTGTTATTGCAGATGGTGTACTA
CAAGATAATATTCATTTTGAGAGGCATGGCACATCATGGGGGTTCTTCT

TCTGCTCTATGTTTTTGTGCAAATATCGCGTGCTGTAGTTGTCGGTGTTT
TGTATCCACTGTTGCGTCACTTTGGGTATGGTATGGACATCAAAGAAGC
CACAGTTCTTGTTTGGTCAGGACTGCGAGGAGCTGTTGCTCTATCACTC
TCTCTGTCCGTAAACGTGCTAGTGATTCAAGTTCAAACCTTATCTGAAAC
CAGAAGTTGGAACAATGTTTGTGTTCTTCACAGGTGGCATTGTGTTTCT
GACATTGATTTTGAATGGTTCTACCACACAATTTTTGTTGCACCTACTTG
GTCTGGGAAAATTGTCAGCAACGAAGCTTCGTGTATTGAAGTATACACA
ATATGAAATGCTAAACAAGGCATTGGAGGCTTTTGGTGATCTCAGGGAT
GATGAGGAACTTGGGCCTGTTGATTGGGTAAATGTGAAGAAATATATCA
CATGTTTGAATAACTTGAAGATGAACAAGCACATCCCCACGATGTTCC
TGACAAGGATGATCACGTACATACCATGAATTTGAAGGATACTCGAGTG
CGGCTTTTGAATGGTGTGCAAGCTGCTTACTGGGGAATGCTTGAAGAG
GGACGAATAACTCAATCTACAGCAAATATTTAATGAGATCAGTTGATGA
AGCTATGGATCTTGTCTCTAG

>JQ063078.1 *Triticum aestivum* cultivar WL 711 salt overly sensitive

ACACACTTATTTTCATACTGAGTGGGGTTGTTATTGCAGATGGTGTACTA
CAAGATAGTATTCATTTTGAGAGGCATGGCACATCATGGGGATTCTTCT
TCTGCTCTATGTTTTTGTGCAAATATCGCGTGCTGTAGTTGTCGGTGTTT
TGTATCCACTGTTGCGTCACTTTGGGTATGGTATGGACATCAAAGAAGC
CACAGTTCTTGTTTGGTCGGGATTGCGAGGAGCTGTTGCTCTATCACTC
TCTTTGTCCGTAAACGTGCTAGTGATTCAAGTTCAAACCTTATCTGAAACC
AGAAGTCGGAACAATGTTTGTGTTCTTCACAGGTGGCATCGTGTTTCTG
ACATTGATTTTGAATGGTTCTACCACACAATTTTTGTTGCACCTACTTGG
TCTGGGAAAATTGTCAGCAACTAAGCTTCGTGTATTGAAGTATACACAA
TATGAAATGCTAAACAAGGCATTGGAGGCTTTTGGTGATCTCAGGGATG
ATGAGGAACTTGGGCCTGTTGATTGGGTAAATGTGAAGAAATATATCAC
ATGTTTGAATAACTTGAAGATGAACAAGCACATCCCCACGATGTTTCT
GACAAGGATGATCACGTACATACCATGAATTTGAAGGATACTCGAGTGC
GGCTTTTGAATGGTGTGCAAGCTGCTTACTGGGGAATGCTTGAAGAGG
GACGAATAACTCAATCTACAGCAAATATTTAATGAGATCAGTTGATGAA
GCTATGGATCTTGTCTCTAG

>KJ832076.1 *Oryza sativa* Indica Group cultivar Kharchia 65 salt overly sensitive
1 (SOS1) gene

GAGAAAATGTTTGAATGCTGGAGGATATATAAGGGCAGAAAGGGTAGT
TAGTCAGTGTTTTATAGCGGGCATTGCGCCAAGATTGTGCGGCATTTTAA
AAAAGAAAATAAAAATACATTGCTTAGTGCCGTATGGTAGACACGCAAC
AGTCAGCAAACGAGGCCACATCATCACACGCGGTTAACCGGGGTGAGC
AGGGACACGTGTCATTGCGTTTGCCTAGTGTTTTCTGGCCTCTACTCGA

CAAAGCACAGGCTTTGCCGAGTGTGCATATTTTGCTGAGTATTTTTGAA
GCGCACTCGGTTTAGGTTTAAAAAGTCCTTCGCCGATGACCTGACAAA
AAACACTTAGGAAAGAGTTGTGCACTCAATAAACTCGCCGATTCCAGT
AGTGACTAAGCGGTATATAGTTTTCCGTGGTTACATAGAGGACATTTGAC
GGCCAAAGTAGAGCTAGCCAAGTGGATCGATTCCCAGGCCGGCAGCTTC
GATATGGAAAGAATGGTCCATTTTAGCGTGCTACCAGCACCGTTGGATA
GCAAAGCATCGTATGCACACCATTTTCAAGGGAGAGTTCAATCTGTCTG
ATCCATATCAACATCGTCCCACCATTAGATAGCAAAGCATCATATGCACA
GCATTTCCGAGGGGTAGTTCAATTTGTATGATCCATGTTACCAAAGCATC
GTATGCGGTCTGTCTCCAACGCGCACATGCATCATTCACTAAGCATAAC
GAAGCAACGGTCGTGAGCGATACGTAAAGCAGTGCGATTCTCAGTTC
CAAGGGGAGAAGAAAACAAAAGCGTACAAATGATCTATATATGCATGA
AGCCAGCTCGTACGTGTTCTTCTCCAGGAAGTGTGGAAGAGCACTAGA
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>KF169799.1 *Triticum aestivum* salt overly sensitive 1 (SOS1) gene

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TTAAATACAGGAAGAAATTTTAAATTCATGAATAGTTTCTGAAATCCAGG
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TTTTCAAAATTGGGAATATTTTCAAATTTAGGATTTTTTTTTGTATTTGCTA
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GAGGCCCCCTTCAAACCCGTCTTCTTAAAAAATTTGGACTGAAAAAAC
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CAAACCAAACACTAATCATCGAGTTTTGGGCTAACTACCACAGACTCCC
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CGCACTATATTTTGAATGGAGGGAGTACAGACTAAACTGGTGCCGTCA
GATGAGATGAGATTCGCATCATAAATAATCTAGCGATTACTCTGCTCTCC
GCCGCCGTCCCATCTGATCTGATGTGTCCACCTCGTCTTGTCTGATCCCA
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CGGCG

>KF169800.1 *Triticum aestivum* salt overly sensitive 1 (SOS1) gene

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GGAGAAGCAAAAAAGAGAAATTCAAATGTGAATAGTGTCATTTTTACAT
TTGTTGTCTTTTAACACTATTCATCATGGATTTGACTTTCTTAGTTATCAA
TGTTTGTTTGAAGTATGGATCTGAGTTTTCTAGCCTTTGTGGCCACATGT
GCTATGAACATTCACGATTTTCTTTAAGTTTTTTGAAACATTTTTACTTTT
TATTTTTTAAATTCGTTGAATTTTTTTGAATTCATGAAAAATAAATTTGTG
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ATTCATGAATATTTTCTGAAAGCCAGGAACAATTTTCCTATTGGTGAACA

TTGTTTAAAGATTCTGAATATTTTTTGCTATTCAGAACCTTTTTCAAGTCTG
TGAGCATTTTTTAAAATTCTGGAACATTTTTCGAAATTGGGAATTTTTTC
AAATTCAGGAATGTTTTTTGTATCTGCTAACATTTATTCGAATTATTGAGC
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ACATAAAAAGAAAAGAAACGAAAGAAAAAAGGCAAGAAATTGGGCCG
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CAAGCGAGTGAGCGTGACGCGCTTGATGGACCAACCCACTTGCTACA
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GGGATCGGTCAGGGATGGTGTCCGTTGCGTAAAATTCGACACTCCTGGC
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CGATGCAGAAGATGAAGCTTGCTCTCCCGTACGATACCTTCACGGTGAT
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CGGCGGCGGTGGC

>JQ063076.1 *Triticum aestivum* cultivar HD 2009 salt overly sensitive

ACACACTTATTTTCATACTGAGTGGGGTTGTTATTGCAGATGGCGTACTA
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TCTGCTCTATGTTTTTGTGCAAATATCGCGTGCTGTAGTTGTCGGTGTTT
TGTATCCACTGTTGCGTCACTTTGGGTATGGTATGGACATCAAAGAAGC
CACAGTTCTTGTTTGGTCAGGACTGCGAGGAGCTGTTGCTCTATCACTT
TCTCTGTCCGTAAACGTGCTAGTGATTCAAGTTCAAACCTTATCTGAAAC
CAGAAGTTGGAACAATGTTTGTGTTCTTCACAGGTGGCATCGTGTTTCT
GACTTTGATTTTGAATGGTTCCACCACACAATTTTGTGTCACCTACTTG
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GATGAGGAACTTGGGCCTGTTGATTGGGTAAATGTGAAGAAATATATCA
CATGTTTGAATAACTTGAAGATGAACAAGCACATCCCCACGATGTTCC
TGACAAGGATGATCACGTACATACCATGAATTTGAAGGATACTCGAGTG
CGGCTTTTGAATGGTGTGCAAGCTGCTTACTGGGGAATGCTTGAAGAG
GGACGAATAACTCAATCTACAGCAAATATTTAATGAGATCAGTTGATGA
AGCTATGGATCTTGTCTCTAG

>JQ063075.1 *Triticum aestivum* cultivar Kharchia65 salt overly sensitive

ACACACTCATTTCATACTGAGTGGGGTTGTTATTGCAGATGGCGTACTA
CAAGATAATATTCATTTTGAGAGGCATGGCACATCATGGGGGTTTCCTTCT
TCTGCTCTATGTTTTTGTGCAAATATCGCGTGCTGTAGTTGTCGGTGTTT
TGTATCCACTGTTGCGTCACTTTGGGTATGGTATGGACATCAAAGAAGC
CACAGTTCTTGTTTGGTCAGGATTGCGTGGAGCTGTTGCTCTATCACTTT
CTCTGTCCGTAAACGTGCTAGTGATTCAAGTTCAAACCTTATCTGAAACC
AGAAGTTGGAACAATGTTTGTGTTCTTCACAGGTGGCATCGTGTTTCTG
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TCTGGGAAAATTGTCAGCAACGAAGCTTCGTGTATTGAAGTATACACAA
TATGAAATGCTAAACAAGGCATTGGAGGCTTTTGGTGATCTCAGGGATG
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ATGTTTGAATAACTTGAAGATGAACAAGCACATCCCCACGATGTTTCT
GACAAGGATGATCACGTACATACCATGAATTTGAAGGATACTCGAGTGC
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GACGAATAACTCAATCTACAGCAAATATTTAATGAGATCAGTTGATGAA
GCTATGGA

>AY326952.3 *Triticum aestivum* putative plasma membrane Na⁺/H⁺ antiporter

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GACGGGGGAGGCCGGCTCCCCAGCCCCGACGACGCGGTGCTCTTCTT
CGGGGTGGCCCTCGTGCTGGGCATCGCCTCCCGCCACCTCCTCCGCGG
CACCCGCGTCCCCTACACCGTCGCCCTCCTCGTCCTCGGCGTCGCCCTC
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CCTCCCCGCCCTCCTCTTCGAGAGCTCCTTCTCCATGGAAGTGCACCAG
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TGATCTCAACAGTTTTGCTTGGCGCCGCTGTAAAGCTCACTTTTCCCTAT
GACTGGAACCTGGAAAACATCATTCTTGTTCACTGGACTGCTTAGTGCAA
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CTGCTATTGTTGTCTATCAGCTATTCTATCGAATGGTGCTTGGAAAACT
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 TCTCGATGATGCTTTGCAGACAGACTTGAAGAAGCTTAAGAGGAATCC
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>KJ563230.1 *Triticum aestivum* cultivar WR704 salt overly sensitive 1 (SOS1)

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CCCTCGGCGGACTAGAGTACGGGACCAAGCATGGCCTCGGCAAGCTCG
GAGCCGGCATCCGTATCTGGGCTGCCATAAATCCTGATCTCCTCCTGGCT
GTCTTCCTCCCCGCCCTCCTCTTCGAGAGCTCCTTCTCCATGGAAGTGC
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CCTTGCAAGTTAGCATGTATGGCAGCATGACGGACGGCATGCACCGTGC
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GCGCGTCGTATCCCAAGGTGCCTTCAAGGGCAGCTGACACGCGGCCTC
TGCTCTCGGTGAGGTCGGAGGGCTCCAATGCGATGAAAAGAAAATCTG
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GCAGCAGGACGGCAACGCCGAGCGGTAGGAGAAGACGACGACTCGAG
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>KJ563229.1 *Triticum aestivum* cultivar Kharchiya 65 salt overly sensitive 1 (SOS1)

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A similar RAS1 gene sequence

>RAS1- TW4

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>RAS1- LW5

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>RAS1-LW6

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>RAS1-LW7

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>MK978170.1 *Triticum aestivum* isolate 22 ABA/WDS induced protein (ASR)
mRNA, complete cds

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>MK978175.1 *Triticum aestivum* isolate 28 ABA/WDS induced protein (ASR)
mRNA, complete cds

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>KX660742.1 *Triticum turgidum* subsp. *durum* abscisic acid stress ripening (ASR1) gene, complete cds

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>KX660744.1 *Triticum turgidum* subsp. *durum* abscisic acid stress ripening (ASR1) mRNA, complete cds

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