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Morpho Physiological Response of Canola (*Brassica napus*) Genotypes to Salt Stress

by

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A thesis submitted in partial fulfillment for the
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I would like to dedicate this work to my parents and teachers

CERTIFICATE OF APPROVAL

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Abstract

Salt stress reduces crop production by affecting the morpho-physiological and metabolic systems. Canola (*Brassica napus* L.), grown worldwide for edible oils and biodiesel, confronts this difficulty while becoming "salt-tolerant." With salt stress, yield and growth fall more. Numerous studies have helped us understand plant salt-response processes, but developing salt-tolerant crops has to be quicker. We know little about how cells and molecules react to salinity, making salt-tolerant canola engineering difficult. Genetic engineering may work, but it's important to understand canola's salt stress response. Bridging these information gaps will enable more focused and effective genetic treatments to improve canola's salt stress tolerance and preserve crop yield.

This research examined the effects of salt stress on five canola genotypes—HC022-B, Gobi, C-777, Desi, and Barani—at four NaCl levels (0mm, 50mm, 100 mm, and 150mm). The purpose was to evaluate these genotypes' morpho-physiological and biochemical responses to salt concentrations.

Among the parameters examined were Proline Content, leaf relative water content (RWC), shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, and chlorophyll content (a, b, a+b). All genotypes showed substantial effects from high salt stress on various morphological parameters.

In a study testing five canola genotypes under different salt concentrations (0, 50, 100, and 150 mmol), a consistent pattern of decreased growth parameters with increasing salt levels was observed. The top-performing genotypes, Desi Canola and Gobi Canola, showed superior characteristics at 150 mmol. such as high shoot length (8.9 cm and 7.3 cm), root length (7.5 cm and 6.8 cm), shoot fresh weight (7.5 gm and 6.8 gm), shoot dry weight (1.32 gm and 1.91 gm), root fresh weight (0.67 gm and 0.86 gm), and root dry weight (0.4 gm and 0.43 gm). As salt levels increased, there was a decrease in chlorophyll content and relative water content, while proline content tended to rise. Desi Canola and Gobi Canola demonstrated elevated proline values (9.1 mol g-1 and 8.6 mol g-1), suggesting an adaptive response to salt stress. The resulting salt tolerance in local canola genotypes is recommended for further crop improvement.

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Abbreviations

B. Canola	Barani Canola
D. Canola	Desi Canola
G. Canola	Gobi Canola
NARC	National Agricultural Research Centre
RDW	Root Dry Weight
RFW	Root Fresh Weight
RL	Root Length
ROS	Reactive Oxygen Specie
RWC	Relative Water Content
SDW	Shoot Dry Weight
SFW	Shoot Fresh Weight
SL	Shoot Length

Chapter 1

Introduction

1.1 Background

In plant biology and agriculture, the term ”salt stress” describes the harmful effect of high concentration of salt, mainly sodium chloride (NaCl), on plant growth and development when it comes to soil or irrigation water [1]. The heightened salt levels can impede the plant’s hydraulic conductance, restricting water uptake and nutrient absorption. This disturbance in osmotic balance leads to cellular dehydration, impacting turgor pressure and overall cell function [1, 2].

The salinity-induced disruption extends to ion homeostasis, resulting in an accumulation of toxic ions, particularly sodium ions (Na^+), within plant tissues. This perturbation adversely affects cellular processes, including enzymatic activities and metabolic pathways. The subsequent imbalance in ion distribution contributes to physiological disorders and compromises plant health. Moreover, salt stress triggers oxidative stress responses in plants, manifested by the enhanced production of reactive oxygen species (ROS) such as hydrogen peroxide and superoxide radicals. The accumulation of ROS leads to the oxidative damage to cellular macromolecules, including proteins, lipids, and nucleic acids. This oxidative stress exacerbates, cellular dysfunction and amplifies the detrimental impact of slat stress on plant growth and development [1]. In agricultural contexts, where optimal plant performance is critical for achieving high yields and ensuring sustainable food production, addressing the challenges posed by salt stress necessitates strategic interventions. Approaches include cultivating salt-tolerant plant varieties, implementing precision irrigation practices, and incorporating soil amendments to enhance soil structure and salinity tolerance. These strategies align with the imperative of sustaining agricultural productivity in the face of salinity-induced constraints [1]. Salinity is a common environmental stressor that significantly hinders the growth and development of canola. First, plants subjected to salt stress, sometimes called osmotic stress, absorbed less water, which stunts their development. Then, too many salty ions are in plant's transpiration stream. In that case, they will harm the cells by interfering with photosynthesis, disrupting the equilibrium of ions, and oxidizing the lipids in the membranes. This would further slowdown the development of the plant and cause ion toxicity. Gaining more knowledge about the physiological mechanisms by which plants respond to salt stress and yield potential decreases is necessary to increase plant tolerance to salt. As a result, salt stress impacts agricultural sustainability and global food security by reducing plant production systems' productivity and efficiency. Plants' ability to absorb water is reduced by increased salt in the soil or irrigation water, while their absorption of Na^+ and Cl^- is increased [2]. An investigation using three replications of hydroponics at the seedling stage was conducted to assess how different Canola genotypes responded to NaCl salinity [3]. The average growth, development, and other physiological activities of plants are retarded by excessive accumulation of Na^+ and Cl^- in the soil, as well as by nutritional deficiency caused by an excess of salt [4].

1.2 Impact of Soil Salinity on Canola (*Brassica napus*)

Agriculture is essential to solving the world's food security problems, but abiotic stressors significantly impact agricultural output. Soil salinity is an element that endangers arable land and inhibits the growth and production of essential crop species. Salt stress can negatively affect Canola (*Brassica napus*), an essential oilseed crop with various uses. Ensuring sustainable agricultural output and food security requires a thorough understanding of the processes influencing the resilience of canola genotypes that are resistant to salt. Providing the growing global population with access to sufficient, wholesome, and sustainable food is one of humanity's most challenging problems [5]. Plants are stressed by salt in two ways. High salt concentrations within plants can be harmful and make it more difficult for roots to get water from the soil. Salts outside of roots affect cell development and related metabolism; harmful salt concentrations take time to build up inside plants, and they have an impact on plant health [1].

Extreme salinity in the soil may lead to problems such as osmotic stress, oxidative damage, and ion toxicity, all of which can impair plant health and development. Plants have evolved chemical, genetic, and physical defense mechanisms against these impacts. These tactics include controlling the flow of ions and water, turning on enzymes that prevent oxidative stress, and eliminating dangerous compounds connected to oxygen. A variety of genes regulate a plant's capacity to tolerate salt. One major environmental problem that has an adverse effect on plant growth and productivity is salt stress [6]. Poor agricultural practices and climate change are predicted to cause a 50% loss of arable land by the middle of the twenty-first century due to the continuous rise in salinity. Salinity is a significant agricultural hazard since it has significantly impacted around 1,125 million hectares of agricultural land [7].

1.3 Salt Stress Causes Osmotic Stress in Plants

Elevated salinity levels within the soil may upset the ion balance and put plants under osmotic strain. Plant development, form, and metabolism are all affected by this stress, which may cause the plant injury or even death. The sodium-to-potassium (Na^+/K^+) ratio rises as the plants absorb greater amounts of sodium (Na^+) and chloride (Cl^-) ions from salted soil. The regular ion processes in the plants are disrupted by this alteration [8]. Many plant species adopted different strategies to overcome these obstacles. The first involves osmotic adjustment to maintain homeostasis by transporting excess Na^+ ions to the vacuoles, and the second involves osmolyte production to address this condition [9]. It is essential for plants to keep a high potassium to sodium ratio in order to regulate the turgor and osmotic pressure and to preserve their membrane potential. Enzyme activation and development of plants patterns depend on this equilibrium as well. In order to shield cells from the damaging impacts of salt stress, plants create osmolytes such as proline and soluble carbohydrates. Increases in these compounds may improve a plant's tolerance to salt through enhancing the osmotic equilibrium [10].

1.4 Regulation of Oxidative Stress Responses

A key component in cells' ability to deal with salt stress is protein. They improve the ability of plants to adapt to salinity and contribute to the regulation of the production of reactive oxygen species (ROS) levels. According to earlier studies, proteins have a variety of functions in lessening the harmful effects of salt on plant cells. One notable aspect is their involvement in osmotic adjustment, a mechanism essential for maintaining cellular turgor and preventing water loss under salt-stress conditions [11]. Furthermore, antioxidant molecules, such as glutathione (GSH), collaborate with proteins in the intricate network of defense mechanisms against ROS. Elevated ROS levels, including hydrogen peroxide (H_2O_2) and superoxide radicals (O_2), are expected consequences of salt stress. Additionally, tiny amounts of transition metals can further contribute to the generation of hydroxide ions (OH). In response to these challenges, plants activate a detoxification process to counteract oxidative damage, with antioxidant enzymes playing a pivotal role in this adaptive response. Antioxidant enzymes work together to neutralize and scavenge ROS, stopping them from damaging cell parts during the detoxification process. Notably, the concentration of OH is regulated through this detoxification process. The upregulation of antioxidant enzymes becomes essential in enhancing plant resilience to drought and salt stress [12].

1.5 Physiological Responses and Adaptations of Plants to Salinity

Plants that are subjected to salt stress may have a number of adverse impacts, including decreased development in both dry and fresh forms and other structural and functional alterations. A large quantity of salt in the soil may cause osmotic stress, which can be harmful to plants and make it difficult for them to absorb water. In order to deal with this, plants create particular compounds, such as proline. Proline facilitates water uptake by plants, maintains the proper osmotic balance inside the cells, and shields them from toxic substances called reactive oxygen species (ROS). When exposed to even low to average quantities of salt, plants that are susceptible to salt have stunted growth, decreased survival rates, and impaired development. In contrast, plants that can withstand salt well may thrive in environments as salinized as the sea. Using ecologically safe and clean methods, such as salt-tolerant plants, is the only option to tackle salinization issues and preserve the sustainability of agricultural and landscape areas [13].

Osmotic stress, ionic stress, and oxidative stress are the two primary factors that might hinder plant development. Reduced leaf area, increased leaf thickness and succulence, abscission of leaves, necrosis of shoots and roots, and shorter internode lengths are just a few of the symptoms that accompany the detrimental effects of salinity on plants' physiological and metabolic processes. The plant takes in a lot of salt, which increases the osmotic stress in the cytosol. In these conditions, a mechanism of osmotic adjustment, including organic osmolytes and the sequestration of large amounts of salt ions in the vacuole, maintains cell homeostasis [14]. Compatible osmoprotectants, including soluble sugars and proline, are created in many animals to shield their cells from the damaging effects of salt stress. High proline accumulation is linked to stress tolerance [10].

Canola oil is the third most significant source of edible oil, behind soybeans and palm seed oil. Salt and other environmental stresses, however, drastically reduce its production [13]. Early in the vegetative development stage, canola is susceptible to salt. Moreover, the juvenile stage is categorized as somewhat susceptible to salty environments. Nonetheless, there has been significant documented interspecific variation in salt tolerance. In many parts of the globe, limits on groundwater usage, salt buildup on soil, and saltwater intrusion into groundwater make salt-tolerant crop types more necessary. Plants tolerant to salt can reduce these adverse effects through various physiological, biochemical, and morphological mechanisms. Studies on plant tolerance to salt stress address morphological, physiological, and molecular alterations, among other elements of how salinity impacts a plant's response. Therefore, the current study aims to examine how salinity affects the physiological and morphological characteristics of canola [13]. Salt stress damages plants by impeding blooming and fruiting, growth and development, and seed germination. The plant's capacity for absorbing nutrients and water is limited by the high amounts of salt found in salty soil. Primary stressors such as osmotic and ionic stress are brought on by dietary imbalance and water shortage. These initial forces have the potential to cause oxidative stress as well as a series of subsequent stressors. Altogether, salt stress causes several physiological and molecular alterations and reduces the resources available to plants by suppressing photosynthesis, which limits cell division and development. Salt stress regulates the change in the state of photosynthesis and affects the formation of light-harvesting complexes. As sessile organisms, plants face the challenge of adapting to salinized environments, necessitating a diverse array of strategies for survival. These strategies encompass intricate signaling transduction pathways that orchestrate ion transport, maintain osmotic balance, and facilitate detoxification processes within plant cells. Furthermore, plants use complex regulatory systems to control the activity of genes susceptible to salt stress, guaranteeing the synchronization of physiological reactions necessary for adapting to high salinity environments [14].

1.6 Challenge of Salt Contamination in Agriculture

Over 800,000,000 hectares of land are damaged by salt contamination globally. Typical physiological state. This quantity makes up over six percent of the entire land area capable of operating. The vast majority of this area damaged by salt is a result of natural processes, namely the long-term deposition of salts in semi-arid and dry regions. As parent rocks weather, various soluble salts are released, mainly calcium, sodium, magnesium chlorides, and smaller amounts of sulfates and carbonates. The most permeable and absorbent salt that has been released is NaCl. The accumulation of sea salts carried by rainfall and wind is the second cause of buildup. Rainwater contains somewhere between 6 and 50 mg/kg of sodium chloride. The level decreases with distance from the coast. For every 100 mm of precipitation in a year, 10 kilograms of salt would be deposited per hectare in rainwater, having 10 milligrams per kilogram of sodium chloride [1]. Canola oil, derived from seeds, is highly nutritious with a well-balanced amino acid profile and desirable functional and technical attributes, making it the second most widely produced oil worldwide. These attributes make it a prospective source of new protein. Rapeseed can also be a viable option in the plant-based protein industry dominated by soy protein. In addition to its high yields, rapeseed adapts well to various farming environments [5].

Salt negatively impacts agricultural plants' growth, development, and production. It is mostly brought on by excessive salt chloride concentrations. It disrupts the delicate balance of ions and water uptake in plant cells, reducing crop productivity [1]. Saline soils and irrigation water sources are increasingly prevalent, posing challenges to agricultural production in various regions. This phenomenon affects numerous crops, from cereals to oilseeds [6]. In several regions of the world, crops must have higher salinity tolerance to continue producing food. Improving a crop's salt resistance may reduce nutrient loss in irrigation-dependent farming settings. Since less fresh water and saltwater disposal are required, this increases the cost-effectiveness of irrigation systems. Farmers suffer decreased agricultural yields and profitability due to salinity stress, which causes significant financial losses in the industry. The financial strain on rural populations is further increased by crop failures brought on by salt stress [7]. The rising salinity of soils and water sources can harm the environment, including reduced biodiversity in saline-affected regions. This aspect highlights the broader ecological implications of salt stress [12].

1.7 Salt Stress Reduces Water Uptake

Saline environments cause plants' water potential to decrease, which can result in water shortages. Plant organelles and the soil solution have unequal solute concentrations caused by salinity, which causes osmotic stress and the loss of turgidity in plant cells. Under severe salt stress, plant cells' ability to express tonoplast aquaporin is impeded, interfering with physiological and metabolic activities, including cell elongation and meristematic activity. Since RWC in plants indicates the amount of water required for artificial complete saturation, it is an essential indicator of a plant's water balance. Plants have a reduction in cell turgidity due to RWC decreasing in salt stress [15]. Different oilseed cultivars of *Brassica sp.* showed varying decreases in RWC in the leaves when subjected to varying saline stress levels. Mahmud et al. demonstrated in one experiment that salinity negatively impacted the water's condition of Brassica napus seedlings, resulting in a drop in leaf RWC. In comparison to unstressed plants, leaf RWC dropped by 6% and 11% at two distinct salinity levels (100 and 150 mM NaCl) [16, 17].

1.8 Problem Statement

The salinity of the soil has emerged as a significant obstacle to the productivity of agriculture worldwide, resulting in considerable financial losses and compromising food security. Despite extensive reports on the effects of salinity on crop species, there is a need for a thorough analysis of the unique reactions of Canola genotypes to salt stress. There is a paucity of research on the detrimental effects of salt stress on significant canola genotypes.

1.9 Scope

The study contributes to our knowledge of how plants react to salt stress at the morphological, physiological, and molecular levels, which benefits the fields of plant biology and agriculture more broadly. Finding genotypes that are resistant to salt may enhance production, which will improve the economy.

1.10 Aim and Objectives

Aim:

The aim of this study is to characterize the local canola genotypes against salt stress.

Objective:

- To study the effect of different concentrations of salt on the morpho physiological traits of economically important locally grown canola genotypes.
- To identify promising salt tolerant canola genotypes for further crop improvement.

Chapter 2

Literature Review

2.1 Salt Resistance

Salt resistance is an intricate tool. Numerous genetic, physiological, and biochemical cycles are involved, from germination to the vegetative stage and then into the trim development. It varies between kinds of the same genus, within harvests, and genotypes of related species [18].

2.2 Understanding the Impact of Salinity Stress on Plants

As environmental conditions vary, plants are exposed to a broad variety of stressors that are both abiotic and biotic. Salinity begins from salty areas at different elevations and severely threatens moderate and tropical agriculture systems. Although potassium and nitrates are naturally occurring elements of soil, agricultural irrigation, and other human activities accelerate soil salinization by adding large amounts of salt [19]. According to Munns [20], there are around 800 million hectares of salinity affected land worldwide *Chatrath et al* [19] estimated that 6 million hectares of this land exist in Pakistan alone. In Pakistan, an arid or semi-arid environment, salinity problems are caused by excessive evapotranspiration, which causes salt to build up on the soil's surface. Salt stress significantly hinders food production by restricting crop yield and limiting the cultivable land, especially in the canal-irrigated areas of Punjab province [19].

Irrigation and inadequate drainage exacerbate salinity-related issues, leading to a build-up of excessive salinity that includes calcium, magnesium, and sodium. Sodium ions become more prevalent in the soil due to water evaporation, which disrupts nutrient-ion interactions and causes imbalances in the ratio of Na^+ to Ca^{2+} or Na^+ to K^+. High cation concentrations prevent water from entering the roots, causing a water shortage like during a drought. Inadequate salt management has the potential to exacerbate soil salinity by weakening the structure of the soil, increasing bulk density, compressing the soil, and lowering total porosity [21].

The USDA Salty Soil Laboratory states that salty soils have an extractable soil electrical conductivity of 4 dS m1 or more, which is detrimental to crop development. Salt tolerance is further influenced by environmental variables such as soil type, temperature, radiation, and vapor pressure deficit. Salt concentrations vary seasonally and geographically in the field depending on conditions unique to each plant [19]. Salinity stress has far-reaching effects beyond decreased crop yields; it also affects soil conditions and ecological balance. The consequences include reduced crop yields, financial gains, and increased soil erosion, resulting from complex interactions between physical, physiological, and biological systems. Critical developmental phases, such as nutrient intake, plant growth, and seed germination, are strongly impacted [22].

The core of adversity brought on by salinity is a series of interrelated physiological processes. Complex processes involved in seed germination are hampered, preventing solid plant populations' growth. Later, plant development is restricted, characterized by reduced biomass accumulation and weakened structural integrity. A cycle of physiological stress is maintained concurrently by inhibiting the absorption of vital nutrients necessary for metabolic activities [22].

The physicochemical properties of the soil matrix regarding soil health are altered by salinity stress. Elevated levels of salt make the soil more prone to erosion and less able to hold onto water. Complex interactions among these variables reduce soil fertility overall, increasing the difficulties encountered by plants trying to survive in salinized settings [22]. From an economic standpoint, the effects show up as lower crop yields and consequent losses for farmers. A decrease in yield has an immediate effect on profitability, but it also ripples across the supply chain for agriculture and affects more general economic dynamics. The need for extra inputs and treatments to ameliorate salinity-related difficulties exacerbates this economic pressure and further constrains financial returns [22].

Numerous issues with the toxicity of ions, osmotic strains, and nutritional deficiencies (Sodium, Calcium, Potassium, Phosphorus, Iron, and Zinc) that impede water intake are among the many consequences of salinity on plant development. Toxic sodium, chlorine, and boron accumulations bring cell death and osmotic stress. Plant health is further disrupted by nutrient imbalances and interference with nutrient absorption [23].

Salinity hurts photosynthesis, lowering stomatal conductance, chlorophyll concentration, and leaf area. Ovule abortion, stamen filament elongation, and microsporogenesis are all negatively impacted by this impaired reproductive development. Plant development is hampered by adverse impacts that appear at the physiological, biochemical, and molecular levels [23]. Measuring growth or survival is one way to evaluate a plant's ability to withstand salt; these metrics reveal complex physiological processes. In saline settings, osmotic equilibrium is essential for plants to avoid cell dehydration and death. Protein conformational changes result from the replacement of Na^+ for K^+ in metabolic processes, which causes ion toxicity. Growth is impeded, and meristem cell division is reduced when salinity stress is on the cell cycle. Adverse impacts on seed germination, seedling development, enzyme function, and basic cellular activities are highlighted in recent investigations [24].

2.3 Salt Affects Photosynthesis

Salt stress curtails growth by reducing resource availability due to decreased photosynthetic activity. This is followed by resource reallocation for osmotic correction. Although there is some healing effect, increasing the carbon dioxide (CO_2) concentration only slightly restores the photosynthetic rate since stomatal closure also reduces the amount of CO_2 that can be fixed. This investigation suggests that, in addition to stomatal closure, there could be an ionic element or, at the very least, a salt-induced influence on photosynthesis.The enzyme activity of carbon dioxide-fixing enzymes decreases under salt stress, and interestingly, in vitro settings reveal species-specific differences in these enzymes' tolerance to sodium ions (Na^+). It is unclear, therefore, how these discoveries will translate to plants' capacity for photosynthetic processes. Precise pH and electro-potential synchronization are required for efficient proton-motive force generation in chloroplasts, which is essential for energy production. The intricate relationships between these components show how salt stress affects the photosynthetic system in numerous ways, including stomata regulation and enzyme activity. It is crucial to understand these intricacies to fully comprehend and deal with how salt stress affects plant photosynthesis [25].

2.4 Salt Tolerance Mechanism in Plants

Plants have three challenges when exposed to high salt levels: ionic, osmotic, and oxidative stress. Plants respond by using a complex combination of chemical and physical tactics to keep their internal environment in balance. This entails regulating reactive oxygen species (ROS), preserving the proper osmotic pressure, and guaranteeing steady ion levels. Ionic stress is a condition that may arise from an excess of sodium ions (Na^+). This stress interferes with the regular functions of cells and jeopardizes the delicate balance of necessary ions. Plants have evolved sophisticated strategies for controlling the flow of ions to address this problem. To maintain a steady internal ionic environment, they carefully control which ions enter and exit. The plant needs these mechanisms to survive in hostile environments. Plants are able to maintain the integrity of their cellular structures and the efficiency of their metabolic activities by managing the quantities of ions in their environment. In addition, controlling osmotic pressure enables plants to effectively absorb water even under adverse environmental circumstances. Last but not least, maintaining a balance in ROS levels shields plant cells against harm that might cause cell death. Plants possess a highly robust and adaptable mechanism. This mechanism enables plants to flourish in situations with high amounts of salt, showcasing their amazing capacity to adapt to and overcome environmental stress. Elevated levels of solutes in the soil may cause osmotic stress, which can result in a water imbalance in plant cells. Plants use osmoregulatory processes, such as the build-up of suitable solutes like proline and carbohydrates, to Osmotic stress is a situation in which plants struggle to keep the proper quantity of water in their cells due to an excess of solute in the soil. Plants respond to this by triggering certain processes that maintain equilibrium. They begin to accumulate certain solutes, such as proline and carbohydrates, which aid in maintaining the integrity and firmness of their cells by allowing them to respond to the osmotic pressure. The plant's ability to survive under stressful circumstances depends on this mechanism. A plant experiences oxidative stress when its defense against oxidation isn't working right and dangerous chemicals called reactive oxygen species (ROS), which include hydrogen peroxide and superoxide radicals, are being made. This circumstance can harm the plant's cells. Plants boost their antioxidant defences, which include enzymes like catalase and superoxide dismutase, to stop this damage. These antioxidants are essential for neutralizing ROS and shielding the plant's cells from the damaging effects of oxidation. Plants use two strategies to guarantee their life and development that are osmoregulation and the enhancement of antioxidant defense systems. This shows how plants cleverly regulate and reduce stress for their survival and growth. All of these adaptive reactions highlight the complex ways that plants deal with the difficulties of excessive salt. Knowledge of the complexities of these biochemical and physiological processes may help us design salt-tolerant crops, advance sustainable agriculture in salty areas, and improve our knowledge of plant stress responses [26].

2.5 Plant Responses to Salt Stress: Decoding Ionic Stress Signaling

Plants experience ionic stress when they are exposed to excessive amounts of salt, particularly sodium ions (Na^{+}). Because of this stress, a complex response mechanism is required to identify and control the presence of salt. The Salt Overly Sensitive (SOS) genes, which are essential, are at the centre of this reaction. These genes play a crucial role in controlling the intricate responses that high Na^{+} levels trigger. A signal involving calcium ions (Ca^{2+}) inside the cell initiates the process. This signal can be picked up by SOS3 and SCaBP8/CBL10, two proteins that can bind calcium because they have special shapes called EF-hand motifs. These proteins recognise the calcium signal and go on to activate another protein called SOS2, which is a member of the serine/threonine protein kinase family. An essential first step is the activation of SOS2, which triggers the activation of the SOS1 protein, a plasma membrane-based $\text{Na}^{+}/\text{H}^{+}$ antiporter. Because it makes it easier for sodium ions to get from within the cell to the apoplast-the area inside the plant structure that is outside the cell membrane-activating SOS1 is essential. This action is necessary to keep the ions in the plant cell in equilibrium so the plant can withstand the stress that comes with excessive salinity levels. This complex system demonstrates the nuanced ways in which plants respond to environmental stressors and preserve internal stability in the face of opposition [27].

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2.6 Osmotic Stress Signaling in Plants: Navigating Cellular Equilibrium

Plants that experience salt stress also experience osmotic stress, which calls for a signaling system to bring the environment back into balance. Recent research has highlighted essential proteins involved in osmotic stress sensing [28]. One is OSCA1, a protein found in the plasma membrane (PM) that forms a hyperosmolality -gated Ca^{2+}-permeable channel and has been suggested as a hyperosmotic stress sensor [29]. The osca1 mutant's impaired Ca^{2+} signalling in response to osmotic stress draws attention to OSCA1's potential role in osmosensing. The mechanosensitive ion channel MSL8, found in the PM of Arabidopsis pollen, serves as a sensor of membrane tension caused by hyperosmotic stress during pollen hydration and germination [30]. KEA1/2 and KEA3, plasmid K^+ exchange antiporters, are involved in the hyperosmotic-induced Ca^{2+} responses brought on by osmotic stress [28].

MAPK cascades, namely MKK4-MPK3 and MKKK20-MPK6, influence salt sensitivity and water loss rates in Arabidopsis via mediating osmotic stress responses.

To find the upstream sensors and downstream effectors in these pathways, further research is required [28]. Osmotic stress mainly activates SnRK2 isoforms, except SnRK2.9, through an ABA-dependent signalling pathway [27]. This activation triggers the transcriptional control of downstream effectors, upon which osmotic stress tolerance depends [31]. ABA-dependent SnRK2-AREB/ABF-BAM1/AMY3 signalling is a key part of osmotic stress adaptation because it controls the breakdown of leaf starch [32]. To combat osmotic stress, plants produce compatible osmolytes, such as proline, glycine betaine, mannitol, and sugars. These osmolytes support cellular stability and osmotic control under salt-induced osmotic stress [28].

2.7 Pathways of Detoxification Signaling

When plants are stressed by salt, they release reactive oxygen species rapidly. The increase in ROS levels is a sign that several redox sensors, which monitor and regulate redox homeostasis, have sprung into action. Heat-stress transcription factors (HSFs) are thought to behave as redox sensors that are reliant on reactive oxygen species (ROS), interpreting ROS signals to activate defence mechanisms. The accumulation of ROS in conditions of salt stress exacerbates oxidative stress, which may be detrimental to plant health. However, ROS are vital signalling molecules that let plants respond to external stimuli in concert. Plant cells must maintain ROS at the right concentrations to balance the harmful effects of ROS with their signalling functions. Because they have evolved sophisticated defence mechanisms to withstand shifting environmental conditions, plants are able to effectively adapt to and manage stressful situations. Reactive oxygen species in cells are important for maintaining cellular homeostasis and reducing potential damage from stress. Detoxification signalling pathways are essential for controlling ROS levels in cells under different stress scenarios. The plant's reaction to salt stress is modulated by a series of signals that are detected by certain sensors or receptors inside the plant's cells. These signals affect the plant's growth and developmental processes when ROS levels rise. The mitogen-activated protein kinase (MAPKKK-MAPKK-MAPK) cascades are particularly important channels for ROS signal transduction among these signalling pathways. These cascades play a critical role in regulating plant growth and development, preserving ROS homeostasis, and modifying ionic balance. This complex signalling network highlights the sophisticated and highly honed systems that plants use to navigate and adapt to their constantly shifting environment, guaranteeing survival and continuous development in spite of the difficulties presented by stressful situations [28]. Plants actively regulate ROS homeostasis to resist oxidative stress caused by salt exposure and transduce stress signals [26].

2.8 Salinity Soil Remediation

By adding calcium ions (Ca^{2+}) to salt-affected soils, gypsum supplements provide divalent cations that may take the place of sodium ions (Na^{+}) and facilitate the leaching of salts. Moreover, the addition of organic matter to the soil increases the availability of exchange sites, facilitating the capture of Ca^{2+} ions. Compared to the weaker interactions with sodium ions, these calcium ions are more likely to form durable organo-mineral bridges with organic materials. Several academic researchers have conducted studies showing that adding organic matter can remediate mildly salinized soils. This tactic highlights the value of using gypsum and organic amendments together as a long-term solution to reduce soil salinity, enhance soil health, and increase agricultural output [33]. The addition of gypsum to three distinct types of organic matter caused the pH of the moderately salinized soil to decrease. This modification is advantageous for salinity-affected soils since they usually have high pH values, which are not appropriate for crop production. This discovery is consistent with past studies that have shown that adding organic amendments might cause the pH of the soil to drop. These kinds of treatments are important because they lessen the alkaline conditions that are often linked to salt stress, which in turn helps to create a more favourable soil environment for plant development. In addition to promoting better plant growth, this increase in soil chemistry also makes agricultural methods in salt-stressed areas more sustainable [34]. The pH of the soil will probably decrease as a result of organic inputs because they increase microbial activity, produce organic acids, and dissolve salts that are then leached down the soil layers. One possible explanation for the pH drop in salt-impacted soils might be the increased concentration of basic-cationic compounds in the additional organic inputs [35].

Chen et al. [36] found that adding peat, fertilizer, and biochar decreased the soil's pH. Organic additions assist in the binding of particles of soil into aggregates by deprotonating fulvic and humic acids. Treating saline-sodic soils with chicken dung increased their CEC and concentrations of exchangeable K^+ ions, as shown in [37]. Exchangeable K^+ ions compete with Na^+ ions to prevent their entry into the soil environment [18]. Adding green manure lowers soil pH and increases $CaCO_3$ solubility due to the increasing partial pressure of carbon dioxide in the soil, enhancing microbiological respiration and breakdown [38].

2.9 Salt Resistance Component

A popular measure for assessing salt tolerance is the decrease in biomass output in salty circumstances compared to non-saline control conditions over a given time period. The degree of this tolerance varies across species and even between genotypes within the same species. For example, at 200 mM of sodium chloride, salt-sensitive species like soybeans may completely stop growing, but salt-tolerant species like sugarbeet may only exhibit a little 20% reduction in dry weight under the same salinity circumstances. Conversely, crops that are somewhat resistant, such as cotton, might show a more marked reduction in biomass accumulation-roughly 60%-when subjected to similar saline levels. This characteristic is essential for comprehending how different crops react to salt stress and for creating plans to increase agricultural output in places impacted by salinity [39]. From a physiological perspective, salt resistance refers to a plant's ability to regulate and move toxic particles through its internal structures without compromising its overall physiological and biochemical functions. Compared to salt-delicate plants, salt-tolerant plants may absorb lower concentrations of sodium and chlorine and transfer them to their leaves. Enclosing these particles in vacuoles also prevents their growth in the cytoplasm and avoids salt toxicity as a result. The accumulating viable solutes outside the vacuole achieve the osmotic equilibrium [37].

One category of plants restricts the amount of salt that can enter them, while the other category limits the amount of salt that can centralise in their cytoplasm. Both are present in halophytes, which 'prohibit' salts and segregate them into vacuoles that mysteriously penetrate the plants. As such, they are actually capable of developing well in salinized environments. In glycophytes, such a mechanism is not genuinely feasible. Some glycophytes also inhibit salts, but the mechanism remains the same. Since most glycophytes cannot forbid salt, the concentration of salts in young, coming-to-pass leaves might reach toxic levels. [40]. Plants primarily depend on their ability to regulate the salt medium in five different locations: Two categories are also included in the specific salts that root cells absorb.

1. The plant restricts the amount of salt that enters and groups salts together in the cytoplasm. Plant cells specifically uptake potassium over sodium through a process called xylem stacking.
2. The stem, roots, or leaves extrude salt from their top portion.
3. There is little sodium and chloride retraction in the phloem, especially in more salt-tolerant plants where phloem stacking occurs. By doing this, it ensures that no salts are discharged into the developing tissues. Certain cell types, such as bladders or salt organs, constantly release salts.

Only halophytes contain this specific kind of cells [41].

The roots are a crucial component of the plant's growth and development as they are the first part to experience salt. The roots are where the salts take up and grow into the occurring stream. Attaches must withstand two types of pressure: osmotic and salt toxicity. These two loads result in reduced water uptake, root growth inhibition, and oxidative pressure activation [1]. The majority of the chloride and broken-down sodium ions present in the soil composition should be rejected by roots, and they should keep the concentrations of sodium ions in the cytosol within a reasonable range. Failure to do so might eventually lead to a continuous build-up of salts in toxic shoots. Salt resistance in completely depleted saline environments differs from that of soil when salinity is associated with water logging [37]. In comparison to non-waterlogged saline conditions, salt intake rises significantly when root area salinity is linked to water logging [42]. Anaerobic conditions that lead to failures in dynamic vehicle and root avoidance procedures might cause this [43].

2.10 Mechanisms of Salt Adaptation in Plants

It is very important to stop the plant from taking in sodium. This includes reabsorption from the xylem sap, sequestration of Na^+ by xylem parenchyma cells, and efflux of Na^+ from the root epidermal and cortical cells' plasma membranes. Plants with enhanced salt tolerance restrict Na^+ ion infiltration and regulate their accumulation within the foliar tissues. In order to lessen the harmful consequences of excess Na^+, plants use intricate physiological systems to redistribute and compartmentalise Na^+ inside vacuoles, avoiding sensitive regions. These tactics, which include partitioning and selective ion transport, are crucial for preserving ionic balance in saline environments and help explain why plants are generally more resilient in them [44]. Reduced salt absorption by plant tissues is the process by which plants reject salt. Under favourable climatic circumstances, plants transpire much more water into the atmosphere (30–70 times the water content needed) than is essential for cellular development. As a consequence of this process, the concentration of solutes within the soil is much greater than the concentration outside by the same amount. Plants use selective ion transport systems across their root membranes to prevent excessive salt build up. This successfully filters out unwanted salts. For example, if the soil has 20% salt content (assumed to be fatal at 98% salt content), plants will control their internal salt concentration so as to keep it below a critical threshold, allowing them to survive and grow under saline soil conditions. But there are certain restrictions on how the plant regulates its salt content. The occurrence where salts are left behind after water evaporates and eventually accumulate in the leaves is known as salt deposition. The long-term sustainability of this build up is questionable; older leaves have an especially high concentration of salt, which causes them to senesce earlier than younger foliage. The plant uses this method to partition and sequester salts away from younger, more important tissues, which allows the older leaves to wither first and extend the plant's overall viability in salty circumstances [37].

2.11 Ion Transport & Homeostasis Mechanisms in Response to Salinity Stress

Transport mechanisms include dynamically moving particles across the tonoplast and into the vacuole, which removes dangerous particles from the cell. These particles serve as osmolytes in the vacuole, allowing water to enter the cell more easily. Na/H antiports attached to plasma films may also be the mechanism by which sodium leaves the cell [45]. Furthermore, it has been demonstrated that the main purpose of Na^+/H antiporters is to promote the growth of hydrogen ion particles within the cell along their electrochemical slope in order to prevent Na^+ from eroding against the electrochemical inclination. A potential Na/H plasma layer antiport is also known [46] to represent SOS in Arabidopsis thaliana. The plasma film H^+-ATPase acts as an instrument for this Na^+ expulsion by producing a protonmotive power for Na^+ expulsion. The H^+-ATPase extracts H^+ from the cell by using the energy of ATP hydrolysis, resulting in an electrochemical H^+ inclination. Cells in the root stele, vascular packs in leaf petioles, or stems exchange potassium for sodium particles, leading to the storage of sodium in the lower shoot and higher root sections of various species [47].

2.12 Salinity Tolerance Studies in Canola Species

Several studies have evaluated the salt tolerance of Brassica species, classifying them as crops with a moderate tolerance [48]. According to these investigations, Brassica species show exceptional endurance to salt, with threshold values as high as 11.5 dSm^{-1}. However, the cultivar Shirallee exhibits noticeably poorer performance when this threshold is exceeded, with tolerance limits reaching as high as 20 dSm^{-1}. Researchers have focused on understanding the salt tolerance of numerous allopolyploid Brassica species, including nigra, oleracea, campestris, and juncea, as well as their diploid progenitors, under controlled glasshouse circumstances.

These 51-day experimental investigations were carried out using sand culture technology. The plants, which were a few days old, were exposed to three different treatment regimens during this time: two experimental groups received 100 and 200 mol m-3 NaCl, respectively, as part of a modified Hoagland solution to simulate saline conditions, and a control group received no salt at all. The aim of this study was to determine the salt threshold that each species could tolerate without experiencing a notable reduction in yield, as well as to clarify the physiological and biochemical reactions of these Brassica species to rising salinity levels. All three amphidiploid species exhibited significantly greater shoot and root loads and seed output in salinized environments than diploid species. Amphidiploids also accumulated more potassium and sodium than diploids because they acquired less sodium but more potassium from their roots and branches. There was no consistent Cl^- collecting architecture in either diploid or amphidiploid species, and there was no discernible variation in Ca^{2+} aggregation between the two types of organisms. They postulated that amphidiploids have adopted greater K/Na, differentiating quality from the A, B, and C genomes, and have gained salt resistance from the A and C genomes. Additionally, they surmised that these characteristics were responsible for the amphidiploids' superior K/Na separation and superior resilience to salt in comparison to their diploid family members [49].

When subjecting five genotypes of *Brassica juncea* to a 100 mM NaCl saltiness treatment using a water system for four weeks, a relative reduction in shoot development ranged from 28% to 72% [50]. In several canola genotypes, salt resistance affects the weight of saltiness in terms of germination, development, ionic uptake, and proline collection. Salinity had a major impact on the length of the shoots and roots, as well as the rate and speed of germination. Additionally noteworthy were the effects of salinity on proline accumulation, ionic content, plant height, leaf area, seed production, and dry matter components. Except for sodium, chloride, and proline levels, all parameters decreased as the degree of salinity increased. During the germination and vegetative growth phases, canola genotypes exhibited various responses. At germination, researchers discovered that genotype Licord exhibited the highest tolerance among cultivars, while they considered genotype Tiptop the most sensitive. The exhibitions 'SLM046' and 'Okcapi' in the pot analysis were comparatively greater than Licord [50].

Assessing salt tolerance in canola varieties during the germination or early seedling stages may not be predictive due to the significant diversity in responses across various developmental periods [18]. Genotypes of canola exposed to salinity and electrical conductivity levels of 16 $\mathrm{dS}m^{-1}$ or higher are unable to grow suitable crops. According to another study, *Brassica napus* is the canola species that is most salt-tolerant, whereas *Brassica compestris* and *Brassica nigra* are the least salt-tolerant. Furthermore, *Brassica napus*, *Brassica carinata*, and *Brassica juncea* demonstrate a relatively higher ability to tolerate saline and alkaline environments compared to their corresponding diploid ancestors, *Brassica compestris*, *Brassica oleracea*, and *Brassica nigra* [51]. Among the four kinds of Brassica species studied for salt tolerance, *B. napus* (canola) and *B. carinata* demonstrated significant resistance to salinity. These species are excellent candidates for rigorous selection and breeding programmes targeted at improving salt resistance since they have shown the ability to tolerate moderate levels of saline in the impacted areas. When exposed to salty conditions, *B. napus* accumulated more potassium than the more salt-sensitive *B. compestris*, which suggests a possible way that it can handle salt [52]. Furthermore, various Brassica species, including canola, *B. cauliflower*, and cabbage, underwent scientific assessments to determine their salt tolerance. In order to ascertain the capacity of these organisms to withstand salt stress, these assessments concentrated on crucial developmental phases, such as germination and early seedling growth [53]. Four salt treatments were applied: 0 dS m^{-1} (control), 4.7, 9.4, and 14.1 dS m^{-1}. Salt had a significant impact on seed germination, germination rate, shoot length (SL) and root length (RL), shoot fresh weight (SFW) and root fresh weight (RFW), leaf area, and number of leaves in canola, cabbage, and cauliflower. All three Brassica species showed a marked decrease in germination (%) with increasing salt levels. Cabbage and canola were less affected by salt compared to cauliflower. Salt also delayed germination. Salt treatments inhibited seedling growth in all three species, with the most significant reduction in root and shoot length observed at the 14.1 dSm^{-1} NaCl level. Salt affected Shoot growth more than root growth in all three plant species. Salt also reduced shoot and root fresh weights, number of leaves, and leaf area at all salt treatments [53].

Researchers looked at whether species of field pea, common vetch, and canola were the most salt-tolerant [54]. Over a 45-day period, we exposed seedlings to four distinct concentrations of NaCl (50, 100, and 150 mM) in a gradient of saline environments to assess how salinity affects their ionic composition and growth dynamics. Using ratios of potassium to sodium (K/Na) and calcium to sodium (Ca/Na), the research sought to evaluate the effects of salinity on seedling development and ion buildup in the shoots. For every examined species, there was a discernible rise in sodium (Na) deposition in the root and shoot tissues as salt levels increased. On the other hand, the amounts of the essential elements, calcium (Ca) and potassium (K), in the plant tissues were much lower. The *Pisum sativum cv. Kirazli* variety outperformed the others in terms of resistance to saline stress. Its resistance to salinity was greatest in conditions where the salinity was less than 100 mM NaCl, making it a good option for farming methods in soils that are prone to salt.Six cultivars of *Brassica campestris* were treated with various NaCl concentrations to investigate the effect of salt on their germination [55]. The researchers increased the salt concentrations, which reduced the germination rate. Germination and seedling growth significantly improved at lower salt levels. These findings suggest Brassica campestris germinates and grows better at lower salt treatments (50 mM NaCl) than in control [55].

According to the findings of research carried out by *Akhtar et al.* [21], the growth and yield of four different species of Brassica (*B. juncea, B. compestris, B. napus, and B. carinata*) were investigated at different salinity levels. The electrical conductivity of the extract (ECe) was used to determine the salinity levels, and the results showed that the salinity levels varied from 3.0 to 9.50 dS m^{-1} [21]. Across the investigated species, the growth measurements showed significant variations in response to the saline environment. These growth measures included plant height, stem girth, number of siliquae per plant, yield per hectare, weight of 1000 grains, and percentage of oil content. These parameters experienced the most substantial declines when the salinity concentration reached its highest level of 9.50 dS m^{-1}. *B. napus* and *B. carinata* exhibited a higher level of resistance to salt compared to the other investigated species, as indicated by the growth indices [56]. *Francois et al.* [57] further highlighted that there is heterogeneity within a species in terms of its ability to tolerate salt. Furthermore, this research observed an amazing capacity of the *Brassica napus* species to rebound from harsh situations. Researchers conducted numerous field tests to examine how salinity affects the growth, productivity, and oil content of various genotypes of *Brassica napus* and *Brassica compestris* trees. These findings demonstrated that *B. compestris Toblin* and *Brassica napus Wester* genotypes exhibited a substantial degree of salt tolerance, making them ideal candidates for cultivation in salty soils [57]. The findings of the study demonstrated that these genotypes displayed adequate levels of tolerance to salt.

In addition, *Qasim et al.* [58] found that there were variations in the genotypes of *Brassica napus* that were present throughout the first stages of seedling development at various times. Under the influence of sodium chloride, the researchers discovered that there were significant changes between two genotypes of *Brassica napus L.*, namely Dunkled and Cyclon. More shoot mass, dry mass, and seeds were produced by the salt-resistant Dunkled genotype than by the salt-sensitive Cyclon genotype in low, medium, and high salinity conditions [58].

A recently published study [59] evaluated the growth-related factors of eight different *Brassica napus* lines. When there was an increase in the amount of salt present in the growth medium, there was a corresponding decrease in the height of the plant as well as the beginning of blooming. The CVs Dunkeld, Cyclon, and Rainbow varieties had the most significant reductions in stem diameter and plant height amongst the three kinds. In addition, increasing salt levels in the growth medium extended the flowering phase of canola for a longer duration. The reduction in salinity significantly impacted the canola plant's growth rate and also resulted in a delay in its flowering [59].

Research was carried out by *Bybordi et al.* [18] to investigate the significant variations in *Brassica napus* variants that were brought about by the presence of salt. They put five distinct genotypes of *Brassica napus* through their paces under a variety of salinity conditions [18]. The *Brassica napus* cultivar SLM046 has shown remarkable performance, particularly in regard to the number of days it takes for the plant to reach maturity and the amount of yield it produces per plant. Analysis of the growth characteristics of the genotypes SLM046 and Okapi revealed their typical resistance to varying degrees of salinity.The genetic variation in twelve distinct genotypes of *Brassica napus* rapeseed was the subject of a study that was conducted by *Murat et al.* [60]. *Murat et al.* [60] demonstrated that salt reduced the weight of the green components in all cultivars harvested 45 days after planting. This was determined through the process of experimentation. When compared to the shoots and roots, the leaves exhibited higher quantities of potassium and calcium. On the other hand, the leaves and shoots exhibited the highest levels of sodium and chloride. The Official and Lira genotypes had the highest levels of potassium and calcium in their leaves when exposed to saline conditions [61].

Siddique and colleagues [61] investigated the physiological responses of certain genotypes of *Brassica napus* to the combined presence of salt stress and drought. A number of physiological parameters, including growth, osmotic potential, ion concentration, and stomatal conductance, were significantly altered as a result of the combined stress. All of the genotypes had a decrease in both their fresh weight and their dry weight as a consequence of this. In comparison to the control group, the stress treatment increased the concentration of sodium while decreasing the concentrations of calcium and potassium in the stressed plants. All of the genotypes had a reduction in stomatal conductance as a consequence of the combined effects of salt stress and drought. We evaluated the germination, growth, and solute concentrations of canola seeds in various genotypes exposed to environmental conditions, including salt [62]. The presence of salt significantly influenced the rate of seed germination, as well as the length of shoots and roots, overall growth, and the quantity of product collected, among the various levels of salinity. Different canola genotypes displayed varying responses depending on the stage of development. For example, the First Class genotype had more resilience during the germination process, while the Licord genotype exhibited the lowest amount of resilience. Compared to the Licord cultivar, the SLM046 and Okapi cultivars exhibited much higher levels of growth and manufacturing [62].

2.13 Impact of Salinity on Crop Yield and Components

Numerous studies have shown that abiotic stresses, such as salt, lead to significant decreases in agricultural yields for essential crops throughout the reproductive phase of their life cycle [63]. When it comes to potassium ions (K^+), the class has a significant affinity to them. The HKT transporter family regulates the entrance of Na^+ ions into leaf blades, affecting the homeostasis of sodium ions during the reproductive stage when the plant is subjected to salt stress [64]. Grain dry matter showed a significant association with the ratio of K^+ to Na^+, as well as with grain filling rate and length, under conditions of salt stress [65]. Changes in water relations, evapotranspiration, nutritional imbalances, stomatal conductance, and oxidative damage can all lead to a drop in yield when plants are under salt stress [66]. Salinity alters morphophysiological and biochemical processes, ultimately reducing agricultural production and productivity [66]. The following things can go wrong with the yield response: reproductive organs breaking down faster, photosynthetic activity going down, biomass accumulation going down, and source-sink activity getting worse [67]. Physiological and biochemical changes affect the leaf area, turgidity, assimilation synthesis, and potential yield [68]. Changes in these areas include mesophyll cells getting smaller, cells getting longer, fewer epidermal cells, and a general loss of thickness in the flag leaf and vascular tissue [69]. These changes are linked to yield quality. For instance, salt stress reduces wheat grain production by 39.1%, 24.3%, and 13.4% during anthesis, early booting, and mid-grain filling, respectively [70]. In addition to having a negative effect on the yield of wheat crops, salinity also has a negative influence on the nutritional quality features and mineral element content [71]. The presence of saltwater has a significant influence on the characteristics that contribute to yield as well as the reproductive responses, which eventually results in a reduction in yield [72]. When exposed to salt stress, a number of crop species, including tomato, *Brassica oleracea var. capitata*, and *Brassica oleracea var. italica*, display a reduction in the components of their production as well as their total yield [71].

2.14 Impact of Salinity on Rice

Researchers have conducted a number of mapping investigations on the resistance of rice to salt. Researchers place a higher priority on the seedling stage because of the immediate influence it has on the quantitative trait loci (QTL) study. This is despite the fact that they acknowledge the relevance of the reproductive stage in connection to grain production. Researchers use this strategy to reduce the amount of time spent on the tedious process of phenotyping. There have been a number of research organisations that have conducted considerable studies and identified the Quantitative Trait Loci (QTL) known as Saltol. This QTL is present in rice throughout the seedling stage. Researchers discovered six distinct sections of the genome, referred to as Quantitative Trait Loci (QTL), during the process of mapping a collection of Recombinant Inbred Lines (RILs). These QTLs are associated with specific characteristics observed during the early development stages of the plants [73]. Researchers discovered these QTLs on chromosomes 1 and 4 of the genome. Researchers used a group of introduced lines (IL) made from the parent plant ”Nona Bokra” to find 18 quantitative trait loci (QTLs) linked to salt tolerance indices. According to the findings of this study, there are three possible routes that might be responsible for salt tolerance in the ”Nona Bokra” variety: Na^+ compartmentalization, shoot Na^+ exclusion, and Na^+:K^+ homeostasis. In another piece of study, sixteen quantitative Trait Loci (QTL) were shown to be related to salt stress. These QTL were isolated from four different linkage groups. The researchers accomplished this by using the method of mapping the QTL on an F2 population during the reproduction phase [74].

2.14.1 Genome Wide Association Study in Rice for Salt Tolerance

Genome-wide SSR markers were used to genotype 180 different accessions of rice during the reproductive stage of the genome-wide association study (GWAS). The researchers evaluated thirteen morpho-physiological variables. These characteristics included ion concentrations in the stem and leaves, grain production, and salt damage rating. Researchers [75] discovered one hundred and nineteen of the twenty-eight significant connections between markers and characteristics related to the equilibrium of ions in stems and leaves. Researchers carried out a Genome-Wide Association Study (GWAS) on 155 different rice cultivars during the early vegetative stage of rice cultivation. The results of this study revealed 151 significant connections between genetic markers and characteristics. The researchers discovered that these linkages were present across ten distinct chromosomes [76]. The researchers measured a variety of agronomic metrics, including shoot and root length, above-ground and below-ground biomass, and relative water content (RWC), to describe the heterogeneous panel. The researchers discovered a genomic region of 11.26 megabase pairs on chromosome 1, which coincided with the SalTol1 copy number variation region. After searching through a large number of chromosomes, the researchers discovered numerous potential genes connected with ion transport and encoding transcription factors. The researchers described the phenotypes of 533 rice accessions. Of them, 297 were of the indica variety, and 257 were of the aus variety. The projected shoot area (PSA), rate of transpiration (TR), and transpiration utilisation rate (TUE) of the accessions were all quantified with the use of RGB imaging. According to [77], the purpose of this research was to investigate how early rice reacts to salt in the soil. Together with this phenotyping data, genome-wide association studies (GWAS) uncovered additional genes on chromosome 11 that have an effect on TUE. A recent genome-wide association study (GWAS) efficiently detected single nucleotide polymorphisms (SNPs) linked with 12 unique features related to salt tolerance in rice during the reproductive phase under field-grown conditions [78]. It was established by the researchers that there is a connection between the ion balance that occurs during the reproductive phase and Saltol, which is a quantitative trait locus (QTL) that is often mentioned and is situated on the first chromosome. Saltol is an important factor in the development of seedlings. In addition, they presented evidence that showed the existence of possible quantitative trait loci (QTL) on chromosomes 4, 6, and 7 [18].

2.14.2 Application of Whole Genome Sequencing to Search for Structural Variations Associated with Tolerance for Salinity

In a study, researchers looked at two distinct varieties of rice plants and found that they reacted differently to the presence of salt. Using a method known as whole-genome resequencing (WGR), they were able to identify a total of 57,656 insertions/deletions (InDels) and 401,683 single nucleotide polymorphisms (SNPs). A total of 614 genes exhibited a higher concentration of nonsynonymous SNPs, while 1247 genes contained 576 large-effect SNPs. Researchers have discovered 266 candidate genes [79] that might be verified and utilised to increase salt tolerance in rice. These genes can improve salt tolerance in rice types that exhibit different responses to stress. Scientists uncovered rice genotypes with various degrees of salt tolerance in a separate experiment. These genotypes had a total of 2,347 nonsynonymous single nucleotide polymorphisms (SNPs) and 51 frameshift mutations. In addition, they found that 396 genes possessing large genetic variants in their coding regions and displaying variable expression are connected with several processes related with salt tolerance [80].

Researchers found 401,683 single nucleotide polymorphisms (SNPs) and 57,656 indels using whole-genome resequencing (WGR) to investigate two rice cultivars with distinct responses to salt stress. The researchers discovered 614 genes that had a greater density of nonsynonymous SNPs and found 576 large-effect SNPs in 1247 genes with a higher density. Different rice cultivars may show a variety of stress responses. Furthermore, the researchers have identified 266 putative genes [79] with the potential to verify and utilize for enhancing salt tolerance. Another study by the same group of researchers found that rice genotypes with varying salt tolerances had 2,347 nonsynonymous SNPs and 51 frameshift mutations. In addition, they demonstrated that 396 genes that have large-effect polymorphisms in their coding areas and variable expression are associated with a number of processes that are associated with salt tolerance [80].

2.15 Effect of Salt Stress on Wheat

Wheat, which is known to be an important agricultural crop on a worldwide scale, is quite susceptible to the negative effects that salt stress may have. An excessive amount of salt in the soil disrupts the germination of wheat seeds and impedes the development of seedlings. When wheat plants are subjected to salt stress, it causes osmotic stress and ion toxicity, which ultimately results in the impairment of various fundamental physiological processes. The research that Munns and Tester carried out in 2008 offers a comprehensive grasp of the effects that salt stress has on wheat. Munns and Tester's research in 2008 comprehensively grasps the detrimental impacts of salt stress on wheat, including decreased crop output, changes in nutritional content, and an overall loss in plant well-being [1]. The article highlights all of these negative effects. Wheat, which is known to be an important agricultural crop on a worldwide scale, is quite susceptible to the negative effects that salt stress may have. The presence of an excessive amount of salt in the soil causes it to be difficult for wheat seeds to germinate and hinders the development of seedlings. Salt stress induces osmotic stress and ion toxicity, ultimately impairing a number of physiological functions in wheat plants. The research that Munns and Tester carried out in 2008 offers a comprehensive grasp of the effects that salt stress has on wheat. Therefore, it is crucial to emphasize the detrimental effects of saltwater exposure, including reduced crop yield, alterations in nutritional composition, and overall decline in plant health [1].

The existence of 90 stable quantitative trait loci (QTL) related with 15 distinct phenotypes was discovered by QTL mapping on a population consisting of 350 recombinant inbred lines (RILs). QTL mapping revealed that these QTLs were dispersed throughout the entire genome, except for chromosomes 4D, 6B, and 7D. A total of eight significant QTLs were verified in a natural population sampled from the four QTL clusters found on chromosomes 2D, 3D, 4B, and 6A. One of these QTLs was discovered to be connected to a gene known as Rht-B1 (Rht-Reduced height), which is responsible for a reduction in the height of the plant as well as an increase in the number of seeds produced. Three Kompetitive Allele-Specific PCR (KASP) markers efficiently targeted three qualitative trait loci (QTL) clusters created from SNPs [81]. An additional research was conducted on a population of 254 people who were referred to as RIL. The results of this inquiry revealed 158 consistent additive QTL (quantitative trait loci) for 27 morphophysiological variables. Except for chromosomes 3A and 4D, we discovered these QTL patterns throughout the entire wheat genome. Out of the 158 QTL, 78 were among the QTL clusters after successful mapping into 9 clusters. In order to evaluate the dependability of these QTL and determine whether or not they may be used in Marker-Assisted Selection (MAS), seven QTL were evaluated in two distinct populations. As a result, KASP indicators established a notably strong connection to stable QTL. Over the course of the germination and early-seedling phases of a study that included 186 F10 RILs, a total of 61 significant impacts QTL were found to be dispersed over fifteen chromosomes. Two major quantitative trait loci (QTLs) were responsible for the weights of the primary leaves and coleoptiles on chromosomes 5 and 2 [82]. During an investigation on a population of wheat created by mating the Excalibur and Kukri types, researchers discovered six quantitative trait loci (QTL) associated with salt tolerance. Characteristics such as the ability to maintain shoot growth in the presence of salt, the accumulation of sodium, the formation of chloride, and the proportion of potassium ions to sodium ions were linked to these quantitative trait loci. Researchers connected the GBS data of the mapping population to both destructive and non-destructive high-throughput imaging data. Some of these data were destructive, like the amount of Na^+, K^+, and Cl^- ions in the leaves [83], as well as projected shoot area (PSA) and relative rate of growth (calculated from PSA).

2.15.1 Genome Wide Association Study in Wheat

A recent genome-wide association study was carried out in 289 elite lines of the Wheat Association Mapping Initiative (WAMI) population under both low and high salt conditions. The analysis used 15,737 SNP markers across the whole plant development cycle. Examination of seven yield-related qualities revealed 120 significant marker-trait associations at high salinity and 118 at low salinity [84]. This was established after examination of seven yield-related features. During the vegetative stage, unique genetic regions for salt tolerance were discovered in 135 distinct wheat lines, as shown by a multi-locus genome-wide association study and published in [85]. Of the 220 quantitative trait nucleotides (QTNs) found for 12 salt tolerance-associated traits, 42 had a significant impact on 10 salt tolerance characteristics. After doing more study, a total of fifty-eight candidate genes for the associated genomic domains were discovered [86].

2.16 Effect of Salt Stress on Maize

During the investigation into salt tolerance, we discovered that out of the 61 quantitative trait loci (QTL) connected with salt tolerance for biomass-related metrics, 41 were present in the 209 doubled haploid (DH) lines. On the chromosomes 1, 3, 7, and 9, quantitative trait loci (QTL) that are unique to salt tolerance have been found. Among these, thirteen main impacts QTLs on chromosome 1 were shown to have the most significant impact on the phenotypic characteristics [87].

A further examination of 240 completely formed individuals in a group with doubled haploid (DH) chromosomes led to the discovery of a substantial genetic area responsible for a quantifiable property known as a quantitative trait locus (QTL). This characteristic is responsible for the height of plants. Researchers discovered this quantitative trait locus on Chromosome 1, which had a significant impact in the presence of salt. The specific location on Chromosome 1 was also home to the most important quantitative trait locus (QTL), which is responsible for determining the salt tolerance index based on the average height of the plant. Two putative genes involved with ion homeostasis were found within the confidence interval of the quantitative trait locus (QTL) [87]. The researchers found that these genes were located within the confidence interval of the QTL. A QTL analysis was conducted using 161 F2:5 RILs during an experiment conducted both inside and outdoors. A total of twenty-nine quantitative trait loci (QTL) were identified, with the majority located on chromosomes 1, 3, and 5. In the whole group of people tested, 14 had important quantitative trait loci (QTL) because of the way the treatment affected the QTL (Q × T) [88].

2.16.1 Genome Wide Association Study in Maize for Salt Tolerance

Transport coefficients and Ca^{2+} concentrations in both above-ground and below-ground regions of maize were measured and analysed using a panel consisting of 305 inbred lines of maize. In addition, Single Nucleotide Polymorphisms (SNPs) were used in order to ascertain the genetic composition of the sample in order to conduct a Genome-Wide Association Study (GWAS), which is a thorough analysis of relationships across the whole genome. Fifty-three significant single nucleotide polymorphisms (SNPs) were found to be genetically associated with five hundred related genes in the investigated areas. Researchers attempted to formulate this particular finding about salt treatment. The gene-based association research findings indicate a significant connection between Ca^{2+} transport and the tetratricopeptide repeat protein encoded by the GRMZM2G123314 gene. This suggests that genetic diversity has an effect on the expression of genes. Researchers investigated the survivability of 445 different kinds of maize plants when exposed to high levels of saline during the first stages of their development. The Genome-Wide Association Study (GWAS) discovered a total of fifty-seven genetic loci that significantly associate with salt tolerance. A total of forty-nine candidate genes were found to be present at these particular loci [88].

Luo et al. [89] conducted a genome-wide association study on maize, identifying genetic markers linked to salt tolerance. They found 57 loci and 49 candidate genes associated with the trait, highlighting mechanisms like stress response and ABA signaling. Two genes, SAG4 and SAG6, were specifically noted for their positive roles in enhancing maize's salt tolerance.

2.17 Effect of Salt Stress in Sorghum

Researchers investigated a mapping population consisting of 181 Recombinant Inbred Lines (RILs) produced from two parents. We concentrated our inquiry on three characteristics that were present during the germination stage, and nine characteristics that were present throughout the seedling stage. We found twelve quantitative trait loci (QTLs) that explain a range of phenotypic variation (PVE) for the germination characteristics, ranging from 5.4% to 6.0%. In addition, we have discovered 29 QTLs that explain a PVE range that extends from 5.3% to 21.9% for the characteristics of the seedlings. A total of six notable quantitative trait loci (QTL) were discovered at the seedling stage. The analysis revealed that the maternal parent exerted the predominant beneficial impacts. The results of this research, which included all stages of plant development, were analysed in great detail, and the results showed that there were a total of 53 quantitative trait loci (QTL) for six different attributes in both the salt and control situations. There were a total of six Quantitative Trait Loci (QTL) that were identified and classified as being very important in terms of statistical significance [90]. The construction of a comprehensive genomic map was accomplished by a recent research that used a population consisting of 177 F3:5 interspecific recombinant inbred lines (RILs).

The map provided a significant amount of accuracy, including all ten chromosomes of Sorghum and composed of markers from 1991. Through the use of the genetic map, the researchers were able to successfully discover ten quantitative trait loci (QTL) that are especially associated with salt stress. In terms of both the growth of plants and their overall health, these QTLs have a substantial influence. Four factors co-localizing on chromosome 4 affect the height of the plant, total biomass, and root biomass. Some genes linked to tolerance to osmotic and ionic stress, as well as a number of aquaporins [91], were found in quantitative trait loci (QTL) that were affected by salt.

2.18 Effect of Salt Salt Stress in Chickpea

In a recent research that was conducted in both controlled glasshouse and outdoor situations, 42 quantitative trait loci (QTL) were shown to have an effect on a number of different growth metrics. Researchers created two hundred recombinant inbred lines (RILs) by breeding two different kinds of Cicer arietinum for this study. These varieties were Rupali, which is sensitive, and Genesis836, which is tolerant. On the fourth, fifth, and sixth chromosomes, researchers discovered six significant quantitative trait loci (QTL) that are associated with salt tolerance. There were a total of 21 Quantitative Trait Loci (QTL) that were successfully found and assigned to two different places on the chromosome [92]. Two genetically different parental lines were crossed in the field: ICCV 10 (which can handle salt) and DCP 92-3 (which can't handle salt). This created a population of 201 Recombinant Inbred Lines (RILs) that were in the reproductive stage. It was discovered that there are 28 Quantitative Trait Loci (QTLs) in the population after conducting an investigation of the genetic makeup of the population as well as the observable attributes. A specific Quantitative Trait Locus (QTL) that is located on chromosome 6 was shown to have the highest effect on the overall variability in yield, accounting for 28.4% of the phenotypic variance that was observed. Researchers discovered that the chromosomes CaLG03 and CaLG07 are the primary locations for significant genetic areas associated with agricultural production and related variables, particularly under salt stress. Studies have shown that genes such as histidine kinase, Ca-dependent protein kinases, antiporter genes, and transcription factors like WRKY and MYB are associated with salt tolerance [93].

2.19 Effect of Salt Stress in Soybean

We evaluated a total of 174 individuals from the F9 generation for their ability to tolerate salt during the vegetative phase [94]. A salt-sensitive cultivar known as Cheongja 3 was crossed with a salt-tolerant landrace known as IT162669 to create this set of individuals. We included a significant number of physiological features that serve as indicators of stress in the phenotypic data collected after subjecting the plants to two weeks of salt stress. These characteristics included vegetative damage as well as the concentrations of Na^+ and K^+ ions. On chromosomes 6 and 10, researchers discovered two quantitative trait loci (QTL) that are both unique and rather significant.

These QTLs, connected to ionic stress and other important physiological characteristics, are associated with the presence of salt in the environment. Functional annotation and an analysis of the changes in gene expression between the parents revealed that qST6 has two prospective candidate genes, while qST10 contains six. A phosphoenolpyruvate carboxylase and an ethylene response factor are the components that make up these products. Do et al. (2018) produced a group of 132 individuals classified as F2:3 by mating the cultivar Williams 82, which has intermediate sensitivity, with the tolerant Fiskeby III (PI 438471). In the next steps, they discovered significant chromosomal areas that were associated with salt tolerance. The plants were examined for their vegetative characteristics and ion concentrations in relation to the presence of salt-induced stress after a time of two weeks had passed. A recent study found a strong link between leaf scorch, chlorophyll content ratio, and sodium and chloride ion levels with a substantial quantitative trait locus (QTL) developed from Fiskeby III and placed on chromosome 03. Furthermore, researchers discovered that Chromosome 13 harbors a supplemental allele linked to the salt content in leaves [8].

2.19.1 Genome Wide Association Study in Soybean for Salt Tolerance

Researchers conducted a genome-wide association study (GWAS) on a broad variety of soybean accessions during the initial phase of plant development [8]. The purpose of this research was to identify and confirm specific genomic areas that are associated with salt tolerance in plants. A comprehensive area on chromosome 3 that demonstrates salt tolerance was validated by the examination of single nucleotide polymorphisms (SNPs) obtained from 305 accessions through the use of SoySNP50K and Whole Genome Resequencing (WGR) of a subset of 234 accessions [95]. The three SNP markers, Glyma03g32900, Salt-20, Salt14056, and Salt11655, which are based on genes, were consistently demonstrated to be connected at the greatest degree of significance in both studies [8]. This was the case in all of the studies. Researchers carried out a subsequent Genome-Wide Association Study (GWAS) on 281 genetically diverse soybean accessions during the germination phase. According to the findings of this research, there are 22 quantitative trait loci (QTL) that are linked to salt tolerance. The researchers examined germination and biomass using four different salt tolerance criteria [96]. Researchers also performed phenotyping. On chromosomes 5 and 16, researchers discovered two quantitative trait loci (QTL) that are particularly noteworthy. Over the course of 121 wild soybean accessions, research was conducted to evaluate the seed germination capacity of these accessions under the influence of salt stress. Our research has led us to discover twenty-one single nucleotide polymorphisms (SNPs) that show significant relationships with salt tolerance characteristics. Our research has led us to discover that these single nucleotide polymorphisms are located on chromosomes 2, 3, 10, 18, and 19. According to *Zhang et. al.,* [97], the Y chromosome 10 showed the greatest significant associations

2.20 Effect of Salt Stress on Cotton

The presence of high amounts of salt in the soil poses a significant threat to cotton crops because it disrupts the physiological and biochemical processes that are taking place. It is possible that excessive salts in the soil might impede the ability of cotton plants to absorb water, which would lead to water stress and a reduction in the plants' overall growth. Furthermore, increased salt levels cause disruptions in the process of nutrient absorption, which ultimately results in nutritional deficiencies that further damage the overall health of the cotton plants. Research, such as the one that was carried out by *Khan et al.* (2019), has highlighted the negative effects that salt stress has on the productivity of cotton, the quality of its fibres, and the efficiency of photosynthesis [98].

We found 66 quantitative trait loci (QTL) in these studies with the help of single nucleotide polymorphism (SNP)-based QTL mapping. An F2-derived F3 population was used for this investigation. This population included a cotton line known as CCRI35, which is tolerant, and a cotton line known as Nan Dan (NH), which is sensitive. We assessed the morpho-physiological features of plants at the seedling stage under three different salt concentrations. Only fourteen of the found QTL displayed stability across three salt environments for six traits. These fourteen QTLs, which ranged from 2.72 to 9.87% of the phenotypic variation explained (PVE), were inherited from the male father and four from the female parent, respectively. In the A sub-genome, there were found to be five QTL identities. Additionally, the Dt subgenome contained nine QTLs, and further analysis identified eight clusters connected to twelve potential key genes associated with salinity [99].

2.21 Salt Stress & Genes Involved in Nutrient Uptake

The osmotic stress that was caused by salt during the salt stress circumstances led to a reduction in plant growth as well as the absorption of nutrients. The capacity of the roots to absorb nutrients and the efficiency with which the leaves carry out photosynthesis are two factors that determine the development and maturity of plants. In addition to inhibiting the process of photosynthesis, the buildup of sodium (Na^+) and chloride (Cl^-) ions in plant cells as a result of soil that has been influenced by salt causes the absorption of nitrogen (N), phosphorus (P), and potassium (K) minerals to be significantly reduced. Several plant organelles, including as hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) superoxide (O^{2-}), produce a greater number of reactive oxygen species (ROS) when they are subjected to increasing levels of salt stress. The mitochondria, chloroplasts, and peroxisomes are all examples of types of organelles that fall within this category. A large amount of damage was caused to proteins, lipids, nucleic acids, and cellular membranes in plants as a result of the increased buildup of reactive oxygen species (ROS). Catalase, ascorbate peroxidase, and superoxide dismutase are examples of antioxidant enzymes that plants produce in order to defend themselves against reactive oxygen species (ROS). The use of extra mineral nutrients and organic amendments has been widespread in recent years [100]. This is done with the intention of improving the capacity of different crop species to tolerate salt and to absorb nutrients. Nitrogen (N), phosphorus (P), and potassium (K) are three nutrients that are completely necessary for plants to have since they are involved in a wide variety of physiological and biochemical processes that have an effect on the development and production of plants. Previous research has shown that macronutrients play an essential part in a number of cellular activities that occur in plants. These processes include photosynthesis, osmoregulation in response to salt stress, energy transfer, and regulation of stomatal aperture, protein synthesis, and enzyme activation [69].

When subjected to salt stress, potassium (K) is believed to be more efficient in controlling osmotic flow compared to nitrogen (N) and phosphorus (P). The greater absorption of potassium compared to sodium is a crucial determinant in the resistance of plants to salt. Research has shown that the application of potassium (K) in combination with humic acid (HA) improves a range of physiological and biochemical processes, including stomatal conductance, water relations, nutrient absorption, and enzyme activation. This helps to offset the detrimental effects of reactive oxygen species (ROS). Humic acids (HAs) are vital organic compounds that play a crucial role in promoting plant growth and improving soil properties. At this point, we don’t really understand how adding HA to soil along with N and P or other molecules, like fulvic acid (FA), improves its properties and helps plants grow in a range of varieties when they are stressed by salt. Identifying important genes may enhance plant yield and nutrient utilisation efficiency [17].

The NRT and NPF gene families have been shown to have a role in the uptake and movement of nitrate inside the plant. The process of NO3- absorption is facilitated by two distinct types of transportation systems: high-affinity transport systems (HATS) and low-affinity transport systems (LATS). While NPF is often considered the primary component of the LATS for NO3- at high concentrations, many NRT family genes have shown significant affinity as well. Studies have shown a connection between certain RNT and NPF family genes and the dual-transport system. In Arabidopsis, genes such as RNT and NPF, specifically NPF6.3, NRT1.1, and CHL1, have been associated with the absorption of nitrate at both high and low affinities. The NPF6.3 gene facilitates the transfer of several substrates such as protein concentration, dipeptides, chloride, glucosinolates, and plant hormones including gibberellins (GAs), jasmonates (JAs), indole-3-acetic acid (IAA), and abscisic acid (ABA). The gene families NRT and NPF played a crucial role in the absorption and delivery of nitrate to various plant regions in Arabidopsis. The discovery of genes with low-to-high affinities for NRT and NPF in other plant species, together with the remarkable performance of the NPF6.3 gene under salt stress, remains unclear [101].

Chapter 3

Material and Methods

3.1 Methodology Flowchart

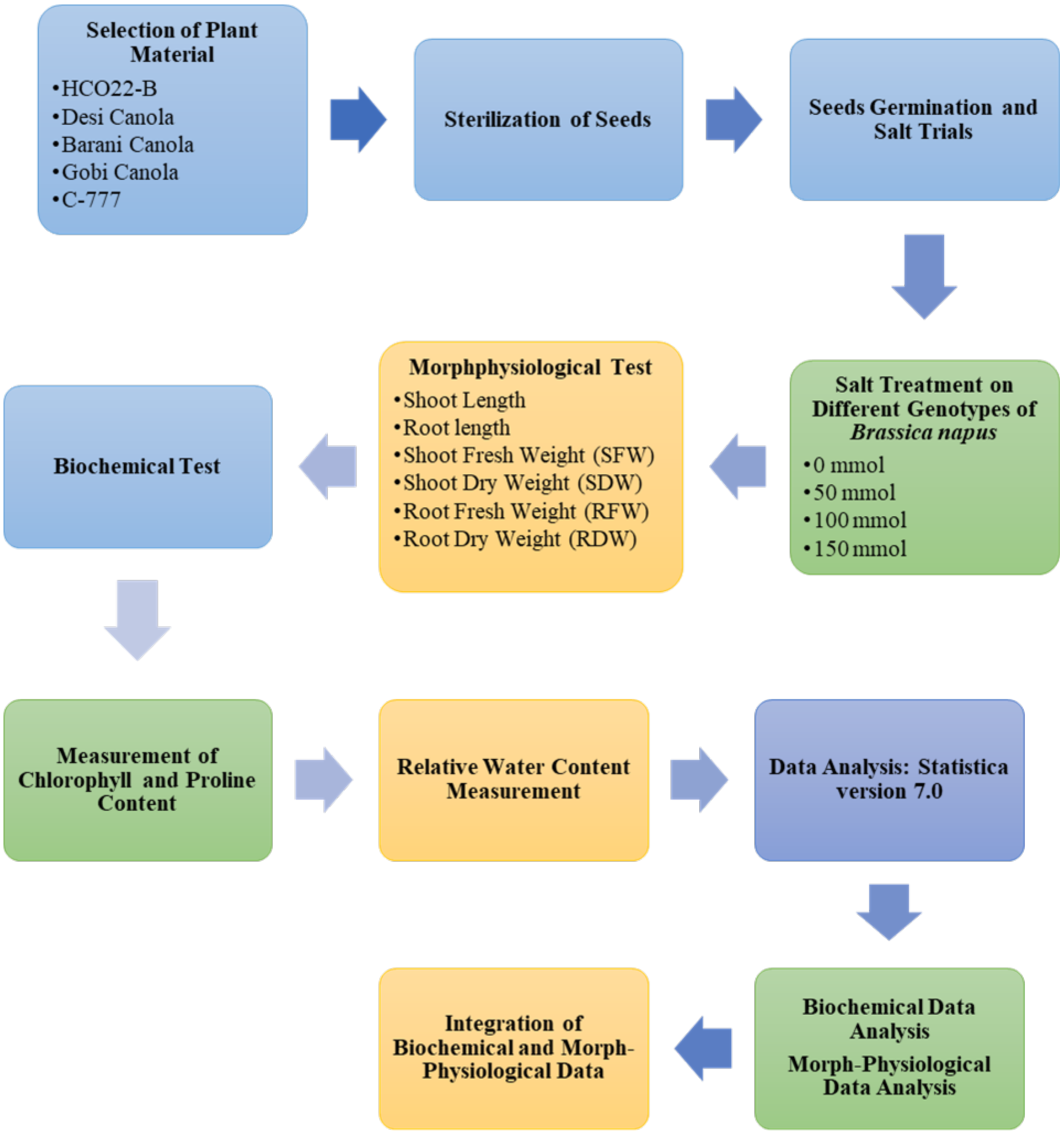

Figure 3.1: Technical route of acquired methodology

3.2 Materials and Chemicals

3.2.1 Seed Acquisition

Seeds of *Brassica napus* genotypes (HC022-B, Gobi Canola, Barani Canola, Desi Canola, and C-777) obtained from the gene bank of National Agricultural Research Centre (NARC), Islamabad, Pakistan and from Barani Agricultural Research Institute in Chakwal, Punjab.

3.2.2 Chemicals

Clorox bleach solution (10% concentration), Acetone (80% v/v), Arnon's [102] methodology for chlorophyll estimation, *Bates et al.* [9] methodology for proline determination, Sulfosalicylic Acid (SSA), Acid Ninhydrin Reagent, Standard Proline Solution, Ethanol, Chloroform, Trichloroacetic Acid (TCA), Hydrochloric Acid (HCl), Potassium Hydroxide (KOH), Barrs and Weatherly [10] methodology for RWC determination.

3.2.3 Experimental Setup

The experiment was conducted in the Department of Bioinformatics and Biosciences, at Capital University of Science and Technology (CUST), Islamabad, Pakistan. The experiment utilized sterilized glass jars, autoclaved filter papers, Petri dishes, forceps, an incubator, in a sterile environment within Wet Lab 1. The list of canola genotypes are mentioned below in Table 3.1.

Table 3.1: List of *Brassica napus* genotypes

Sr. No.	Genotypes	Origin	Source
1	HC022-B	Chakwal, Punjab, Pakistan	BARI, Chakwal
2	Gobi Canola	Chakwal, Punjab, Pakistan	BARI, Chakwal
3	Barani Canola	Chakwal, Punjab, Pakistan	BARI, Chakwal
4	Desi Canola	Chakwal, Punjab, Pakistan	BARI, Chakwal
5	C-777	Chakwal, Punjab, Pakistan	NARC, Islamabad

3.3 Sterilization of Seeds

The seeds were surface sterilized by rinsing them for ten minutes with double-distilled water to remove external contaminants. Following this, they underwent agitation for ten to fifteen minutes while being subjected to a 10% Clorox bleach solution. This step aimed to eradicate potential microbial contaminants on the seed surface.

To ensure the effectiveness of the sterilization process, the seeds were subsequently rinsed again for ten minutes with double-distilled water, facilitating the removal of any residual bleach solution. This dual rinsing procedure aims to minimize the chemical impact on the seeds. The final stage involved drying the sterilized seeds in Petri dishes, utilizing autoclave filter paper. This method ensures a controlled and sterile environment for the seeds during drying, preventing post-sterilization contamination.

3.4 Seed Germination and Salt Trials

Sterilized glass jars, equipped with autoclave filter papers, were the vessels for conducting seed germination experiments under varying salinity levels. This controlled environment was essential to elucidate the impact of sodium chloride (NaCl) concentrations on the germination process. The glass jars, pre-sterilized to eliminate potential contaminants, provided a sterile and standardized substrate for seed germination studies.

This experimental design employed four distinct NaCl concentrations: 0 mmol (control), 50 mmol, 100 mmol, and 150 mmol. Each glass jar was meticulously filled with 2 ml of the respective salt concentration, ensuring precise and uniform exposure to the salinity treatments. The inclusion of varying NaCl concentrations aimed to create a gradient of salinity stress, allowing for the systematic observation of germination responses under different physiological conditions.

Sterilized seeds were carefully introduced into each jar using forceps to initiate germination. This step ensured the controlled placement of seeds within the salted environment, minimizing external influences on the experimental setup. The use of forceps maintained sterility and facilitated precision in seed placement, a critical factor in obtaining reliable germination data.

Subsequently, the prepared glass jars with seeds were placed in an incubator at 28 ± 3^oC. This controlled temperature provided an optimal and consistent germination environment, and the incubation period adhered to a light/dark cycle of 16/8 hours, simulating natural day-night conditions. The provision of a regulated photoperiod is crucial for observing the influence of light on seed germination, a key aspect in understanding the physiological responses of the seeds to salinity stress.

Control plants, used for baseline comparison, were regenerated under the same experimental conditions but received distilled water instead of NaCl solutions. This allowed for a comparative analysis, isolating the effects of salinity on seed germination from other potential environmental factors. Including control groups is a standard practice in scientific research, providing a reference point for evaluating the specific impact of the variable under investigation.

3.5 Salt Treatment on Different Genotypes of Canola

In a controlled experiment, seeds from five distinct genotypes of canola were subjected to a rigorous salt treatment regimen. The seeds were placed in glass jars for ±28 days, creating a conducive environment for pre-germination growth. Subsequently, the salt treatment was initiated, involving the application of 2 mmol NaCl concentrations at varying levels: 0 mmol (control), 50 mmol, 100 mmol, and 150 mmol. This systematic treatment occurred every three days, ensuring a dynamic exposure to salt stress. The experiment was conducted at a constant temperature of 25^oC, maintaining a stable and controlled environment for the plants.

The examined morphophysiological parameters included shoot length, root length, shoot fresh weight, shoot dry weight, root dry weight, and root fresh weight as well. These parameters served as indicators of the plant's response to salt stress, capturing the visible growth and physiological changes occurring within the plant tissues. The assessment was carried out using established scientific protocols and instruments to ensure precision and accuracy in the measurements.

Additionally, biochemical tests were conducted to delve into the molecular responses of the plants to the salt treatment. This encompassed the analysis of various biochemical constituents such as enzymatic activity, chlorophyll content, and antioxidant levels. These biochemical markers provided insights into the underlying physiological mechanisms activated by the plants to counteract the detrimental effects of salinity stress.

3.6 Measurement of Chlorophyll & Proline Contents

A systematic approach was employed in the biochemical analysis of fresh leaves from salt-treated and control plants to extract crucial information regarding chlorophyll content and proline levels. By the methodology outlined by Arnon [102], chlorophyll quantification in canola leaf samples was conducted using a meticulous procedure. Fresh leaves of canola were meticulously prepared by chopping them into small segments. The subsequent chlorophyll extraction involved the immersion of leaf samples in 80% v/v acetone, followed by an incubation period in an environment set at -10°C overnight (24 hours).

Following the incubation, the extract, enriched with chlorophyll pigments, underwent centrifugation at 5000 rpm. The resulting supernatant was then subjected to spectrophotometric analysis at 645 nm and 663 nm wavelengths to estimate the concentrations of chlorophyll-a, chlorophyll-b, and Chlorophyll a+b.

This rigorous methodology ensures precise quantification of chlorophyll pigments, providing valuable insights into the photosynthetic activity of Brassica plants under investigation. For measuring proline levels, the methodology established by *Bates et al.* was employed with minor adjustments to suit the experimental conditions [9].

Proline, a well-known osmoprotectant, is a crucial indicator of plant stress response. The chlorophyll analysis and proline quantification, provides a comprehensive understanding of the biochemical responses of plants to salt stress. The choice of well-established methods, Arnon's [102] for chlorophyll estimation and Bates et al. [9] for proline determination, ensures the results' reliability and comparability.

3.7 Relative Water Content Measurement

The determination of Relative Water Content (RWC) in leaves, following the Barrs and Weatherly [10] procedure, involves a meticulous series of steps designed to assess the water status of plant tissues.

3.7.1 Leaf Disk Collection and Water Soaking

Leaf disks were carefully collected, and to achieve full hydration, they are soaked in distilled water in the absence of light for eight hours

3.7.2 Calculation of Fresh Weight (FW)

Following the duration of immersion, the leaf discs were measured to ascertain their fresh weight (FW). This measurement represents the overall moisture content inside the leaf tissues.

3.7.3 Determination of Turgid Weight (TW)

To eliminate excess water and calculate turgor pressure, the leaf disks were dried, yielding the turgid weight (TW). This step involves removing the surface moisture without affecting the cell turgor.

3.7.4 Drying to Obtain Dry Mass (DW)

The leaf disks were then dried at 80^oC for two days to obtain their dry mass (DW). This process ensures the removal of all water content from the leaves.

3.7.5 Calculation of Relative Water Content (RWC)

The RWC is calculated using the formula:

$$RWC(\%) = \frac{(FW - DW)}{(TW - DW)} \times 100$$

Where:

- FW is the fresh weight of the leaf disks.
- TW is the turgid weight of the leaf disks.
- DW is the dry mass of the leaf disks.

3.8 Data Analysis

The biochemical and morph-physiological data were analyzed using 'Stastistica version 7.0' software.

Chapter 4

Results and Discussion

The presence of an excessive amount of salt in the soil has the effect of significantly disrupting the normal morphological, physiological, and biochemical processes that occur in plants. In the current investigation, the impact of salt stress was systematically examined across five distinct Canola genotypes, namely HC022-B, Gobi Canola, Barani Canola, Desi Canola, and C-777. Each of these genotypes exhibited distinct morphophysiological and biochemical responses when subjected to varying salinity levels, ranging from 0 to 150 mmol.

Across the diverse genotypes, a spectrum of morpho-physiological and biochemical traits unfolded in response to the gradient of salinity levels. The investigation focused on key characters, encompassing morphological features, physiological responses, and biochemical indicators, at the designated salinity tiers (0, 50, 100, and 150 mmol). These observations aimed to unravel the nuanced variations in the adaptive strategies employed by the canola genotypes to contend with the challenges imposed by elevated salt concentrations. The multifaceted nature of the responses underscores the intricacy of plant interactions with salinity stress and paves the way for a more comprehensive understanding of salt tolerance mechanisms within the canola species.

4.1 Salinity Effects on Shoot Length of Canola Genotypes

Whether the amount of NaCl is low or high, it influences the shoot length of canola genotypes. The shoot length response of these genotypes changes depending on the severity of salt stress. Under the control condition (0 mmol), all genotypes had the highest shoot length compared to other salt stress levels, except for Barani Canola, which displayed a shoot length of 7.4 cm at a concentration of 50 mmol, indicating it had the largest shoot length at that level. During the 50 mmol salt experiment, the genotype Gobi Canola had a maximum shoot length of 10.5 cm, whereas the genotype Desi Canola had a shoot length of 10.4 cm. The genotypes HCO22-B, Barani canola, and C-777 exhibited excellent shoot length at this concentration. At a salt concentration of 100 mmol, the genotype Gobi Canola had a maximum shoot length of 10.3 cm, whereas the genotype Desi Canola had a shoot length of 10.2 cm. The Desi Canola genotype exhibited a significant increase in shoot length with a very high salt concentration (150 mmol), surpassing other genotypes, including C-777, as shown in Fig. 4.6. The results indicated that Gobi Canola, Desi Canola, and C-777 exhibited superior tolerance to varying degrees of salt in comparison to HCO22-B and Barani Canola. The Gobi Canola and Desi Canola exhibited greater salt stress resistance, maintaining their green and healthy appearance across all degrees of salt stress. The Barani Canola genotype exhibited a low germination rate and reduced growth under a salt stress of 150 mmol (Fig. 4.1 - 4.5).

4.2 Salinity Effects on Root Length of Canola Genotypes

The root length of canola genotypes is affected by the different amounts of sodium chloride, regardless of whether they are low or high. There is a variance in the root length response across these genotypes when subjected to varying degrees of salt stress. With the exception of Desi Canola, which had a length of 7.2 centimetres, and HCO22-B, which had a length of 6 centimetres, all genotypes displayed the longest root lengths when compared to other salt stress levels when the control condition was equal to zero millimoles. Under the conditions of the 50 mmol salt experiment, the maximum shoot length of the Desi Canola genotype was 8.3 centimetres. At the concentration in question, the genotypes HCO22-B, Barani canola, and C-777 had a satisfactory length of shoots overall. In Fig. 4.7, it can be shown that Desi Canola had a maximum shoot length of 9 centimetres when exposed to a high quantity of salt (100 mmol).

When compared to other genotypes, the Gobi Canola genotype had a considerably longer shoot length of 8.5 centimetres when subjected to circumstances with a very high salt concentration (150 mmol). According to the findings, Gobi Canola, Desi Canola, and C-777 demonstrated a higher level of tolerance to different concentrations of salt when compared to HCO22-B and Barani Canola. Both Gobi Canola and Desi Canola had a higher resilience to salt stress, as seen by their ability to keep their green and healthy looks regardless of the level of salt stress they were subjected to. A poor germination rate and limited growth were seen in the Barani Canola and HCO22-B genotypes when subjected to conditions of salt stress of 150 mmol (Fig. 4.1 - 4.5).

4.3 Salinity Effects on Shoot Fresh Weight of Canola Genotypes

When the effect of salt on shoot fresh weight was carefully examined, different genotype variations of canola exhibited distinct responses. All five genotypes have shown significant variances in their response patterns at varying degrees of salt stress. The Desi Canola and Gobi Canola genotypes recorded the highest shoot fresh weights, measuring 8.9 gm and 8.6 gm, respectively. In contrast, the Barani Canola genotype had the lowest shoot fresh weight, measured at 3.1 gm, when subjected to a significant salt stress of 150 mmol Fig. 4.8.

The results highlight the contrasting degrees of salt tolerance present in different varieties of canola plants. Gobi Canola and Desi Canola have greater capacity to adapt to a range of salt concentrations compared to HCO22-B, C-777, and Barani Canola. Significantly, Gobi Canola and Desi Canola have shown strong resilience as genotypes, exhibiting a high degree of resistance to salt stress. This was evident from their continuously healthy condition at all levels of salinity stress, as shown in Fig. 4.8.

On the other hand, the genotypes Barani Canola, C-777, and HCO22-B exhibited impaired physiological responses, characterised by reduced germination rates and stunted development, which were especially noticeable at the highest salinity level of 150 mmol. This empirical research demonstrates that salt stress affects different canola genotypes to varying degrees. The aforementioned trio of genotypes, in particular, show a significantly decreased ability to adapt to the high salinity conditions (Fig. 4.8).

4.4 Salinity Effect on Shoot Dry Weight (SDW) of Canola Genotypes

Canola genotypes displayed varied patterns in the evaluation of shoot dry weights when subjected to a variety of salt-stress situations. Barani Canola, Desi Canola, and genotype C-777 exhibited the highest shoot dry weights under salt stress conditions with a salt concentration of 0 mmol. C-777 performed the best in terms of dry weight yield among all. Genotype C-777 exhibited the lowest shoot dry weight of 0.79 grams under enhanced salt stress of 150 millimoles (Fig. 4.9).

The data suggests that Barani Canola and Desi Canola genotypes outperform HCO22-B and C-777 genotypes in terms of dry weight performance. These data indicate that the investigated genotypes exhibited a variety of responses to salt stress. As shown in Figure 4.9, the shoot dry weights of Barani Canola and Desi Canola were much higher than those of HCO22-B and C-777.

4.5 Effect of Salinity on Root Fresh Weight (RFW)

Using fresh weight measurements of the roots under varying salt stress conditions, distinct patterns among the canola genotypes were found. Notably, at the 0 mmol salt stress level, genotypes Desi Canola, Barani Canola, and C-777 showed the greatest root fresh weights. In contrast, when exposed to a salt stress level of 150 mmol, genotype HCO22-B showed the lowest root fresh weight of 0.48 gm. This suggests that there is a direct inverse link between the severity of salt stress and the fresh weight of the roots, with the fresh weight of the roots decreasing as the salt stress increased (Fig. 4.10).

Moreover, genotypes HCO22-B, Barani Canola, and C-777 showed either very low or insignificant root fresh weights at the maximum salt stress level of 150 mmol, highlighting the detrimental effects of increased salt stress on root growth in these genotypes (Fig. 4.10).

The root dry weight data showed consistent patterns, with genotypes Gobi Canola and Desi Canola responding better than the other genotypes at all salt stress levels. This highlights the potential of Gobi Canola and Desi Canola to withstand stress caused by salt, as seen by their increased root dry weights under difficult circumstances.

4.6 Salinity Effects on Root Dry Weight (RDW) of Canola Genotypes

The analysis of root dry weights (RDW) across different genotypes of canola revealed unique reactions to different salt concentrations. Notably, genotypes Desi Canola, Barani Canola, and C-777 showed better root dry weights at baseline salt level of 0 mmol; they registered values of 0.46 gm, 0.43 gm, and 0.35 gm, respectively. At this stress level, however, genotype HCO22-B showed a somewhat lower root dry weight of 0.37 gm, indicating variation in the initial response among the genotypes under study (Fig. 4.11).

For every genotype, a tendency of declining root dry weights was seen as salt concentrations rose. Genotypes HCO22-B, Barani Canola, and C-777 showed significantly lower root dry weights at the increased salt concentration of 150 mmol; their values were 0.31 gm, 0.33 gm, and 0.30 gm, respectively. Interestingly, under these demanding salt stress circumstances, Desi Canola retained a comparatively greater root dry weight of 0.40 gm, suggesting a degree of resistance compared to other genotypes (Fig. 4.11).

Examining the genotypes' relative performance, root dry weight responses for Gobi Canola and Desi Canola were consistently higher than those of HCO22-B, Barani Canola, and C-777 at all salt stress levels. This implies that the genotypes under study exhibit a complex range of adaptation and tolerance to salt stress. Notably, Desi Canola and Gobi Canola demonstrated increased resistance, especially when it came to maintaining root dry weights in unfavorable salinity circumstances (Fig. 4.11).

4.7 Relative Water Content of Leaf (RWC)

Different salt concentrations (mmol) were used to examine how salt stress affected the relative water content (RWC) of different genotypes of canola. Notably, the findings showed that genotype Desi Canola had the maximum RWC (82.5%) under 0 mmol salt stress, followed by Barani Canola (55.5%) and C-777 (61.5%). In contrast, the RWC for genotype HC022-B was 65.5% (Fig. 4.12).

All genotypes showed a steady decrease in RWC with increasing salt stress levels. The RWC for HC022-B dropped to 55.5%, Gobi Canola to 68.5%, Barani Canola to 51.2%, Desi Canola to 71.5%, and C-777 to 50.2% at the maximum salt concentration of 150 mmol. These results show a negative relationship between the relative water content and the degree of salt stress, with greater salt concentrations causing the plant tissues' ability to store water to decrease. Notably, at all salt stress levels, Gobi Canola and Desi Canola showed comparatively greater RWC values than the other genotypes, indicating a superior capacity to retain water content in salinized environments (Fig. 4.12).

4.8 Effect of Salinity on Relative Proline Content

When several genotypes of canola were examined to see how salt stress affected proline levels, the findings showed varied tendencies related to varying salt concentrations. Desi Canola genotype had the greatest Proline content at the baseline salt concentration of 0 mmol, followed by Barani Canola and C-777. At this stress level, genotype HCO22-B had the lowest proline concentration (5.2 mol g-1) (Fig. 4.13).

All genotypes showed a general tendency of increasing Proline content as the concentration of salt rose. Gobi Canola had the greatest Proline level at 50 mmol, exceeding the values of the other genotypes. At 100 mmol and 150 mmol, this increasing trend persisted, and Gobi Canola continuously showed the greatest Proline concentration in all salt stress levels as shown in (Fig. 9).

Notably, different genotypes responded differently to salt stress, and each genotype showed a distinct profile of Proline concentration. On the whole, nonetheless, the pattern suggested that Proline content rose as salt concentrations increased. These results highlight the function of proline as a putative marker for Brassica napus genotypes' responses to stress generated by salt, with Gobi Canola exhibiting an especially strong reaction in terms of proline accumulation.

4.9 Chlorophyll Content

The results shows differential reactions to varying salt concentrations when analyzing the effect of salt stress on chlorophyll content in different genotypes of canola.

4.9.1 Chlorophyll a

The genotypes D. Canola (0.86 mg g-1), G. Canola (0.82 mg g-1), C-777 (0.73 mg g-1), B. Canola (0.71 mg g-1), and HCO22-B (0.75 mg g-1) showed the greatest amounts of chlorophyll an at 0 mmol salt stress (Fig. 4.14).

All genotypes showed a steady decrease in chlorophyll a concentration as salt stress rose (to 50, 100, and 150 mmol).

4.9.2 Chlorophyll b

Genotypes D. Canola, G. Canola, and C-777 showed the greatest amounts of chlorophyll b under 0 mmol salt stress (0.84, 0.78, and 0.72 mg g-1, respectively) (Fig. 4.15).

All genotypes showed an overall decrease in chlorophyll b concentration as salt stress increased.

4.9.3 Chlorophyll a+b

In D. canola (1.7 mg g-1), G. canola (1.6 mg g-1), C-777 (1.45 mg g-1), B. canola (1.4 mg g-1), and HCO22-B (1.46 mg g-1) had the greatest total chlorophyll (a+b) concentration under 0 mmol salt stress.

All genotypes showed a steady reduction in total chlorophyll content as salt stress levels increased (Fig. 4.16).

All things considered, the findings show that there is a negative relationship between chlorophyll concentration and salt stress in *Brassica napus* genotypes, with D. Canola often displaying greater chlorophyll preservation than other genotypes under salt stress circumstances.

FIGURE 4.1: The effect of salt stress on HC022-B genotype, poor morphogenic response of genotype HCO22-B at 100 mmol and 150 mmol

FIGURE 4.2: The effect of salt stress on G. Canola genotype, excellent morphogenic response is shown by G. Canola to different salinity levels

FIGURE 4.3: The effect of salt stress on B. Canola, Better morphogenic response of Barani Canola at 50 and 100 mmol but shows poor response at 150 mmol

FIGURE 4.4: The effect of salt stress on D. Canola genotype excellent morphogenic response of genotype Desi Canola to different salinity levels

Figure 4.5: The effect of salt stress on C-777 genotype better morphogenic response of genotype C-777 to different salinity levels

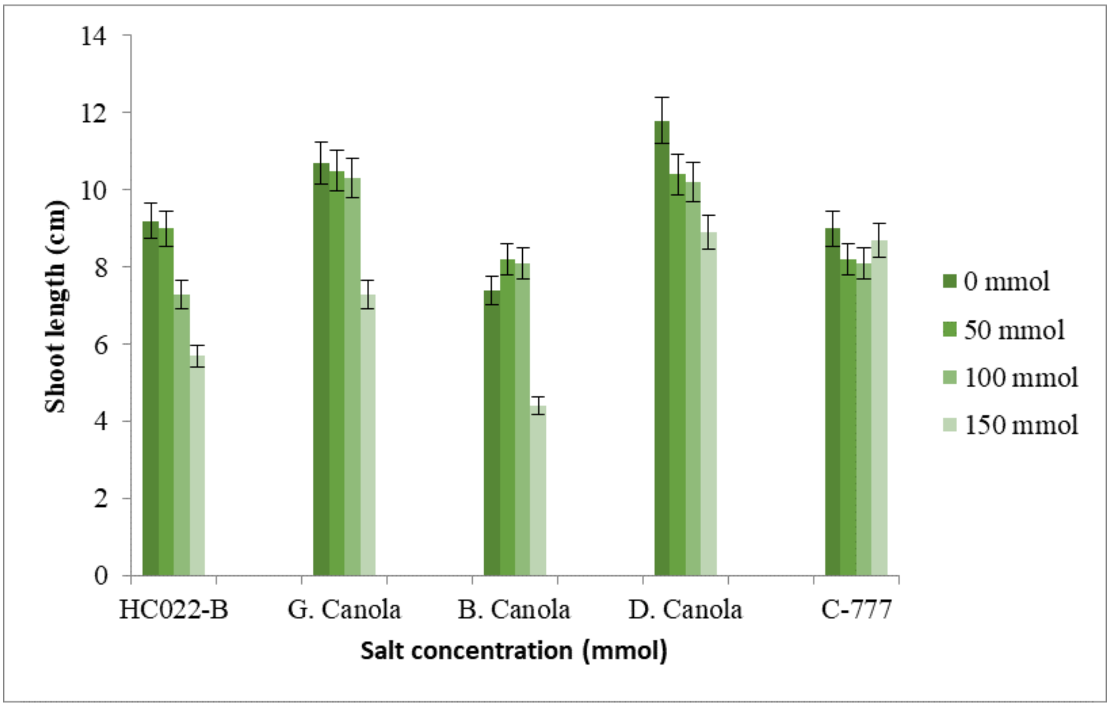

Figure 4.6: Effect of Salinity on Shoot Length on *B. napus* Genotypes

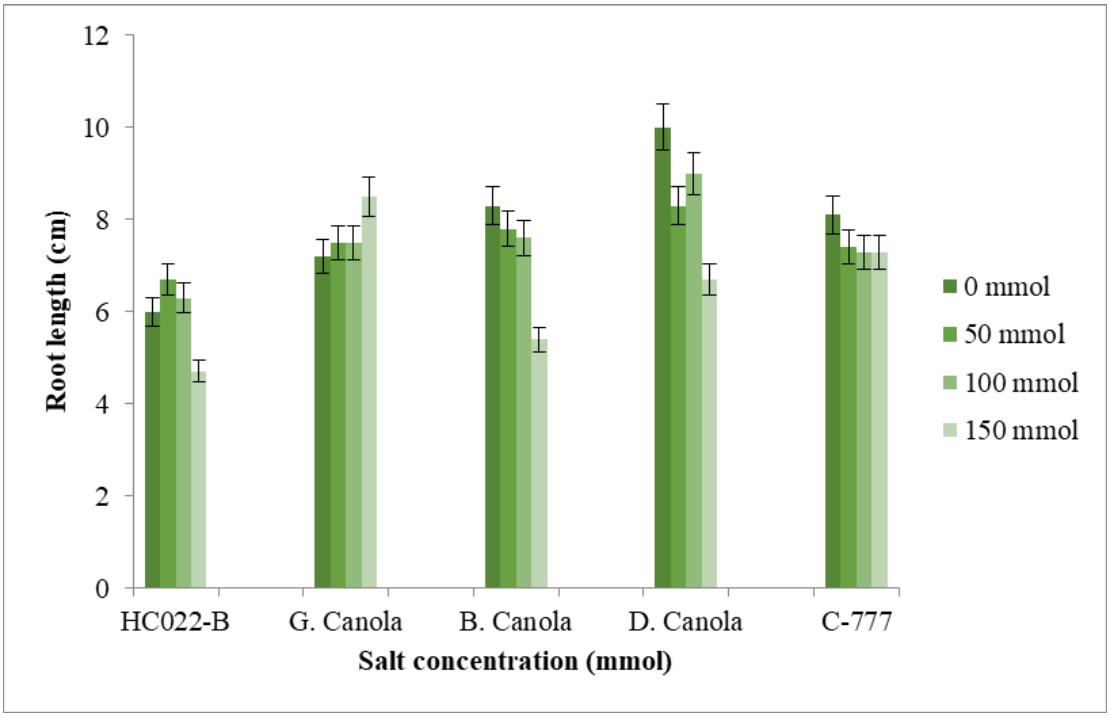

FIGURE 4.7: Effect of Salinity on Root Length on *B. napus* Genotype

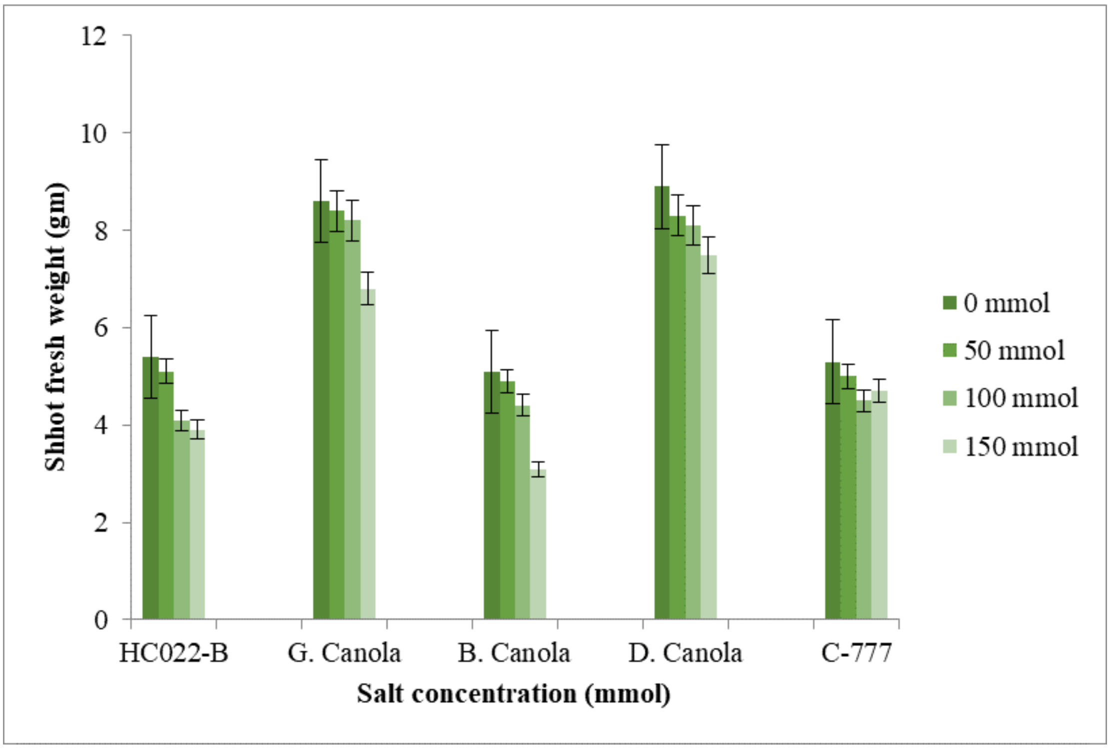

FIGURE 4.8: Effect of Salinity on Shoot Fresh Weight (SFW) on *B. napus* Genotypes

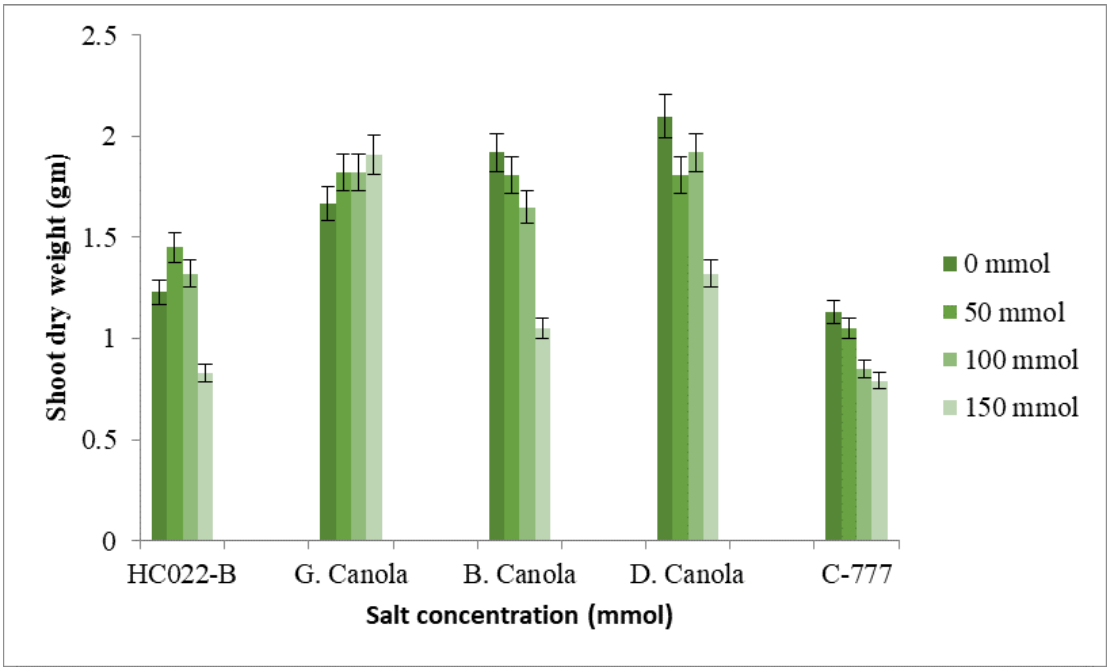

FIGURE 4.9: Effect of Salinity on Shoot Dry Weight (SDW) on *B. napus* Genotypes

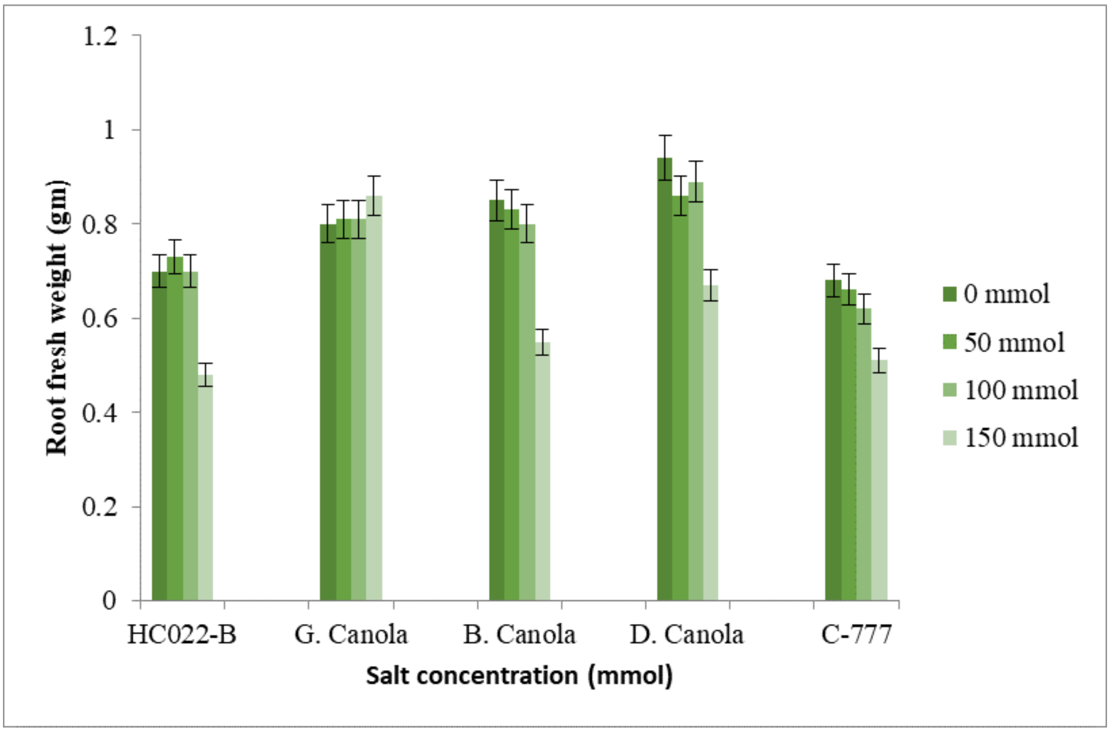

FIGURE 4.10: Effect of Salinity on Root Fresh Weight (RFW) on *B. napus* Genotypes

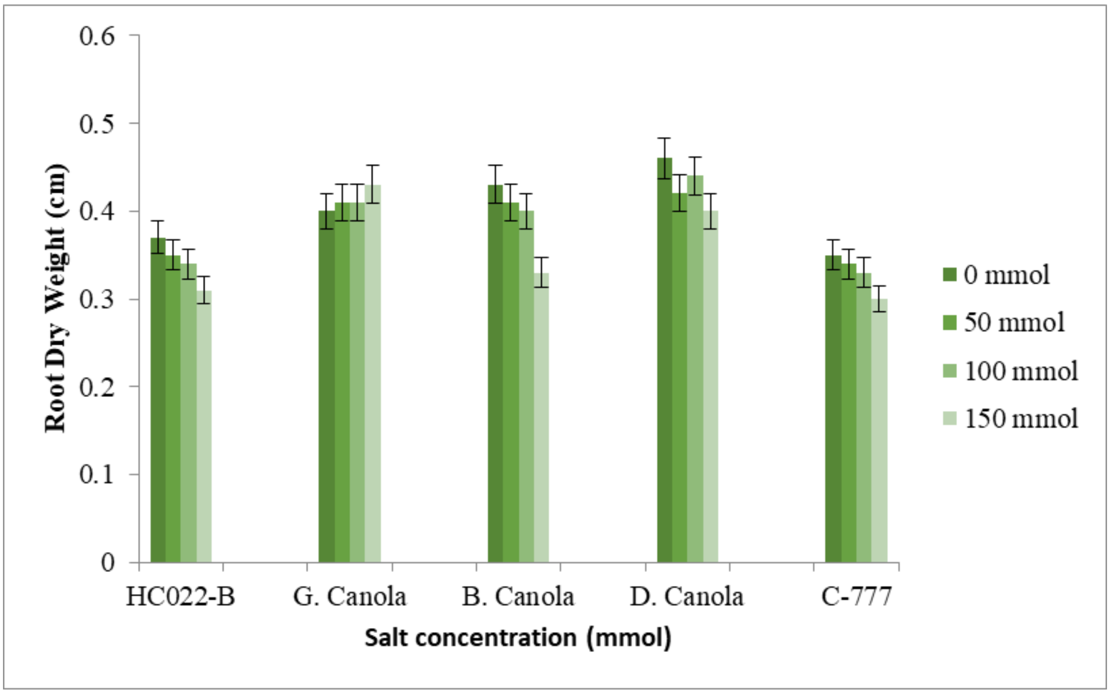

FIGURE 4.11: Effect of Salinity on Root Dry Weight (RDW) on *B. napus* Genotypes

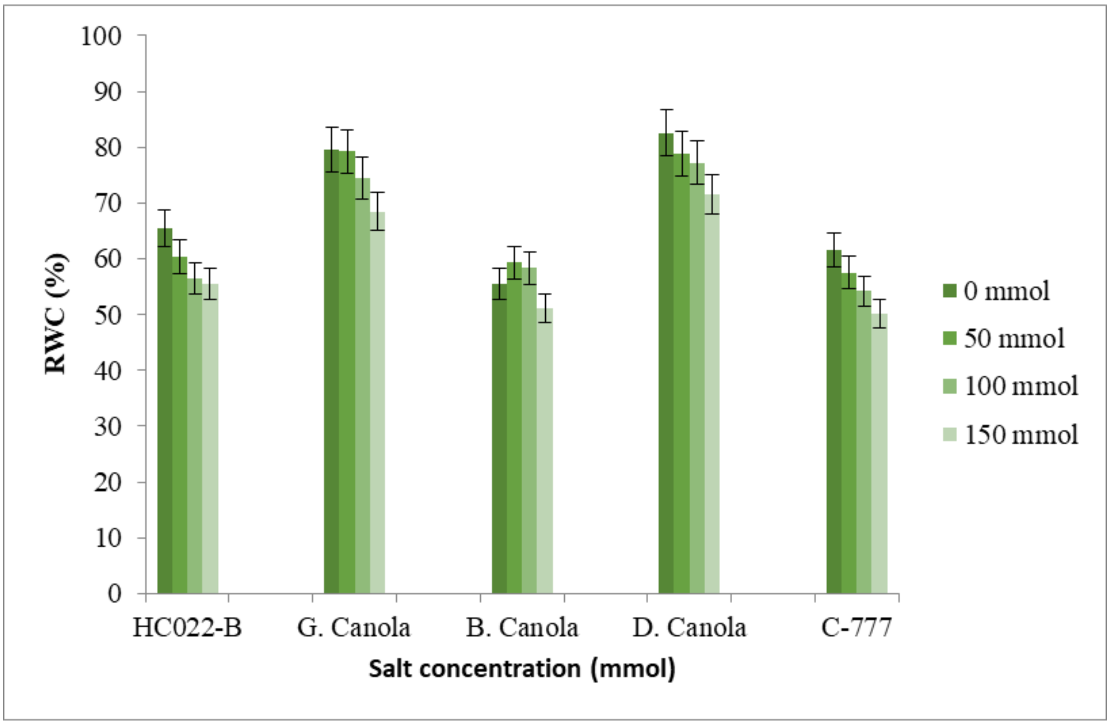

FIGURE 4.12: Effect of Salinity on Relative Water Content (RWC) on *B. napus* Genotypes

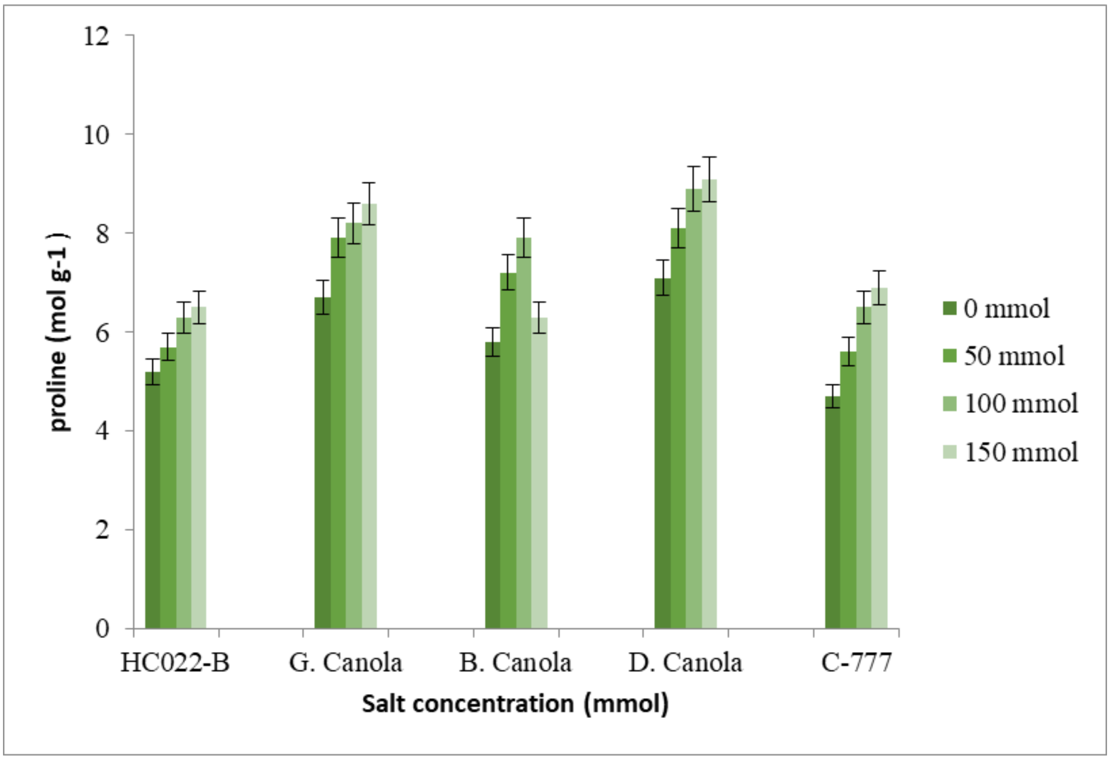

FIGURE 4.13: Effect of Salinity on Proline Content on *B. napus* Genotypes

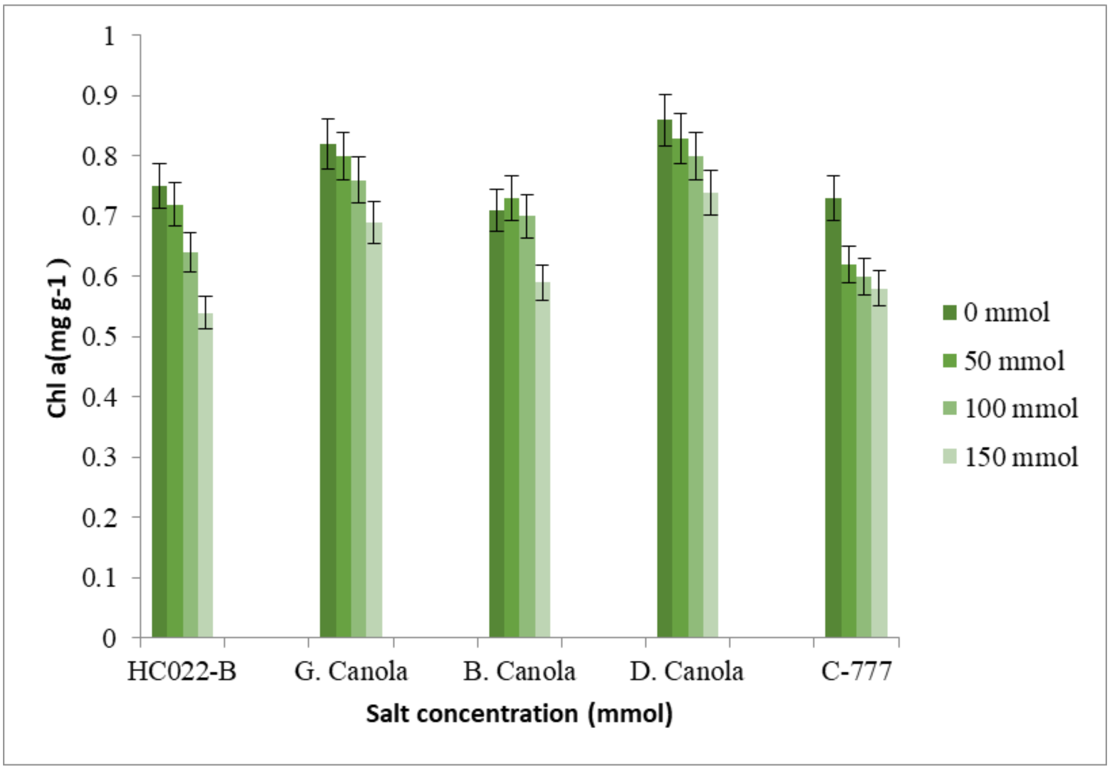

FIGURE 4.14: Effect of Salinity on Chlorophyll a on *B. napus* Genotypes

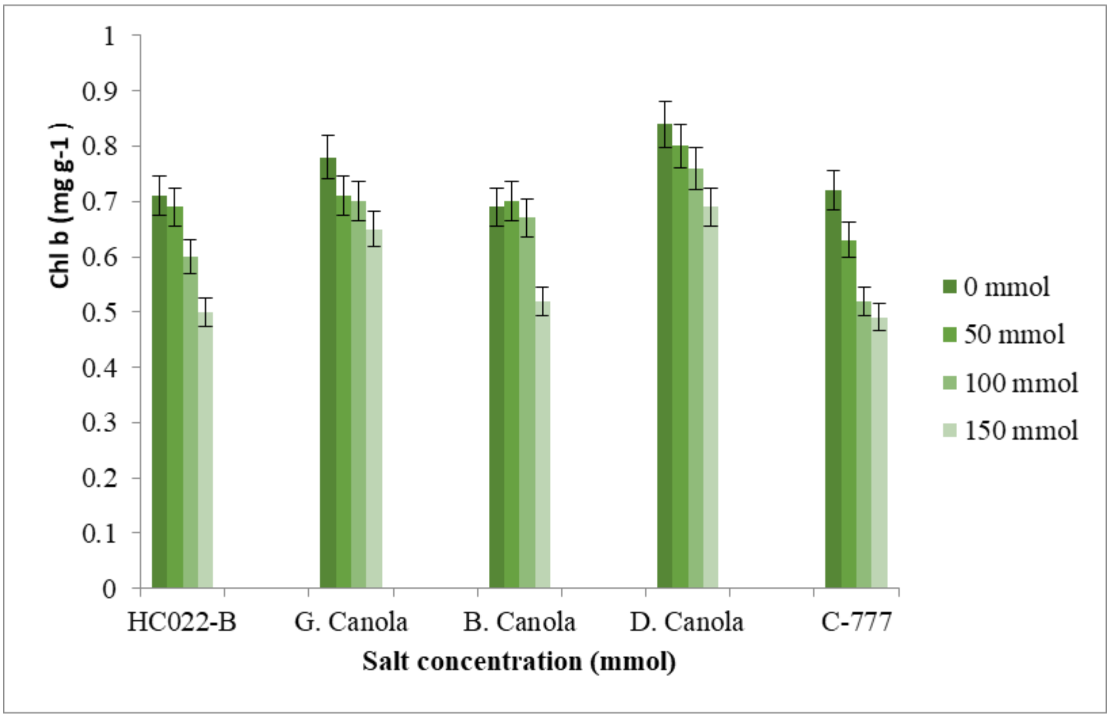

FIGURE 4.15: Effect of Salinity on Chlorophyll b on *B. napus* Genotypes

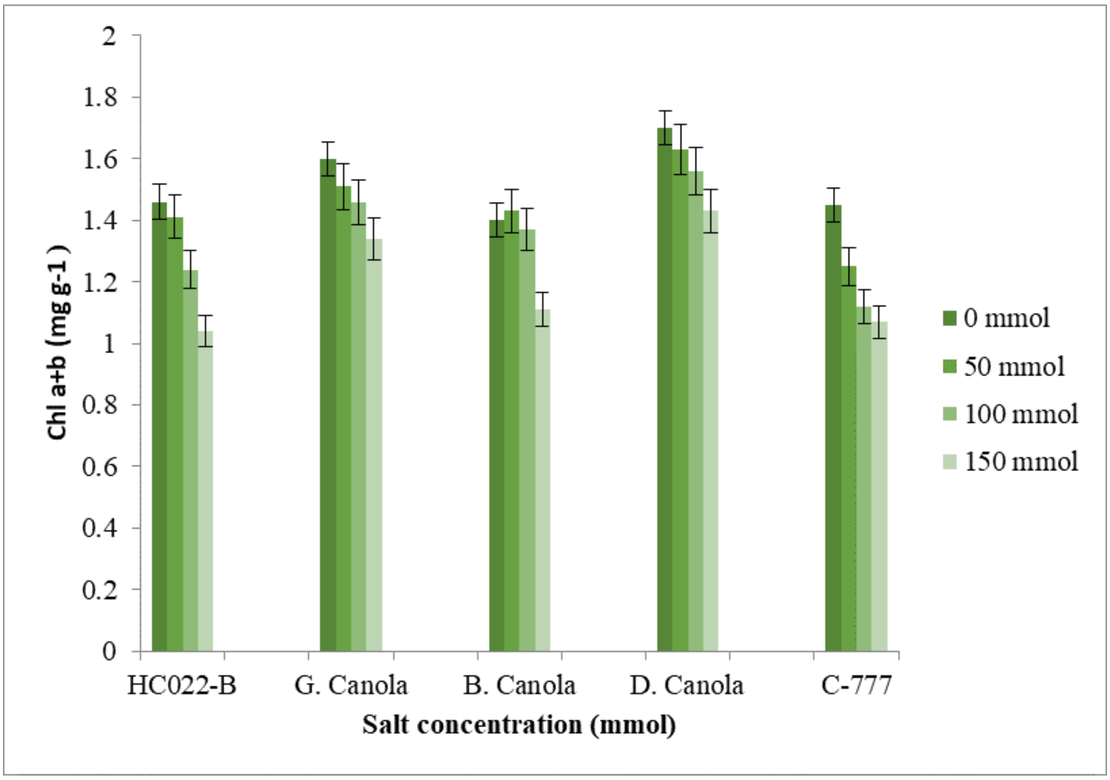

FIGURE 4.16: Effect of Salinity on Chlorophyll a+b on *B. napus* Genotypes

Chapter 5

Discussion

Salinity is a significant element that negatively impacts the development and metabolism of plants. Complex and varying processes linked to distinct metabolic pathways of different organs are involved in salinity stress. A number of metrics have been created in this research to assess the two pistachio cultivars' resistance to salt stress. Growth has been attributed to a variety of physiological processes, and several studies have extensively shown its decrease after salt therapy [1]. The current research examined the effects of salinity on the morphology, physiology, and metabolic processes of five significant B. napus genotypes. Four different degrees of salt stress (0, 50, 100, and 150 mmol) were applied to the canola genotypes HCO22-B, G. Canola, B. Canola, D. Canola, and C-777. Each genotype had a unique response at different salt concentrations. At elevated salt concentrations, all genotypes exhibited a substantial decrease in both growth and development. The study investigated the fundamental morphological and physiological changes in five important canola genotypes (HCO22-B, G. Canola, B. Canola, D. Canola, and C-777) under varying amounts of salt, ranging from low to moderate and even high. Figure 4.6 - 4.16 illustrates the impact of salinity on the lengths of shoots and roots in five different genotypes of canola during the first stage of germination. The results showed that genotype D. Canola had the highest shoot and root length, as well as root fresh and dry weight, at salt levels of 50, 100, and 150 mmol, 28 days after planting. Genotype G. Canola had the second highest value in these parameters.

Performance for the HCO22-B genotype was very subpar under all stress conditions. Compared to HCO22-B and Barani Canola, the brown D. Canola, G. Canola, and C-777 varieties of B. napus exhibit greater salt tolerance and maintain their green coloration under varying salinity conditions. The findings of this research align with the studies conducted by *Jan et al.* [4] and *Shahbazi et al.* [103], which demonstrated that elevated salt levels (50, 100, and 150 mmol) hinder the initial germination of seeds in all examined genotypes of *Brassica napus* and Brassica rapa. The findings of this research align with the studies conducted by *Jan et al.* [4] and *Shahbazi et al.* [103], which demonstrated that elevated salt levels (50, 100, and 150 mmol) hinder the initial germination of seeds in all examined genotypes of *Brassica napus* and *Brassica rapa.*

Jamil et al. [53] suggest that salinity may affect plant growth via many mechanisms, such as disrupting the normal ion exchange process, decreasing the availability of essential nutrients, and promoting the accumulation of detrimental chemicals. Also investigating the impact of salt in *Brassica campestris* was *Bilgili et al.* [54]. Every genotype that was analyzed exhibited inhibited growth when exposed to a high salt concentration of 180 mmol. The results we obtained align with the studies conducted by *Munns & Tester* [1] and *Wang et al.* [90]. These studies found that higher salt concentrations led to a decrease in the dry mass of leaves, shoots, and the whole plant compared to lower salt levels. Normal photosynthetic and other biological functions are inhibited by high salt levels (*Munns et al.* [20]. The impact of salinity on *B. napus* physiological processes was investigated in the current investigation. The percentage of relative water contents (%RWC) was considerably reduced by the high salt concentration, often by several orders of magnitude. In comparison to other genotypes, the genotypes G. and D. Canola exhibited the highest percentage of RWC at a low salt concentration of 50 mmol. However, the amount was decreased in all the genotypes analyzed at high levels of salt (50 and 100 mmol) (Fig. 4.12). *Alam et al.* [74] found that under drought stress, *B. napus, B. campestris,* and *B. juncea* had low RWC values.

Gupta et al. [99] also reported results that were similar. Plant water status is immediately reflected by RWC, and a decrease in RWC suggests that salt caused a water deficit in the plants. An increase in soluble salts had the detrimental impact on plant water relations because they slow down the absorption of nutrients and water, leading to osmotic effects and toxicity.Frost stress reduced the tomato genotypes Riogrande, Roma, and Money Maker's %RWC value by many orders of magnitude. *Shah et al.* [104]. According to *Bates et al.* [9], proline is essential for maintaining normal osmotic potential, signal transduction, and antioxidant characteristics. All examined *B. rapa* genotypes in this investigation had significant amounts of proline contents at high salt stress levels. Proline values rose multiple times at high salt levels as compared to plants under control (Fig. 4.13). *Alam et al.* [74] reported that *B. campestris* had a high proline concentration during a drought. Our results concur with those of earlier research *Ali et al* [104]. *Bates et al.* [9] also observed a significant proline concentration in the leaves of brassica species. The impact of salinity on chlorophyll contents was investigated in the current research. The findings demonstrate that salt stress dramatically reduced the amounts of chlorophyll a, b, and (a+b) in five of the B. napus subspecies (HCO22-B, G. Canola, B. Canola, D. Canola, and C-777) (Fig. 4.8). All five kinds' responses, however, differ from low to severe salt stress. In all genotypes, there were no overt signs of salt damage during the early stages of stress. The most significant pigments produced by plants via photosynthesis, chlorophyll a, chlorophyll b, and chlorophyll a+b, were found in lower concentrations in the leaves of the various treatments as the stress duration increased. This resulted in yellowing, withering, curling, and shedding of the leaves. D. Canola has the highest total chlorophyll (a+b) content of all five genotypes when compared to the other four. According to many studies *Siddiqui et al.* [61] *Elsayed et al.* [2], damage to significant photosynthetic pigments is the cause of the decline in photosynthetic rate). Chlorophyll concentration has been proposed in a number of studies as a biological indicator of plant salt tolerance. It is well known that although salt-sensitive plants experience a loss in chlorophyll contents, salt-tolerant plants exhibit increasing or stable chlorophyll levels under salinity conditions. Generally speaking, it is thought that either rapid or sluggish cellular pigment production is the cause of these pigments' decline under salt stress [50]. Chlorophyll, the primary pigment responsible for photosynthesis in plants, plays a crucial role in absorbing, transmitting, and converting light energy. The concentration of chlorophyll within a plant's tissues serves as a fundamental indicator of photosynthetic efficiency. However, under conditions of salt stress and prolonged stress durations, the levels of chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (Chl a+b) exhibit a consistent decreasing trend.This decline in chlorophyll content can be attributed to the detrimental effects of salt stress on the structural integrity of chloroplasts. Salt stress disrupts the organization and function of chloroplasts, leading to damage in their structure and membranes. Additionally, the increase in enzyme activity within chloroplasts, possibly triggered by stress response mechanisms, accelerates the breakdown of photosynthetic pigments. As a consequence of reduced chlorophyll levels, plants experience a significant decrease in the rate of CO_2 absorption during photosynthesis. This reduction in CO_2 uptake is not solely attributed to stomatal closure (non-stomatal limitation), but also to the impaired functioning of chloroplasts due to oxidative damage caused by salt stress. The findings corroborated those of *Akbarimoghaddam et al* [23], who found that when NaCl concentrations rose, chlorophyll decreased. Many significant plant species experience a direct reduction in chlorophyll concentration as a result of abiotic stressors like salt and drought. *Robinson et al.* [51], *Zhu et al.* [78]. *Alam et al.* [74] observed that during drought stress, there was a low concentration of chlorophyll a, b, and a+b. additionally, our results are consistent with those of *Sintaha et al.* [87].

Chapter 6

Conclusion and Future Work

In conclusion, the comprehensive investigation on the impact of salt stress across five distinct Canola genotypes elucidates the intricate dynamics of plant responses to varying salinity levels. The study unveiled diverse morphophysiological and biochemical adaptations employed by the genotypes to cope with elevated salt concentrations, showcasing nuanced variations in salt tolerance mechanisms. The differential responses observed in shoot and root growth, shoot fresh and dry weights, relative water content, proline accumulation, and chlorophyll content underscore the genotype-specific resilience to salt stress. Gobi Canola and Desi Canola emerged as promising candidates with superior adaptability to salinity gradients, while HCO22-B, C-777, and Barani Canola exhibited compromised physiological responses under high salt stress. These findings underscore the importance of understanding genotype-specific responses to salt stress for developing targeted breeding strategies aimed at enhancing salt tolerance in *Brassica napus* cultivars, thus contributing to sustainable crop production in saline environments.

6.1 Future Recommendations

To enhance our understanding and tackle the challenges posed by salt stress in Brassica napus cultivation, several future recommendations emerge from extensive findings on various morphophysiological and biochemical parameters in different genotypes. Firstly, comprehensive genetic diversity studies across a broader range of Brassica napus genotypes are crucial. This would help identify novel genetic loci associated with salt tolerance traits using advanced genomic tools like GWAS and NGS. Furthermore, implementing marker-assisted breeding approaches is essential to introgress salt tolerance traits from tolerant genotypes like Gobi Canola and Desi Canola into elite cultivars. Molecular markers linked to key genes and QTLs associated with salt tolerance can expedite the development of salt-tolerant varieties. Conducting transcriptomic and proteomic analyses will elucidate molecular mechanisms underlying salt tolerance, identifying potential targets for genetic manipulation and crop improvement.

Metabolic profiling can characterize metabolic responses to salt stress, providing insights into biochemical mechanisms of adaptation. Detailed physiological studies focusing on ion transport, water relations, and photosynthesis will reveal mechanisms conferring salt tolerance in different genotypes. Field evaluations of salt-tolerant genotypes under real-world conditions are essential to assess agronomic performance and adaptability. Integrated management strategies, including soil amendments and irrigation management, can mitigate salt stress effects and enhance productivity. Investing in capacity building initiatives to train stakeholders on best practices for salt-affected agriculture is crucial. Lastly, fostering collaborative research between institutions, academia, industry, and government agencies will facilitate knowledge exchange and technology transfer, addressing the challenges of salt stress in Brassica napus cultivation.

By implementing these recommendations, we can advance our understanding of salt tolerance mechanisms and develop sustainable strategies to mitigate adverse effects on crop productivity, ensuring food security and agricultural sustainability in salt-affected regions.

Bibliography

[1] R. Munns and M. Tester, “Mechanisms of salinity tolerance,” *Annu. Rev. Plant Biol.*, vol. 59, pp. 651–681, 2008.

[2] A. I. ElSayed, A. H. Mohamed, M. S. Rafudeen, A. A. Omar, M. F. Awad, and E. Mansour, “Polyamines mitigate the destructive impacts of salinity stress by enhancing photosynthetic capacity, antioxidant defense system and upregulation of calvin cycle-related genes in rapeseed (brassica napus l.),” *Saudi Journal of Biological Sciences*, vol. 29, no. 5, pp. 3675–3686, 2022.

[3] N. Dolatabadi and M. Toorchi, “Rapeseed (brassica napus l.) genotypes response to nacl salinity,” *J. Biodivers. Environ. Sci*, vol. 10, pp. 265–270, 2017.

[4] S. A. Jan, Z. K. Shinwari, M. A. Rabbani *et al.*, “Agro-morphological and physiological responses of brassica rapa ecotypes to salt stress,” *Pak. J. Bot*, vol. 48, no. 4, pp. 1379–1384, 2016.

[5] A. Chmielewska, M. Kozłowska, D. Rachwał, P. Wnukowski, R. Amarowicz, E. Nebesny, and J. Rosicka-Kaczmarek, “Canola/rapeseed protein–nutritional value, functionality and food application: a review,” *Critical Reviews in Food Science and Nutrition*, vol. 61, no. 22, pp. 3836–3856, 2021.

[6] T. J. Flowers and T. D. Colmer, “Salinity tolerance in halophytes,” *New phytologist*, pp. 945–963, 2008.

[7] R. Munns, R. A. James, and A. Läuchli, “Approaches to increasing the salt tolerance of wheat and other cereals,” *Journal of experimental botany*, vol. 57, no. 5, pp. 1025–1043, 2006.

[8] T. D. Do, T. D. Vuong, D. Dunn, S. Smothers, G. Patil, D. C. Yungbluth, P. Chen, A. Scaboo, D. Xu, T. E. Carter *et al.*, "Mapping and confirmation of loci for salt tolerance in a novel soybean germplasm, fiskeby iii," *Theoretical and Applied Genetics*, vol. 131, pp. 513–524, 2018.

[9] L. S. Bates, R. a. Waldren, and I. Teare, "Rapid determination of free proline for water-stress studies," *Plant and soil*, vol. 39, pp. 205–207, 1973.

[10] H. Barrs and P. Weatherley, "A re-examination of the relative turgidity technique for estimating water deficits in leaves," *Australian journal of biological sciences*, vol. 15, no. 3, pp. 413–428, 1962.

[11] T. F. Bhuiyan, K. U. Ahamed, K. Nahar, J. Al Mahmud, M. B. Bhuyan, T. I. Anee, M. Fujita, and M. Hasanuzzaman, "Mitigation of peg-induced drought stress in rapeseed (brassica rapa l.) by exogenous application of osmolytes," *Biocatalysis and Agricultural Biotechnology*, vol. 20, p. 101197, 2019.

[12] S. Wang, S. Ge, C. Tian, and W. Mai, "Nitrogen–salt interaction adjusts root development and ion accumulation of the halophyte suaeda salsa," *Plants*, vol. 11, no. 7, p. 955, 2022.

[13] K. K. So and R. W. Duncan, "Breeding canola (brassica napus l.) for protein in feed and food," *Plants*, vol. 10, no. 10, p. 2220, 2021.

[14] S. Zhao, Q. Zhang, M. Liu, H. Zhou, C. Ma, and P. Wang, "Regulation of plant responses to salt stress," *International Journal of Molecular Sciences*, vol. 22, no. 9, p. 4609, 2021.

[15] M. R. H. Raihan, K. Nahar, F. Nowroz, A. Siddika, and M. Hasanuzzaman, "Oilseed brassica responses and tolerance to salt stress," in *Oilseed Crops-Uses, Biology and Production.* IntechOpen, 2023.

[16] J. A. Mahmud, M. Hasanuzzaman, M. I. R. Khan, K. Nahar, and M. Fujita, "β-aminobutyric acid pretreatment confers salt stress tolerance in brassica napus l. by modulating reactive oxygen species metabolism and methylglyoxal detoxification," *Plants*, vol. 9, no. 2, p. 241, 2020.

[17] M. K. Sharma, "Plants stress: Salt stress and mechanisms of stress tolerance." *Current Agriculture Research Journal*, vol. 11, no. 2, 2023.

[18] A. Bybordi, "The influence of salt stress on seed germination, growth and yield of canola cultivars," *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, vol. 38, no. 1, pp. 128–133, 2010.

[19] R. Chatrath, B. Mishra, G. Ortiz Ferrara, S. Singh, and A. Joshi, "Challenges to wheat production in south asia," *Euphytica*, vol. 157, pp. 447–456, 2007.

[20] R. Munns, "Genes and salt tolerance: bringing them together," *New phytologist*, vol. 167, no. 3, pp. 645–663, 2005.

[21] A. K. Parida and A. B. Das, "Salt tolerance and salinity effects on plants: a review," *Ecotoxicology and environmental safety*, vol. 60, no. 3, pp. 324–349, 2005.

[22] A. M. Bohnert, J. W. Aikins, and J. Edidin, "The role of organized activities in facilitating social adaptation across the transition to college," *Journal of Adolescent Research*, vol. 22, no. 2, pp. 189–208, 2007.

[23] H. Akbarimoghaddam, M. Galavi, A. Ghanbari, N. Panjehkeh *et al.*, "Salinity effects on seed germination and seedling growth of bread wheat cultivars," *Trakia journal of Sciences*, vol. 9, no. 1, pp. 43–50, 2011.

[24] P. Shrivastava and R. Kumar, "Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation," *Saudi journal of biological sciences*, vol. 22, no. 2, pp. 123–131, 2015.

[25] A. Jamil, S. Riaz, M. Ashraf, and M. Foolad, "Gene expression profiling of plants under salt stress," *Critical Reviews in Plant Sciences*, vol. 30, no. 5, pp. 435–458, 2011.

[26] E. Van Zelm, Y. Zhang, and C. Testerink, "Salt tolerance mechanisms of plants," *Annual review of plant biology*, vol. 71, pp. 403–433, 2020.

[27] H. Fu and Y. Yang, "How plants tolerate salt stress," *Current Issues in Molecular Biology*, vol. 45, no. 7, pp. 5914–5934, 2023.

[28] W.-Y. Kim, Z. Ali, H. J. Park, S. J. Park, J.-Y. Cha, J. Perez-Hormaeche, F. J. Quintero, G. Shin, M. R. Kim, Z. Qiang *et al.*, "Release of sos2 kinase from sequestration with gigantea determines salt tolerance in arabidopsis," *Nature communications*, vol. 4, no. 1, p. 1352, 2013.

[29] N. S. Mishra, R. Tuteja, and N. Tuteja, "Signaling through map kinase networks in plants," *Archives of Biochemistry and Biophysics*, vol. 452, no. 1, pp. 55–68, 2006.

[30] Y. Yang and Y. Guo, "Unraveling salt stress signaling in plants," *Journal of integrative plant biology*, vol. 60, no. 9, pp. 796–804, 2018.

[31] F. Yuan, H. Yang, Y. Xue, D. Kong, R. Ye, C. Li, J. Zhang, L. Theprungsirikul, T. Shrift, B. Krichilsky *et al.*, "Osca1 mediates osmotic-stress-evoked ca2+ increases vital for osmosensing in arabidopsis," *Nature*, vol. 514, no. 7522, pp. 367–371, 2014.

[32] E. S. Hamilton, G. S. Jensen, G. Maksaev, A. Katims, A. M. Sherp, and E. S. Haswell, "Mechanosensitive channel msl8 regulates osmotic forces during pollen hydration and germination," *Science*, vol. 350, no. 6259, pp. 438–441, 2015.

[33] M. Thalmann, D. Pazmino, D. Seung, D. Horrer, A. Nigro, T. Meier, K. Kölling, H. W. Pfeifhofer, S. C. Zeeman, and D. Santelia, "Regulation of leaf starch degradation by abscisic acid is important for osmotic stress tolerance in plants," *The Plant Cell*, vol. 28, no. 8, pp. 1860–1878, 2016.

[34] M. He, X. Xiong, L. Wang, D. Hou, N. S. Bolan, Y. S. Ok, J. Rinklebe, and D. C. Tsang, "A critical review on performance indicators for evaluating soil biota and soil health of biochar-amended soils," *Journal of hazardous materials*, vol. 414, p. 125378, 2021.

[35] J. Urra, I. Alkorta, and C. Garbisu, "Potential benefits and risks for soil health derived from the use of organic amendments in agriculture," *Agronomy*, vol. 9, no. 9, p. 542, 2019.

[36] S. Amini, H. Ghadiri, C. Chen, and P. Marschner, "Salt-affected soils, reclamation, carbon dynamics, and biochar: a review," *Journal of Soils and Sediments*, vol. 16, pp. 939–953, 2016.

[37] P. Trivedi, K. Singh, U. Pankaj, S. K. Verma, R. K. Verma, and D. Patra, "Effect of organic amendments and microbial application on sodic soil properties and growth of an aromatic crop," *Ecological engineering*, vol. 102, pp. 127–136, 2017.

[38] L. Yang, X. Bian, R. Yang, C. Zhou, and B. Tang, "Assessment of organic amendments for improving coastal saline soil," *Land Degradation & Development*, vol. 29, no. 9, pp. 3204–3211, 2018.

[39] R. Munns, "Comparative physiology of salt and water stress," *Plant, cell & environment*, vol. 25, no. 2, pp. 239–250, 2002.

[40] Y. Liang, Y. Yang, C. Yang, Q. Shen, J. Zhou, and L. Yang, "Soil enzymatic activity and growth of rice and barley as influenced by organic manure in an anthropogenic soil," *Geoderma*, vol. 115, no. 1-2, pp. 149–160, 2003.

[41] A. Abdul Qadir, G. Murtaza, M. Zia-ur Rehman, and E. A. Waraich, "Application of gypsum or sulfuric acid improves physiological traits and nutritional status of rice in calcareous saline-sodic soils," *Journal of Soil Science and Plant Nutrition*, vol. 22, no. 2, pp. 1846–1858, 2022.

[42] I. S. Møller, M. Gilliham, D. Jha, G. M. Mayo, S. J. Roy, J. C. Coates, J. Haseloff, and M. Tester, "Shoot na+ exclusion and increased salinity tolerance engineered by cell type–specific alteration of na+ transport in arabidopsis," *The Plant Cell*, vol. 21, no. 7, pp. 2163–2178, 2009.

[43] H. Wu, "Plant salt tolerance and na+ sensing and transport," *The Crop Journal*, vol. 6, no. 3, pp. 215–225, 2018.

[44] D. West and J. Taylor, "Response of six grape cultivars to the combined effects of high salinity and rootzone waterlogging," *Journal of the American Society for Horticultural Science*, vol. 109, no. 6, pp. 844–851, 1984.

[45] T. Hiramatsu, K. Kodama, T. Kuroda, T. Mizushima, and T. Tsuchiya, "A putative multisubunit na+/h+ antiporter from staphylococcus aureus," *Journal of bacteriology*, vol. 180, no. 24, pp. 6642–6648, 1998.

[46] H. Ji, J. M. Pardo, G. Batelli, M. J. Van Oosten, R. A. Bressan, and X. Li, "The salt overly sensitive (sos) pathway: established and emerging roles," *Molecular plant*, vol. 6, no. 2, pp. 275–286, 2013.

[47] M. Sussman, "Molecular analysis of proteins in the plant plasma membrane," *Annual Review of Plant Biology*, vol. 45, no. 1, pp. 211–234, 1994.

[48] S. J. Roy, S. Negrão, and M. Tester, "Salt resistant crop plants," *Current opinion in Biotechnology*, vol. 26, pp. 115–124, 2014.

[49] J. W. Fahey, A. T. Zalcmann, and P. Talalay, "The chemical diversity and distribution of glucosinolates and isothiocyanates among plants," *Phytochemistry*, vol. 56, no. 1, pp. 5–51, 2001.

[50] M. Iqbal and M. Ashraf, "Seed preconditioning modulates growth, ionic relations, and photosynthetic capacity in adult plants of hexaploid wheat under salt stress," *Journal of plant nutrition*, vol. 30, no. 3, pp. 381–396, 2007.

[51] S. J. Robinson, I. A. Parkin, and J. Singh, "Abiotic stress tolerance of the brassica oilseeds," *'Genetics, genomics and breeding of oilseed brassicas'.*, p. 230, 2011.

[52] R. Setia, P. Rengasamy, and P. Marschner, "Effect of exchangeable cation concentration on sorption and desorption of dissolved organic carbon in saline soils," *Science of the Total Environment*, vol. 465, pp. 226–232, 2013.

[53] M. Jamil, C. C. Lee, S. U. Rehman, D. B. Lee, M. Ashraf, and E. S. Rha, "Salinity (nacl) tolerance of brassica species at germination and early

seedling growth," *Electronic Journal of Environmental, Agricultural and Food Chemistry*, vol. 4, no. 4, pp. 970–976, 2005.

[54] U. BİLGİLİ, E. B. Carpici, B. B. AŞIK, and N. Celik, "Root and shoot response of common vetch (vicia sativa l.), forage pea (pisum sativum l.) and canola (brassica napus l.) to salt stress during early se," *Turkish Journal of Field Crops*, vol. 16, no. 1, pp. 33–38, 2011.

[55] S. A. Memon, X. Hou, and L. J. Wang, "Morphlogical analysis of salt stress response of pak choi." *Electronic Journal of Environmental, Agricultural & Food Chemistry*, vol. 9, no. 1, 2010.

[56] C. Vera, R. Downey, S. Woods, J. Raney, D. McGregor, R. Elliott, and E. Johnson, "Yield and quality of canola seed as affected by stage of maturity at swathing," *Canadian journal of plant science*, vol. 87, no. 1, pp. 13–26, 2007.

[57] L. Bernstein, L. Francois, and R. Clark, "Interactive effects of salinity and fertility on yields of grains and vegetables 1," *Agronomy journal*, vol. 66, no. 3, pp. 412–421, 1974.

[58] M. Qasim, M. Ashraf, M. A. Jamil, M. Ashraf, and E. S. RHA, "Water relations and leaf gas exchange properties in some elite canola (brassica napus) lines under salt stress," *Annals of applied biology*, vol. 142, no. 3, pp. 307–316, 2003.

[59] I.-u. Haq, M. K. Saeed, I. Ahmed, M. Ashraf, and N. Ejaz, "Chemical analysis and characterization of (brassica napus) canola oil seed," in *2009 ICME International Conference on Complex Medical Engineering.* IEEE, 2009, pp. 1–4.

[60] M. Tunçtürk, R. Tunçtürk, B. Yildirim, and V. Çiftçi, "Effect of salinity stress on plant fresh weight and nutrient composition of some canola (brassica napus l.) cultivars," *African Journal of Biotechnology*, vol. 10, no. 10, pp. 1827–1832, 2011.

[61] M. H. Siddiqui, F. Mohammad, M. N. Khan, M. H. Al-Whaibi, and A. H. Bahkali, “Nitrogen in relation to photosynthetic capacity and accumulation of osmoprotectant and nutrients in brassica genotypes grown under salt stress,” *Agricultural Sciences in China*, vol. 9, no. 5, pp. 671–680, 2010.

[62] S. Zamani, M. Nezami, D. Habibi, and M. Khorshidi, “Effect of quantitative and qualitative performance of four canola cultivars (brassica napus l) to salinity conditions,” *Advances in environmental biology*, pp. 422–428, 2010.

[63] N. A. Kalhoro, I. Rajpar, S. A. Kalhoro, A. Ali, S. Raza, M. Ahmed, F. A. Kalhoro, M. Ramzan, F. Wahid *et al.*, “Effect of salts stress on the growth and yield of wheat (triticum aestivum l.),” *American Journal of Plant Sciences*, vol. 7, no. 15, p. 2257, 2016.

[64] K. Suzuki, N. Yamaji, A. Costa, E. Okuma, N. I. Kobayashi, T. Kashiwagi, M. Katsuhara, C. Wang, K. Tanoi, Y. Murata *et al.*, “Oshkt1; 4-mediated na+ transport in stems contributes to na+ exclusion from leaf blades of rice at the reproductive growth stage upon salt stress,” *BMC plant biology*, vol. 16, no. 1, pp. 1–15, 2016.

[65] K. Poustini and A. Siosemardeh, “Ion distribution in wheat cultivars in response to salinity stress,” *Field crops research*, vol. 85, no. 2-3, pp. 125–133, 2004.

[66] H. Kaveh, H. Nemati, M. Farsi, and S. V. Jartoodeh, “How salinity affect germination and emergence of tomato lines,” *Journal of Biological and Environmental Sciences*, vol. 5, no. 15, 2011.

[67] M. Khataar, M. H. Mohammadi, and F. Shabani, “Soil salinity and matric potential interaction on water use, water use efficiency and yield response factor of bean and wheat,” *Scientific Reports*, vol. 8, no. 1, p. 2679, 2018.

[68] S. Farouk, “Ascorbic acid and α-tocopherol minimize salt-induced wheat leaf senescence,” *Journal of Stress Physiology & Biochemistry*, vol. 7, no. 3, pp. 58–79, 2011.

[69] M. A. Ashraf and M. Ashraf, “Growth stage-based modulation in physiological and biochemical attributes of two genetically diverse wheat (triticum aestivum l.) cultivars grown in salinized hydroponic culture,” *Environmental Science and Pollution Research*, vol. 23, pp. 6227–6243, 2016.

[70] M. Nadeem, M. N. Tariq, M. Amjad, M. Sajjad, M. Akram, M. Imran, M. A. Shariati, T. A. Gondal, N. Kenijz, and D. Kulikov, “Salinity-induced changes in the nutritional quality of bread wheat (triticum aestivum l.) genotypes,” *AGRIVITA, Journal of Agricultural Science*, vol. 42, no. 1, pp. 1–12, 2020.

[71] K. Parvin, M. Fujita, and M. Hasanuzzaman, “Salicylic acid enhances growth and productivity in cabbage (brassica oleracea var. capitata l.) grown under saline condition,” *Focus Sci*, vol. 3, no. 1, 2017.

[72] P. Khursheda, K. U. Ahamed, M. M. Islam, M. N. Haque *et al.*, “Response of tomato plant under salt stress: role of exogenous calcium.” *Journal of Plant Sciences*, vol. 10, no. 6, pp. 222–233, 2015.

[73] V. R. R. Puram, J. Ontoy, and P. K. Subudhi, “Identification of qtls for salt tolerance traits and prebreeding lines with enhanced salt tolerance in an introgression line population of rice,” *Plant molecular biology reporter*, vol. 36, pp. 695–709, 2018.

[74] H. Hossain, M. Rahman, M. Alam, and R. Singh, “Mapping of quantitative trait loci associated with reproductive-stage salt tolerance in rice,” *Journal of Agronomy and Crop Science*, vol. 201, no. 1, pp. 17–31, 2015.

[75] A. S. Warraich, S. Krishnamurthy, B. S. Sooch, N. Vinaykumar, B. Dushyanthkumar, J. Bose, and P. C. Sharma, “Rice gwas reveals key genomic regions essential for salinity tolerance at reproductive stage,” *Acta Physiologiae Plantarum*, vol. 42, pp. 1–15, 2020.

[76] L. Nayyeripasand, G. A. Garoosi, and A. Ahmadikhah, “Genome-wide association study (gwas) to identify salt-tolerance qtls carrying novel candidate genes in rice during early vegetative stage,” *Rice*, vol. 14, pp. 1–21, 2021.

[77] N. Al-Tamimi, C. Brien, H. Oakey, B. Berger, S. Saade, Y. S. Ho, S. M. Schmöckel, M. Tester, and S. Negrão, "Salinity tolerance loci revealed in rice using high-throughput non-invasive phenotyping," *Nature communications*, vol. 7, no. 1, p. 13342, 2016.

[78] J.-K. Zhu, "Abiotic stress signaling and responses in plants," *Cell*, vol. 167, no. 2, pp. 313–324, 2016.

[79] M. Jain, K. C. Moharana, R. Shankar, R. Kumari, and R. Garg, "Genomewide discovery of dna polymorphisms in rice cultivars with contrasting drought and salinity stress response and their functional relevance," *Plant Biotechnology Journal*, vol. 12, no. 2, pp. 253–264, 2014.

[80] P. K. Subudhi, R. Shankar, and M. Jain, "Whole genome sequence analysis of rice genotypes with contrasting response to salinity stress," *Scientific Reports*, vol. 10, no. 1, p. 21259, 2020.

[81] Q. Luo, Q. Zheng, P. Hu, L. Liu, G. Yang, H. Li, B. Li, and Z. Li, "Mapping qtl for agronomic traits under two levels of salt stress in a new constructed ril wheat population," *Theoretical and Applied Genetics*, vol. 134, pp. 171–189, 2021.

[82] M. Luo, Y. Zhao, R. Zhang, J. Xing, M. Duan, J. Li, N. Wang, W. Wang, S. Zhang, Z. Chen *et al.*, "Mapping of a major qtl for salt tolerance of mature field-grown maize plants based on snp markers," *BMC plant biology*, vol. 17, no. 1, pp. 1–10, 2017.

[83] E. Rezaei, E. M. Hervan, A. Azadi, A. Etminan, and H. Ramshini, "Prioritisation of candidate genes in qtl regions for seed germination and early seedling growth in bread wheat (triticum aestivum) under salt-stress conditions," *Crop and Pasture Science*, vol. 72, no. 1, pp. 1–16, 2021.

[84] M. A. Asif, M. Garcia, J. Tilbrook, C. Brien, K. Dowling, B. Berger, R. K. Schilling, L. Short, C. Trittermann, M. Gilliham *et al.*, "Identification of salt tolerance qtl in a wheat ril mapping population using destructive and

non-destructive phenotyping," *Functional Plant Biology*, vol. 48, no. 2, pp. 131–140, 2020.

[85] F. S. Alotaibi, R. N. Al-Qthanin, M. Aljabri, T. Shehzad, M. Albaqami, and S. F. Abou-Elwafa, "Identification of genomic regions associated with agronomical traits of bread wheat under two levels of salinity using gwas," *Plant Molecular Biology Reporter*, vol. 40, no. 3, pp. 595–609, 2022.

[86] S. Chaurasia, A. K. Singh, L. Songachan, A. D. Sharma, R. Bhardwaj, and K. Singh, "Multi-locus genome-wide association studies reveal novel genomic regions associated with vegetative stage salt tolerance in bread wheat (triticum aestivum l.)," *Genomics*, vol. 112, no. 6, pp. 4608–4621, 2020.

[87] Y.-S. Ku, M. Sintaha, M.-Y. Cheung, and H.-M. Lam, "Plant hormone signaling crosstalks between biotic and abiotic stress responses," *International Journal of Molecular Sciences*, vol. 19, no. 10, p. 3206, 2018.

[88] D. Cui, D. Wu, Y. Somarathna, C. Xu, S. Li, P. Li, H. Zhang, H. Chen, and L. Zhao, "Qtl mapping for salt tolerance based on snp markers at the seedling stage in maize (zea mays l.)," *Euphytica*, vol. 203, pp. 273–283, 2015.

[89] M. Luo, Y. Zhang, J. Li, P. Zhang, K. Chen, W. Song, X. Wang, J. Yang, X. Lu, B. Lu *et al.*, "Molecular dissection of maize seedling salt tolerance using a genome-wide association analysis method," *Plant Biotechnology Journal*, vol. 19, no. 10, pp. 1937–1951, 2021.

[90] H. Wang, R. Wang, B. Liu, Y. Yang, L. Qin, E. Chen, H. Zhang, and Y. Guan, "Qtl analysis of salt tolerance in sorghum bicolor during whole-plant growth stages," *Plant breeding*, vol. 139, no. 3, pp. 455–465, 2020.

[91] A. N. Hostetler, R. Govindarajulu, and J. S. Hawkins, "Qtl mapping in an interspecific sorghum population uncovers candidate regulators of salinity tolerance," *Plant Stress*, vol. 2, p. 100024, 2021.

[92] J. Atieno, T. D. Colmer, J. Taylor, Y. Li, J. Quealy, L. Kotula, D. Nicol, D. T. Nguyen, C. Brien, P. Langridge *et al.*, "Novel salinity tolerance loci in

chickpea identified in glasshouse and field environments," *Frontiers in Plant Science*, vol. 12, p. 667910, 2021.

[93] K. R. Soren, P. Madugula, N. Kumar, R. Barmukh, M. S. Sengar, C. Bharadwaj, P. C. Sharma, S. Singh, A. Bhandari, J. Singh *et al.*, "Genetic dissection and identification of candidate genes for salinity tolerance using axiom® cicersnp array in chickpea," *International journal of molecular sciences*, vol. 21, no. 14, p. 5058, 2020.

[94] K.-H. Cho, M. Y. Kim, H. Kwon, X. Yang, and S.-H. Lee, "Novel qtl identification and candidate gene analysis for enhancing salt tolerance in soybean (glycine max (l.) merr.)," *Plant Science*, vol. 313, p. 111085, 2021.

[95] Q. Song, D. L. Hyten, G. Jia, C. V. Quigley, E. W. Fickus, R. L. Nelson, and P. B. Cregan, "Development and evaluation of soysnp50k, a high-density genotyping array for soybean," *PloS one*, vol. 8, no. 1, p. e54985, 2013.

[96] Y. Cao, X. Zhang, S. Jia, B. Karikari, M. Zhang, Z. Xia, T. Zhao, and F. Liang, "Genome-wide association among soybean accessions for the genetic basis of salinity-alkalinity tolerance during germination," *Crop and Pasture Science*, vol. 72, no. 4, pp. 255–267, 2021.

[97] M.-q. Shi, X.-l. Liao, Y. Qian, W. Zhang, Y.-k. Li, J. A. Bhat, G.-z. Kan, and D.-y. Yu, "Linkage and association mapping of wild soybean (glycine soja) seeds germinating under salt stress," *Journal of Integrative Agriculture*, vol. 21, no. 10, pp. 2833–2847, 2022.

[98] M. S. Nawaz, S. A. Sami, M. Bano, M. R. Q. Khan, Z. Anwar, A. Ijaz, and T. Ahmed, "Impact of salt stress on cotton." 2023.

[99] S. Verma, S. Gupta, N. Bandhiwal, T. Kumar, C. Bharadwaj, and S. Bhatia, "High-density linkage map construction and mapping of seed trait qtls in chickpea (cicer arietinum l.) using genotyping-by-sequencing (gbs)," *Scientific reports*, vol. 5, no. 1, p. 17512, 2015.

[100] M. Adil, S. Bashir, S. Bashir, Z. Aslam, N. Ahmad, T. Younas, R. M. A. Asghar, J. Alkahtani, Y. Dwiningsih, and M. S. Elshikh, "Zinc oxide nanoparticles improved chlorophyll contents, physical parameters, and wheat yield under salt stress," *Frontiers in plant science*, vol. 13, p. 932861, 2022.

[101] I. Ahmad, G. Zhu, G. Zhou, M. U. Younas, M. E. Suliman, J. Liu, M. Zhuyi, and E. I. S. Gabralla, "Integrated approaches for increasing plant yield under salt stress," *Frontiers in Plant Science*, vol. 14, p. 1215343, 2023.

[102] D. I. Arnon, "Copper enzymes in isolated chloroplasts. polyphenoloxidase in beta vulgaris," *Plant physiology*, vol. 24, no. 1, p. 1, 1949.

[103] E. Shahbazi, A. Arzani, G. Saeidi *et al.*, "Effects of nacl treatments on seed germination and antioxidant activity of canola (brassica napus l.) cultivars." *Bangladesh Journal of Botany*, vol. 40, no. 1, pp. 67–73, 2011.

[104] S. H. Shah, S. Ali, S. A. Jan, and G. M. Ali, "Piercing and incubation method of in planta transformation producing stable transgenic plants by overexpressing dreb1a gene in tomato (solanum lycopersicum mill.)," *Plant Cell, Tissue and Organ Culture (PCTOC)*, vol. 120, pp. 1139–1157, 2015.