CALCIUM INDUCED CHANGES IN EARLY SEEDLING GROWTH OF RICE (*Oryza sativa* L.) UNDER SALT STRESS

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December, 2021

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REGISTRATION NO. 19-10391

A Thesis
Submitted to the Faculty of Agriculture
Sher-e-Bangla Agricultural University, Dhaka
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
IN
AGROFORESTRY AND ENVIRONMENTAL SCIENCE

SEMESTER: JULY-DECEMBER/2021

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CERTIFICATE

This is to certify that the thesis entitled ***"CALCIUM INDUCED CHANGES IN EARLY SEEDLING GROWTH OF RICE (Oryza sativa L.) UNDER SALT STRESS"*** *submitted to the Department of Agroforestry And Environmental Science, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka in partial fulfillment of the requirements for the degree of* ***Master of Science (MS) in Agroforestry and Environmental Science*** *embodies the result of a piece of bona fide research work carried out by* ***MOST. MAHMUDA ASRAFI, Registration No. 19-10391*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that the Author duly acknowledges any help or source of information, as has been availed of during this investigation.

Dated:

Place: Dhaka, Bangladesh

Dr. Dr. Jubayer-Al-Mahmud
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DEDICATED TO MY
WELL WISHERS

ABBREVIATIONS

Full word	Abbreviation	Full word	Abbreviation
Percent	%	Kilogram	kg
Degree Celsius	^{0}C	Least Significant Difference	LSD
Agro-Ecological Zone	AEZ	Maximum	Max
Bangladesh Agricultural Research Institute	BARI	Minimum	Min
Bangladesh Bureau of Statistics	BBS	Muriate of Potash	MOP
Percentage of Coefficient of Variance	CV%	Nitrogen, Phosphorus and Potassium	NPK
Days after Sowing	DAS	Not significant	NS
And others	*et al.*	Completely Randomized Design	CRD
Food and Agricultural Organization	FAO	Sher-e-Bangla Agricultural University	SAU
gram (s)	g	Soil Resources and Development Institute	SRDI
Per hectare	ha^{-}1	Triple Super Phosphate	TSP
Horticulture Research Centre	HRC	Weight	Wt

ACKNOWLEDGEMENTS

All praises goes to Almighty Allah, the Supreme Ruler of the universe who enabled the Author to complete the present piece of work.

I would like to express my heartiest respect, my deep sense of gratitude and sincere, profound appreciation to my supervisor, ***Professor Dr. Jubayer-Al-Mahmud****, Department of Agroforestry and Environmental Science, Sher-e-Bangla Agricultural University, Dhaka for his sincere guidance, scholastic supervision, constructive criticism and constant inspiration throughout the course and in preparation of the manuscript of the thesis.*

I would like to express my heartiest respect and profound appreciation to my Co-supervisor, ***Professor Dr. Ferzana Islam****, Department of Agroforestry and Environmental Science, Sher-e-Bangla Agricultural University, Dhaka for her utmost cooperation and constructive suggestions to conduct the research work as well as preparation of the thesis.*

I express my sincere respect to the Chairman, ***Associate Prof. Dr. Nasrin Sultana****, and all the teachers of Department of Agroforestry and Environmental Science, Sher-e-Bangla Agricultural University, Dhaka for providing the facilities to conduct the experiment and for their valuable advice and sympathetic consideration in connection with the study.*

Mere diction is not enough to express my profound gratitude and deepest appreciation to my well-wisher and friends for their ever ending prayer, encouragement.

The Author

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ABSTRACT

Salt stress is a severe limiting factor for rice production worldwide. So a pot experiment was conducted at the research field of the Department of Agroforestry and Environmental Science, Sher-e-Bangla Agricultural University, Dhaka during the period from February 2021 to April 2021 to find out the effect of calcium in mitigation of salt stress in BRRI dhan 28 (inbreed) with nine treatments in combination with three doses of NaCl (0 mM, 50 mM and 100 mM) and three doses of $CaCl_2$ (0 mM, 2.5 mM and 5 mM) in completely randomized design with three replications. Salt stress drastically damaged morpho-physiological attributes and growth performance of rice in dose dependent manner. In contrast use of 2.5 mM $CaCl_2$ under salt stress had positive impact on plant height, relative growth rate, number of leaves per plant, length of flag leaf, width of flag leaf, fresh weight of plant, dry weight of plant, relative water content (RWC) and SPAD value at 25, 40 and 55 at days after sowing (DAS) under both level of salt stresses. But addition of 5 mM $CaCl_2$ showed good result under 50 mM NaCl stress only. So, addition of 2.5 mM $CaCl_2$ in fertilization process might be a solution for rice production under salt stress.

CHAPTER 1
INTRODUCTION

To meet the food and nutritional requirements of the increasing global population, which is predicted to reach 9.6 billion by 2050, there is an urgent need to enhance crop productivity (Chen *et al.,* 2021). Rice, *Oryza sativa L*. (2n = 24) is an economically valuable plant group in the grass family Poaceae. Because it includes the world's most-important food crop that is a primary food source for more than one-third of the global population (Khush, 1997). Rice is the most important cereal crops in the world both agronomically and nutritionally. It is a major source of food for more than 2.7 billion people on a daily basis and is planted on about one-tenth of the earth's cultivable land. It is the single largest source of food energy to half of humanity. (Nagaraju *et al*., 2002). More than 90 percent of rice is produced and consumed in Asia (FAO, 2004a). Considering its important position, the United Nations designated year 2004 as the International Year of Rice. Devoting a year to a commodity was unprecedented in United Nations history. However, the 57th session of the United Nations General Assembly noted that rice is the staple food of more than half the world's population, affirmed the need to heighten the awareness of the role of rice in alleviating poverty and malnutrition and reaffirmed the need to focus world attention on the role rice can play in providing food security and eradicating poverty and declared the year 2004 as the International Year of Rice (adopted from December16, 2002; www.fao.org/ag/irc). With a growing global population, with an expectation of 9 billion people for the year 2050, the world food demand is expected to increase as well (FAO 2004b; Sakamoto *et al*., 2009; Shen *et al*., 2009).

Salinity is one of the major obstacles in increasing production of rice growing areas worldwide, which is an ever-presented threat to crop production. Nearly 20% of the world's cultivated areas and half of the world's irrigated lands are affected by salinity, making salt stress as the most serious environmental factors limiting the productivity of cultivated crops (Sairam and Tyagi, 2004). In Bangladesh, about 2.8 million hectares of coastal soil has become saline due to heavy withdrawal of surface and groundwater for irrigation and intrusion of seawater. The total saline area forms one third of the nine million hectares of total national cultivated area in Bangladesh

(ABSPII, 2006). Agriculture is major sector of Bangladesh economy, and the coastal area of Bangladesh is potential for growing rice. Increase of salinity due to poor water management, high evapotranspiration, and submerged irrigation and also due to pre-exposure of lands to sea water (Jin *et al*., 2007) will have serious negative impact on Agriculture. Salinity, whether natural or induced, is a widespread environmental stress that limits the growth and development of salt-sensitive plants (Patel *et al*., 2010). The food production does not seem to have a better future in the event of climate change. In Bangladesh, rice production may fall by 10% and wheat by 30% by 2050 (IPCC, 2007). Plants exposed to higher levels of salinity are affected by both hyperionic and hyperosmotic stress through accumulating Na^+ and Cl^- which causes membrane damage, nutrient imbalance, enzymatic inhibition, metabolic dysfunction, photosynthesis inhibition, and hampers other major physiological and biochemical processes that ultimately leads to growth inhibition or death of the plant (Mahajan and Tuteja, 2005; Munns and Tester, 2008). Higher levels of salt in plant growth medium decrease K^+ content and increase Na^+. With higher levels of NaCl, Na displaces Ca from membranes, which also increases intracellular Na. As a result, under salt-stress conditions Na content exceeds that of K, resulting in a higher Na/K ratio as well as nutrient imbalance (Shabala *et al*., 2006).

Rice is a salt sensitive crop species. Soil salinity, particularly due to NaCl, can be considered as the single most widespread soil toxicity problem that global rice production faces at present (Hong *et al*., 2007). Vegetative growth becomes more resistant to salinity (Pearson, 1959). Some toxic effects of salt stress include decreased germination and seedling growth (Zeng and Shannon, 2000a; Ashraf, 2010), and suppressed leaf expansion which ultimately reduces photosynthetic area and dry matter production (Mansour and Salama, 2004). K^+ in plant tissues evidently decreases when plants are exposed to salt stress, especially rice genotypes (Basu *et al*., 2002). Translocation of salt into roots and to shoots is an outcome of the transpirational flux required to maintain the water status of the plant and unregulated transpiration may cause toxic levels of ion accumulation in the shoot (Yeo,1998). Lower transpiration rate, coupled with reduced ion uptake by the roots, or reduced xylem loading, may cause poor supply via the xylem. So it is possible that an inadequate supply of ions to the expanding region may restrict cell division and/or expansion when plants are grown at high levels of NaCl (Berstein *et al*., 1995). Many

reports show salt induced reduction in photosynthetic pigments in many plant species such as rice (Cha-um *et al*., 2007). Plants also show the high chlorophyll degradation symptom, chlorosis, as a common morphological and physiological characteristic in response to salt stress (Harinasut *et al*., 2000). Chlorophyll content of salt stressed rice can be described as a function of the leaf sodium content (Yeo and Flowers, 1983). The response of plants to excess NaCl is complex and involves changes in their morphology, physiology and metabolism (Hilal *et al*., 1998). Keeping in mind all these observations, experiments were designed to study the effect of NaCl stress on germination percentage, seedling growth, ion content (Na, K and Ca), protein content and photosynthetic pigments i.e. Chl a, Chl b and carotenoids.

As an essential macronutrient, calcium (Ca) plays important roles including stabilizing cell walls and membranes, improving the metabolic processes of other nutrients, regulating enzymatic and hormonal processes, and other essential functions. Calcium also acts as a secondary messenger that mediates many aspects of cell and plant development, as well as the stress-resistance response. Higher plants have distinctive behaviors when faced with will be conducted to know the function of calcium in salt accumulation, morpho-physiological and yield attributes of rice under different level of salinity stress. Most plants, including the majority of agricultural crops and tree species, are glycophytes and cannot tolerate high salinity. In glycophytes, salinity imposes ionic stress (Munns and Tester, 2008), osmotic stress (Thiyagarajah *et al*., 1996) and secondary stresses such as nutritional disorders (Forde *et al*., 2004) and oxidative stresses (Desikan *et al*., 2001). The low osmotic potential (more negative) of saline solutions hampers plant water uptake, resulting in "physiological drought" (Munns and Tester, 2008). In halophytic plants that are tolerant to sodium toxicity, osmotic stress may be the main cause of growth inhibition. Plants such as *Seidlitzia rosmarinus* accumulate high levels of free proline (Hadi *et al*., 2008) in response to osmotic stress under salinity conditions. Competition and interactions between sodium (Na^+) and other inorganic nutrients in the substrate as well as within the plants frequently lead to ion imbalances that may result in nutrient deficiencies (Silva *et al*., 2003). Excess NaCl in saline soils can directly affect nutrient uptake, for example, reduction in calcium and nitrate absorption by sodium and chloride ions respectively. Addition of calcium to the soil (as gypsum or lime) displaces Na^+ from clay particles. This prevents the clay from

swelling and dispersing (Sumner, 1993) and also makes it possible for Na^+ to be leached deeper into the soil. Thus, exogenously supplied calcium not only improves soil structure, but also alters soil properties in various ways (Shabala *et al.*, 2003) which benefits the plant growth. Moreover, an improved Ca/Na ratio in the soil solution enhances the capacity of roots to restrict Na^+ influx. Exogenously supplied calcium may significantly alleviate detrimental effects of Na^+ on the physiological performance of hydroponically grown plants.

Salinity inhibits plants growth in three principle ways: by ion toxicity (mainly of Na^+ and Cl^-), osmotic stress, and by nutritional imbalance (Patel *et al*., 2010). The extent to which any one of these factors can affect growth is difficult to determine, because many factors can be involved. These include genetic variability among both species and cultivars within species, and also duration and timing of exposure to salinity (Sairam and Tyagi, 2004). Recently, it was shown that SOS2 also affected CAX1 (a vacuolar $Ca^{2+/}H^+$ antiporter) thereby linking cellular Ca^{2+} with Na^+ transport (Cheng *et al*., 2004). Understanding the mechanisms of Na^+/Ca^{2+} interaction, ameliorative effects of Ca^{2+} on Na^+ toxicity in plants and molecular basis effects of Ca^{2+} on salt-stress signaling is essential for breeding and genetic engineering of salt tolerance in crop plants. This review focuses on recent progresses in understanding the physiological characterization of Ca^{2+} transporter involved in salt uptake, compartmentalization, its effects on Na^+ uptake and cellular transduction of the salt-stress signal in regulation of Na^+ transport in plants.

The general objective of this study is to reduce the effect of salinity using calcium under salt stress. Considering above constrains the following specific objectives were under taken:

- **i.** To evaluate the morpho-physiology and growth performance of BRRI dhan 28 under salinity stress and
- **ii.** To assess the performance of calcium to confer salinity stress in BRRI Dhan 28.

CHAPTER 2

REVIEW OF LITERATURE

Rice is the most important cereal crop in Bangladesh and as well as many countries of the world. It is very important crop for conducting several types of agricultural researches. Various types of resources are accessible now for research on rice, the researchers gave much attention on various aspects of its production under different adverse condition especially in salt stress. Salinity is genetically and physiologically complex process and the most important abiotic stresses for agricultural crops. High concentrations of salt cause hyper osmotic and ionic stresses. Which in turn, may generate secondary stresses including oxidative stress, Coastal saline soils, inland saline soil are the examples of saline soil. Salt injury depends on species, variety, growth stage, environmental factors etc. It has been shown that, calcium (Ca^{2+}) is an important determinant for plant salt tolerance. Many studies have done on calcium induced changes on growth and yield of rice. The work so far done in Bangladesh is not adequate and advanced. However, some of the important and informative works and research findings so far been done at home and abroad on this topic have been reviewed in this chapter under the followings:

2.1 Rice

Rice is the seed of the grass species *Oryza sativa* (Asian rice) or less commonly *Oryza glaberrina* (African rice). The name wild rice is usually used for species of the genera *Zizania* and *Porteresia*, both wild and domesticated, although the term may also be used for primitive or uncultivated varieties of *Oryza*.

As a cereal grain, domesticated rice is the most widely consumed staple food for over half of the world's human population, especially in Asia and Africa. It is the agricultural commodity with the third-highest worldwide production, after sugarcane and maize. Since sizable portions of sugarcane and maize crops are used for purposes other than human consumption, rice is the most important food crop with regard to human nutrition and caloric intake, providing more than one-fifth of the calories consumed worldwide by humans. There are many varieties of rice and culinary preferences tend to vary regionally. The varieties of rice are typically classified as long-grained, medium-grained, and short-grained. Rice, a monocot, is normally grown

as an annual plant, although in tropical areas it can survive as a perennial and can produce a ratoon crop for up to 30 years. Rice cultivation is well-suited to countries and regions with low labor costs and high rainfall, as it is labor-intensive to cultivate and requires ample water. However, rice can be grown practically anywhere, even on a steep hill or mountain area with the use of water-controlling terrace systems. Although its parent species are native to Asia and certain parts of Africa, centuries of trade and exportation have made it commonplace in many cultures worldwide.

2.2 Salt Stress

Hazell and Wood (2008) stated that, Soil salinization is a major environmental challenge that is threatening agriculture across the world.Wahid *et al*., (2007) stated that,increased salinity is a stringent problem and a major limiting factor for crop production around the globe. Zaki (2011) stated that, salinity is defined as the presence of an excessive concentration of soluble salts in the soil which suppresses plant growth. Most of the water on the Earth contains about 30 g of sodium chloride per liter. This can make the Earth a really salty planet. Parvaiz (2014) stated in a study that, the salt stresses affect badly to the plant morphology, functioning and homeostasis, and decrease the plant biomass. Khan and Srivastava (1998) conducted an experiment and found that, high levels of soil salinity can significantly inhibit seed germination and seedling growth, due to the combined effects of high osmotic potential and specific ion toxicity. Salt stress had adverse effects on the functioning and metabolism of plants considerably hinders the productivity.

Qadir *et al.* (2014) stated that, plants are sessile and thus have to develop suitable mechanisms to adapt to high-salt environments. Salt stress increases the intracellular osmotic pressure and can cause the accumulation of sodium to toxic levels. Approximately 7% of total earth and 20% of the world's irrigated agricultural lands are adversely affected by soil salinization. Park *et al*. (2016) and Ziska *et al*. (2012) stated that, issues with soil salinization are aggravated by natural environment deterioration, poor irrigation practices, and climate changes. Thus, to effectively improve crop yields, it is critical to address the increasingly serious threat of soil salinization.

2.3 Calcium (Ca^{2+})

Calcium is a chemical element with the symbol Ca and atomic number 20. As an alkaline earth metal, calcium is a reactive metal that forms a dark oxide-nitride layer when exposed to air. Its physical and chemical properties are most similar to its heavier homologues strontium and barium. It is the fifth most abundant element in Earth's crust, and the third most abundant metal, after iron and aluminum. Calcium compounds are widely used in many industries, in foods and pharmaceuticals for calcium supplementation, in the paper industry as bleaches, as components in cement and electrical insulators, and in the manufacture of soaps. On the other hand, the metal in pure form has few applications due to its high reactivity; still, in small quantities it is often used as an alloying component in steelmaking, and sometimes, as a calcium–lead alloy, in making automotive batteries.

According to Linus Pauling Institute, Oregon State University (2017), calcium is the most abundant metal for plant growth. As electrolytes, calcium ions (Ca^{2+}) play a vital role in the physiological and biochemical processes of organisms and cells. In signal transduction pathways where they act as a second messenger and as cofactors in many enzymes in fertilization. Calcium ions outside cells are important for maintaining the potential difference across excitable cell membranes and protein synthesis.

2.4 Effect of salinity on Plant growth

Parihar *et al.* (2015) reported that, the environmental stress is a major area of scientific concern because it constraints plant as well as crop productivity. This situation has been further worsened by anthropogenic activities.

Safdar *et al*. (2019) found that, Plants are affected by salt stress in two main ways: osmotic stress and ionic toxicity. These stresses affect all major plant processes. They examined the ways in which salt inhibits plant function and the correlating responses of plants to salt stress including photosynthesis, cellular metabolism, and plant nutrition.

According to Munns and Tester (2008), salinity imposes detrimental effects on plant growth through low osmotic potential of soil solution and nutritional imbalance. Zhu, (2001) stated that, as the consequence primary effects of salt stress, caused by its hyperosmotic effect, secondary stresses, such as oxidative damage, often occur.

Several investigators have reported plant growth reduction as a result of salinity stress, e.g. in tomato Romero-Aranda *et al.* (2001), in cotton Meloni *et al*. (2001) and in sugar beet Ghoulam *et al*. (2002). However, there are differences in tolerance to salinity among species and cultivars as well as among the different plant growth parameters recorded. For instance, Aziz and Khan (2001) found that the optimum growth of *Rhizophora mucronataplants* was obtained at 50% seawater and declined with further increases in salinity while in *Alhagi pseudoalhagi* (a leguminous plant) and Kurban *et al*. (1999) found that, total plant weight increased at low salinity (50 mM NaCl) but decreased at high salinity (100 and 200 mM NaCl). Ghoulam *et al*. (2002) found in sugar beet leaf area that, fresh and dry mass of leaves and roots were dramatically reduced at 200 mM NaCl, but leaf number was less affected. Fisarakis *et al*. (2001) recorded a larger decrease in accumulation of dry matter in shoots than in roots, particularly at high NaCl concentration, indicating partitioning of photo assimilates in favor of roots while working with sultana vines. They proposed that the results may be due to a greater ability for osmotic adjustment under stress by the roots.

2.5 Physiological responses of plants to salinity

Volkmar *et al.* (1998) found in an experiment that, root-zone salinization presents a challenge to plant productivity that is effectively countered by salt-tolerant halophytic plants, but unfortunately, much less successfully by major crop plants. The way in which salt affects plant metabolism is reviewed. Cellular events triggered by salinity, namely salt compartmentation, osmotic adjustment and cell wall hardening are connected to the whole plant responses, namely leaf necrosis, altered phenology and ultimately plant death. The roles of ion exclusion and K/Na discrimination in mediating crop response to salt appear to be central to the tolerance response, but they are by no means essential. The processes involved in regulating ion uptake at the membrane level are considered.

2.6 Effects of salinity on photosynthesis

According to Taiz and Zeiger (1998), growth of plants is dependent on photosynthesis. Therefore, environmental stresses affecting growth also affect photosynthesis. Studies conducted by a number of authors with different plant species, Romero-Aranda *et al*. (2001), showed that photosynthetic capacity was

suppressed by salinity. A positive association between photosynthetic rate and yield under saline conditions has been found in different crops. Fisarakis *et al*. (2001) found that inhibition of vegetative growth in plants submitted to salinity was associated with a marked inhibition of photosynthesis. There are many studies in which no or little association between growth and photosynthetic capacity is evident. In *Triticum repens* Rogers and Noble (1992) and in *Triticum aestivum* Hawkins and Lewis (1993) found that, the effect of salinity on photosynthetic rate depends on salt concentration and plant species. There is evidence that at low salt concentration salinity may stimulate photosynthesis. For instance in *Bruguiera parviflora*, Parida *et al*. (2004) reported that photosynthetic rate increased at low salinity and decreased at high salinity, whereas stomatal conductance was unchanged at low salinity and decreased at high salinity. Iyengar and Reddy (1996) recognized that decreases in photosynthetic rate as a result of salinity to a number of factors:

(1) Dehydration of cell membranes which reduce their permeability to CO_2. High salt concentration in soil and water create high osmotic potential which reduces the availability of water to plants. Decrease in water potential causes osmotic stress, which reversibly inactivates photosynthetic electron transport via shrinkage of intercellular space.

(2) Salt toxicity caused particularly by Na^+ and Cl ions. According to Banuls *et al*. (1990), Cl inhibits photosynthetic rate through its inhibition of NO_3-N uptake by the roots. Fisarakis *et al*. (2001) found that NO_3-N was significantly reduced in salt-stressed sultana vines and this reduction was correlated with photosynthetic reduction. The reduced NO_3-N uptake combined with osmotic stress may explain the inhibitory effect of salinity on photosynthesis.

(3) Reduction of CO_2 supply because of closure of stomata. The reduction in stomatal conductance results in restricted availability of CO_2 for carboxylation reactions stated by Brugnoli and Bjorkman (1992). Iyengar and Reddy (1996) reported that stomatal closure minimizes loss of water by transpiration and this affects chloroplast light harvesting and energy-conversion systems thus leading to alteration in chloroplast activity. Higher stomatal conductance in plants is known to increase CO_2 diffusion into the leaves and thereby favor higher photosynthetic rates. Higher net assimilation rates could in turn favor higher crop yields as was found by Radin et al. (1994) in Pima cotton (*Gossypium barbadense*). Though, the results for photosynthetic rate and

stomatal conductance offered by Ashraf (2001) for six Brassicaspecies did not show any significant relationship. There are also reports of nonstomatal inhibition of photosynthesis under salt stress. Iyengar and Reddy (1996) reported that this nonstomatal inhibition is due to increased resistance to CO_2 diffusion in the liquid phase from the mesophyll wall to the site of CO_2 reduction in the chloroplast, and reduced efficiency of RuBPC-ase.

Other causes of reduced photosynthetic rates due to salinity have been identified by Iyengar and Reddy (1996) as:

(1) Enhanced senescence induced by salinity,

(2) Changes of enzyme activity induced by changes in cytoplasmic structure, and

(3) Negative feedback by reduced sink activity. Although the rate of photosynthesis is reduced under salt stress, this is not the cause of reduction in the rate of cell expansion as suggested by several lines of evidence.

2.7 Effects of salinity on plant water uptake and ion homeostasis

Yadav *et al*. (2011) stated that, salt has two major effects on plants: osmotic stress and ionic toxicity, both of which affect all major plant processes. According to Kader (2010), plants are able to take up water and essential minerals because they have a higher water pressure than the soil under normal conditions. When salt stress occurs, the osmotic pressure of the soil solution is greater than that in plant cells. Thus, the plant cannot get enough water.Parida and Das (2005) added that, plant cells will have decreased turgor and its stomata will close to conserve water. Stomatal closing can lead to less carbon fixation and the production of Reactive Oxygen Species (ROS) such as superoxide and singlet oxygen. ROS disrupts cell processes through damage to lipids, proteins, and nucleic acids. Ionic toxicity occurs when concentrations of salts are imbalanced inside cells and inhibit cellular metabolism and processes. Sodium ions at the root surface disrupt plant nutrition of the similar cation potassium by inhibiting both potassium uptake and enzymatic activities within the cell. Kader and Lindberg (2010) Stated that Potassium is an important nutrient in a plant, regulating over 50 enzymes. Essential for maintaining cell turgor pressure, creating membrane potential, and regulating enzymatic activities, potassium must be maintained at 100-200mM in the cytosol. Sodium, on the other hand, causes stress at concentrations higher than 10mM in the cytosol. Parida and Das (2005) stated that,

Na^+ is a cation similar to K^+ and easily crosses the cell membrane. It also acts as an inhibitor to many enzymes, affecting metabolic processes. Calcium cations, however, protect some plants through signaling pathways that regulate potassium sodium transporters. Again according to Kader (2010) when a plant senses salt stress through transmembrane proteins or enzymes in the cytosol, the amount of calcium in the cytosol increases. Zhu (2001) stated that, calcium is a second messenger important to many biochemical pathways and can aid plants in responding to salt stress. The osmotic and ionic stress induced by salinity can halt plant growth as the plant focuses its energy on conserving water and improving ionic balance. In order for plants to return to normal functioning and photosynthesis, the plant must facilitate its own detoxification – damage must be prevented or lessened, homeostasis must be reestablished, and growth must resume.

2.8 Effect of salinity on rice

Jamil *et al*. (2012), stated that, Seeds of three different varieties (Shaheen Basmati, Basmati-385 and NIAB-IR 9) of rice were exposed to increasing concentrations (0, 50, 100 and 150mM) of NaCl to investigate the effect of salinity on seed germination and seedling growth, ion content, photosynthetic pigments and total protein content . There was a regular decrease in seed germination and seedling growth raised in Petri dishes for ten days with increasing salt concentration.

Hasanuzzaman *et al.* (2009) observed in an experiment that seed germination, plant height, tiller number and leaf area index are negatively influenced by different salinity levels in all the rice varieties. All the yield components that is number of panicles, panicle length, spikelets per panicle, filled grain and grain weight also significantly decrease with the increased salinity stress. An increase of NaCl concentration up to 150 mM decreased 36-50% of the grain yield of all the four rice varieties. Among the varieties BRRI dhan 41 showed better performance at salinity stress up to a certain level.

Ali and Awan (2004) stated that salinity influence root-shoot ratio of rice at seedling stage. Two lines of rice showed higher root-shoot ratio and were found tolerant which appeared that screening at the seedling stage and higher root-shoot ratio provides a clue about the salt tolerance potential of a genotype. Based on the result, further comparative studies for salt tolerance in different genotypes were made under the

artificial salinity conditions. Generally, salinity caused a marked reduction in yield and yield components in all the genotypes.

Ponnamperuma and Deturck (1994) observed that rice is moderately sensitive to salinity. The degree of injury however depends on the nature and concentrations of salts, soil pH, water regime, method of planting, seedling age, growth stage, duration of exposure and temperature. Rice which is tolerant during germination becomes very sensitive during the early seedling stage, gains tolerance during vegetative growth, becomes sensitive during pollination and fertilization and becomes more tolerant at maturity.

Rahmanzadeh *et al*. (2008) evaluated the response to salinity stress of rice cultivars. Three salinity levels (0, 75 and 150 mM NaCl) were used. The result indicated that all cultivars were influenced by increasing salinity level from 0 to 150 mM NaCl in all traits.

Gregorio (1997) reported that salt quantity, adversely affects plant growth, yield and quality. The salt suppressing plant growth at low concentration can cause a detrimental effect to the plants or even death of the plants. The Na^+ and Cl^-, usually most prevalent ions in saline soil and water, account for most of deleterious effect to the plant that can be related to the ion toxicity. Salt injury depends on species, variety, growth stage environmental factors and characteristics which include salt source, nature and content of salts, water regime and other soil related toxicities.

Chinnusamy *et al*. (2005) reported that most grain crops and vegetables are glycophytes and are highly susceptible to soil salinity even when the soilis <4 dS m^{-1}. Different threshold tolerance EC_e indicate that there were variations in mechanisms of salt tolerance among crop species.

Gregorio *et al.* (2002) pointed that salt stress in soils is often associated with other abiotic stress such as mineral deficiencies, flooding, soil alkalinity, or drought. It is therefore important to consider breeding for multiple stress tolerance when breeding rice for saline environment.

Bhowmik *et al*. (2007) studied 11 rice genotype to evaluate salinity tolerance phenotypically and Geno typically and five genotypes were identified as salt tolerant. In rice salinity breeding study.

2.8.1 Effects of salinity on seed set in rice

Khatun and Flowers (1995) conducted an experiment and found that,increase in salinity in the medium resulted in a decrease in the number of fertile florets and in the viability of pollen as determined both by pollen germination and by pollen staining with the tetrazolium salt (3-4,5-dimethylithyazolyl) and (2,5-diphenyl monotetrazolium hromide). In order to assess the effects of salt on stigmas, seed production was measured for salt-grown and non-salt-grown female plants pollinated with viable pollen (from plants grown in the absence of salt). The percentage of seed set was reduced by 38% when the female plants were grown in 10mM Na and by 72% at 25mM Na, no seed setting was recorded for plants grown in 50mM Na. Comparisons between crosses involving male and female parents grown at different salinities indicated that effects on the female plants dominated those on pollinator plants. Mineral analysis of leaves of different ages showed that there was a gradient of K concentration from leaf to leaf which was opposite to that of Na and Cl at all levels of applied salinity: K was maximal in the flag leaf, where Na and Cl were minimal. Analysis also revealed that there was an increase in the concentrations of Na and Cl and a decrease in the concentration of K in the pollen grains and stigmas of plants subjected to saline conditions. Correlations between the concentration of Na and Cl in pollen and pollen staining and pollen germination in vitro suggest that Na and Cl were responsible for the poor viability. The change in ionic concentrations in pollen and stigmas was much larger than that in the younger leaves, and in particular very much larger than that in the lemmas and pleas.

2.8.2 Effects of salinity on leaf growth in rice

Yeo et al. (1991) stated that 50mM NaCl to *Oryza sativa* L. had little effect upon the time of leaf initiation, but leaf mortality prior to the normal phase of senescence was increased and the onset of senescence was advanced. There was no significant effect upon the day-to-day pattern of growth, nor upon the ultimate length, of leaves that were developing at the time of, or shortly after, salinization with 50mM NaCI. Leaves that developed after prolonged exposure of the plants to salinity were shorter. Addition of NaCl, KC1 or mannitol to the root medium brought about a cessation of leaf elongation within one minute. Growth at a reduced rate restarted abruptly after a lag period that depended upon the external concentration. Elongation rate recovered its original value within 24 h after exposure to 50mM NaCl, though not at higher

concentrations. Addition of NaCl at concentrations up to 100mM elicited no short-term effects upon photosynthetic gas exchange. Na uptake contributed to osmotic adjustment of the growing zone. When plants were rapidly exposed to 50mM NaCl, no change in turgor pressure was detectable in the growing zone with the resolution of the miniature pressure probe used (about 70kPa). It is concluded that the initial growth reduction in rice caused by salinization is due to a limitation of water supply. A clear distinction is made between the initial effects of low salinity which are recoverable and the long-term effects which result from the accumulation of salt within expanded leaves.

2.8.3 Effects of salinity on grain yield and yield components of rice

Zeng and Shannon (2000b) investigated thatsalinity effects were highly significant on grain yield, plant stand, seed weight per plant, seed weight per panicle, and spikelet's per panicle, but not significant on panicle density, kernel weight, and shoot weight per plant at seeding densities tested. Grain yield was not significantly increased with an increase of seeding density. Plant stand and panicle density were significantly increased, while seed weight per plant, fertility, and harvest index were significantly decreased with increases of seeding densities. The density-dependent seed weight per plant under salinity was explained by the competition within and among plants at high-density populations affected by salinity. Seed weight per panicle accounted for 62% of total variation and contributed more than panicle density to the grain yield under salinity. It was concluded that yield loss under moderate salinities may not be compensated for by increasing seeding density above normal density levels.

2.8.4 The effect of salinity upon photosynthesis in rice

Yeo*et al*. (1985) investigated that, the effect of salinity upon net photosynthesis and transpiration by individual leaves of rice has been investigated by gas exchange measurements in seedlings at the five to six leaf stage. Salinity did not, initially, reduce net photosynthesis in the whole plant but only in the older leaves in which sodium accumulated. Analysis of the course of events in leaf four following salinization of the medium showed that net photosynthesis was inversely correlated with the sodium concentration in the leaf tissue. There was no evidence of a threshold effect; net photosynthesis declined linearly with increasing leaf sodium concentration and was reduced by 50% at only 05 mM sodium per gram dry weight. The

relationship between transpiration rate and leaf sodium concentration closely paralleled that for photosynthesis; there was no effect of leaf sodium concentration on the carbon dioxide concentration in the intercellular spaces, showing that sodium accumulation in the leaf affected stomatal aperture and carbon dioxide fixation simultaneously. Photosynthesis was reduced by half at a sodium concentration in the leaf which did not reduce the concentration of chlorophyll. The nature of the effect of salinity upon leaf gas exchange is discussed.

2.8.5 Effect of salinity on chlorophyll concentration, leaf area, yield and yield components of rice

Ali *et al*. (2013) found that the yield per plant, chlorophyll concentrations, fertility percentage, and number of productive tillers, panicle length and number of primary braches per panicle of all the genotypes were reduced by salinity. However, genotypes viz. Jhona-349 x Basmati-370, NR-1, DM-59418, DM-63275, DM-64198 and DM-38-88 showed better salinity tolerance than others.

2.8.6 Effects of salinity on ion accumulation, ion homeostasis, membrane injury and sugar contents of rice

Siringam *et al*. (2011) stated that excess salt induced ionic and osmotic stresses that disturbed metabolism and led to reduction of plant development. Previous studies reported that sugars in stressed plants were involved in stress tolerance. However, the role of sugars in salt-stressed plants against only ionic effects is still unclear. The objective of this research was to investigate accumulation and homeostasis of ions, membrane injury, water content, growth characters and sugar contents in roots, in-response to salt stress under iso-osmotic conditions. Salt-sensitive rice, Pathumthani1 (PT1) was grown on MS culture medium for 7 days and was adjusted to salt stress under iso-osmotic conditions (-1.75 ± 0.20 MPa) by mannitol for 4 days. Additionally, growth characters, including number, length, fresh weight and dry weight of roots, were inhibited. Sugar accumulations in PT1 roots were enhanced by increases in NaCl. The increase in Na^+ was positively related to total soluble sugars, resulting in an osmotic adjustment of the membrane that maintained water availability. The accumulation of sugars in PT1 roots may be a primary salt-defense mechanism and may function as an osmotic control.

2.9 Effect of calcium on plant

Hasanuzzaman *et al*. (2019) studied and found that Calcium is a macronutrient for plant growth and development as it takes part in many physiological and metabolic processes. But, Ca also acts as second messenger for making intracellular stimulation under different abiotic stress conditions to give protection. Abiotic stresses cause nutritional, hormonal, metabolic, and physiological disorders result in crop loss. In this condition, plants suffer from acute oxidative damage caused by excess ROS and disruption of antioxidant defense system. Apart from being toxic, ROS also gives signal to mediate stress tolerance governed by the signal from Ca. Therefore, stressor-induced cytosolic Ca involves in protection response through activation of stress tolerance gene and plays vital role in anti-oxidative defense response. Hence, many research reports suggested that exogenous Ca increases the plant tolerance against abiotic stresses and stimulates different physiological and metabolic processes, which further increase crop production. Hence, in this chapter we focused the role of Ca against devastating effect of abiotic stresses in plant growth, development, physiology, and yield.

Xu *et al*. (2013) studied the alleviating effects of signal molecules on zoysiagrass (*Zoysia japonica*) under drought stress. Calcium chloride has been shown to ameliorate the adverse effects of drought stress on many plants. It is necessary to investigate how to enhance drought tolerance of zoysiagrass using calcium chloride. The study elucidated the effects of calcium chloride on zoysiagrass under drought conditions by investigating the following parameters: biomass, chlorophyll (Chl) content, net photosynthetic rate (Pn), chlorophyll fluorescence, antioxidant enzymes, proline content, and malondialdehyde (MDA) content.

Afsana *et al*. (2017) conducted a study to find out the role of exogenous foliar application of salicylic acid (SA) and calcium (Ca^{2+}) on growth, reproductive behavior and yield of tomato. The morphological and yield contributing characters as well as yield of tomato were positively influenced with single and combined application salicylic acid (SA) and calcium ($Ca^{2+)}$. Significant increase of plant height and number of leaves plant-1 at 20, 40 and 60 DAT was observed with the application of A3 treatment. Application of A3 treatment also showed significant influence on production of cluster plant-1 (20.44), flowers plant-1 (168.1), and fruits plant-1

(99.42) as well as fruit yield (72.57 t ha^{-1}). However application of A4 treatment failed to improve the morphological and yield contributing characters as well as yield of tomato over the A0 treatment (control). Results suggested that combined application of SA and Ca^{2+} successfully increase the tomato fruit yield by altering the morphological and reproductive characters.

2.10 Effect of Calcium on soil salinity

Burstrom (1968) stated that Calcium as a plant nutrient is characterized by its relatively high content in the plant coupled with a requirement not much higher than that of a micro nutrient element and an exceedingly uneven occurrence in soils. The difficulties in defining its actions are accentuated by a weak biochemical activity. In ecological conditions the secondary consequences of variations in calcium content may be more striking than the direct ones.

Electron-microscopical studies have revealed that calcium is required for formation and maintenance of lamellar systems in cell organelle, a fact which might suffice to explain its indispensability for meristematic growth.

Calcium is required for cell elongation in both shoots and roots; the common experience that it inhibits shoot elongation is certainly due to calcium additions far above actual requirement.

It must be assumed for a rational interpretation of cell elongation that the fundamental mechanism is the same in shoots and roots. The one action which can be ascribed with certainty to calcium is a stabilizing of the cell wall with an increase in rigidity, an effect which, with over-optimal supply, may lead to growth inhibitions. The function is, however, necessary for the normal organization of cell walls. Calcium has, on the contrary, no significant effect on the synthesis of cell wall compounds but appears to act on their proper incorporation into the cell wall.

The growth-active calcium may be bound not only to pectins but also to proteins and nucleoproteids in or in close contact with the cell wall.

The supposition that calcium interacts directly with auxin in the cell wall has not been verified and does not seem very probable. There are reasons to believe that the points of action of calcium and auxin in the cell wall differ, auxin inducing growth by wall loosening and calcium establishing new wall parts.

For submerged organs it may be necessary to consider an indirect effect of calcium on growth by its regulation of cytoplasmic permeability and thus affecting the exudation of growth-active compounds.

The ecological problem is to characterize calcifuges (acid soil plants) from calcicoles (base soil or calcareous soil plants). Growth inhibitions on acid soils depend upon poisoning by $A1^{3+}$ and Mn^{2+}. Opinions differ as to what extent this can be antagonized by calcium. Lime-induced chlorosis in calcifuges depends upon iron deficiency or iron inactivation in the plant. No acceptable explanation is given, but it might be related to an interaction of calcium carbonate, phosphorus, and iron. A hypothesis that it is linked to formation of organic acids is not tenable in the given form.

Plants react to the calcium ions in the concentrations found in soils. Calcifuges have a low calcium-optimum for growth and show growth inhibition at high concentrations. Calcicoles have a high optimum for growth. Calcifuges are resistant to aluminium poisoning. Attempts made to explain the differences in calcium uptake and generally in salt uptake are tentative only, and relevant data are lacking.

Grossi *et al.* (2022), found that the salinity of soils affects plant development and is responsible for great losses in crop yields. Calcium-dependent protein kinases (CDPKs) are sensor–transducers that decode Ca^{2+} signatures triggered by abiotic stimuli and translate them into physiological responses.

Kaya*et al.* (2002) stated that, dry matter, fruit yield and chlorophyll content of plants grown at high NaCl were less than those at normal nutrient solution. Supplementary Ca ameliorated the negative effects of salinity on plant growth and fruit yield. Water use by plants decreased with elevated NaCl and increased with supplementary Ca. Membrane permeability increased with high NaCl application and these increases in membrane permeability were decreased with supplementary Ca. Sodium (Na) concentration in plant tissues increased in both cultivars in the high NaCl level. Concentrations of Ca were at deficient ranges in the plants grown at high NaCl levels and these deficiencies were corrected by supplementary Ca.

Al-Whaibi *et al*. (2012) showed that, Application of 90 mM of NaCl reduced plant growth (plant height, fresh weight (FW) and dry weight (DW), chlorophyll (Chl) *a*,

Chl *b*, CA activity) and enhanced malondialdehyde (MDA) and Pro concentration. However, the application of SA or Ca alone as well as in combination markedly improved plant growth, photosynthetic pigments, Pro concentration, CA activity and activities of antioxidant enzymes peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and ascorbate peroxidase (APX) under salt stress. It was, therefore, concluded that application of SA and Ca alone as well as in combination ameliorated the adverse effect of salinity, while combined application proved more effective to reduce the oxidative stress generated by NaCl through reduced MDA accumulation, Chl *a*/*b* ratio and Chls degradation and enhanced activities of antioxidant enzymes on *Triticum aestivum* L. (cv. Samma) under salt stress.

Cachorro *et al.* (1994) proved that when NaCl concentration was increased germination and early seedling growth was decreased. The addition of Ca^{2+} to the media increased both germination percentage and seedling growth. Chloride concentrations were not affected by the level of Ca^{2+}. Potassium and Ca^{2+} concentrations and transport from roots to shoots were decreased by NaCl, but were restored by increasing Ca^{2+} in the medium. The opposite was true for Na^{+}. Leakage of NO_3^- and $H_2PO_4^-$ was increased by salinity and reduced by high Ca^{2+} in the medium. The results are discussed in terms of the beneficial effects of calcium for plant growth under saline conditions.

Patel *et al*. (2011) conducted a Greenhouse experiments and found the effects of supplemental calcium in salinized soil on the response of germination and seedling growth of *Caesalpinia crista*, L. (Fabaceae). NaCl and $CaSO_4 \cdot 2H_2O$ were added to the soil and 0:0, 1:0, 1:0.25, 1:0.50, 1:0.75, 1:1, 1:1.25, and 1:1.50 Na/Ca ratios were maintained. Salinity significantly retarded the seed germination and seedling growth, but the injurious effects of NaCl on seed germination were ameliorated and seedling growth was restored with calcium supply at the critical level (1:0.50 Na/Ca ratio) to salinized soil. Calcium supply above the critical level further retarded the seed germination and seedling growth due to the increased soil salinity. Salt stress reduced N, P, K and Ca content in plant tissues, but these nutrients were restored by addition of calcium at the critical level to saline soil. The opposite was true for Na^{+}. The results are discussed in terms of the beneficial effects of calcium supply on the seedling growth of *C. crista* grown under saline conditions.

Vaghela *et al*. (2009) also conducted a similar type of greenhouse experiments and found almost same result, and the findings were the effects of supplemental calcium in salinized soil on the response of germination and seedling growth of *Salvadora oleoides*, Decne. (Salvadoraceae). NaCl and $CaSO_4 \cdot 2H_2O$ were added to the soil and 0:0, 1:0, 1:0.25, 1:0.50, 1:0.75, 1:1, 1:1.25, and 1:1.50 Na/Ca ratios were maintained. Salinity significantly retarded the seed germination and seedling growth, but the injurious effects of NaCl on seed germination were ameliorated and seedling growth was restored with calcium supply at the critical level (1:0.50 Na/Ca ratio) to salinized soil. Calcium supply above the critical level further retarded the seed germination and seedling growth due to the increased soil salinity. Salt stress reduced N, P, K, and Ca content in plant tissues, but these nutrients were restored by the addition of calcium at the critical level to saline soil. The opposite was true for Na^+. The results are discussed in terms of beneficial effects of calcium supply on the seedling growth of *Salvadora oleoides* grown under saline conditions.

Parvin *et al*. (2016) studied the variation of ion uptake in tomato cv. BARI Tomato-5 under different levels of salinity (0, 2, 4, 6 and 8 dSm^{-1}) and their mitigation by different concentration of Ca^{2+} (0, 5, 10 mM). The results showed that salt stress significantly affects the stomatal conductance of tomato. Salt treatment markedly increased the uptake of Na^+ and decreased both K^+ and Ca^{2+} uptake in the leaves of tomato. The uptake of Na^+ decreased and uptake of Ca^{2+} and K^+ increased in tomato when salt-stressed plants were treated with Ca^{2+}. Our results revealed that Ca supplementation can effectively reduce the salt-induced ionic toxicity in tomato plants. Exogenous application of Ca^{2+} significantly mitigates the adverse effects of salt-induced ionic toxicity.

Guo *et al*. (2019) conducted a growth chamber study to assess the interactive effects of salinity and Ca on the emergence and early seedling growth of castor bean (*Ricinus communis* L.). Seedlings were cultured in wet sands filled with one-half Hoagland solution containing salts either at 0, 50 or 100 mM NaCl. Supplemental Ca was added at molar mass ratio of NaCl and $CaCl_2$ of 20:0, 20:1, 20:2 and 20:3. Increasing salinity level reduced emergence rate, height and leaf area by up to 34.0%, 26.1% and 46.0% respectively. Calcium amendment increased emergence, height, leaf area, dry plant weight, chlorophyll a, b, chlorophyll (a + b) and soluble protein by up to 22.1%, 13.7%, 21.3%, 30.3%, 28.6%, 24.0%, 25.8% and 42.4% respectively.

Islam (2020) conducted an experiment in Horticulture Farm of Sher-e-Bangla Agricultural University, Dhaka to mitigate salt stress in chili. Seedlings of 30 days of BARI Morich-3 were used as planting material. The two factors experiment was laid out in Randomized Complete Block Design with three replications. Factor A: Four levels of sodium chloride (Na and (iv) S 3 : 12 dSm^{+}) salt viz. (i) S -1 0 : Control, (ii) S 1 : 4 dSm^{-1} , (iii) S ; Factor B: Three levels of calcium nitrate as mitigating agent (i) M_0: Control, (ii) M_1: 6 mM and (iii) M_2: 12 mM Ca. The results of this experiment showed that, the salt stress reduced the morphological parameters and yield (kg) of chili with the increased level of salinity. The shortest plant height (35.28 cm), number of branches per plant (8.22), number of leaves per plant (18.33), individual fruit weight (1.29 g) and yield per plant (23.11 g) was recorded at S whereas the highest value (68.66 g) was recorded. The results also showed that Ca^{2+} significantly increased the growth contributing characters as well as yield of chili in both saline and non-saline conditions. For combined effect, the tallest plant (54.26 cm) was found from S 0 M at 75 DAT and highest number of fruits per plant (44.33) counted from S 0 1 M, highest weight of individual fruit (2.22 g) and the highest yield per plant (99.33 g) was recorded from S 2 0 M; whereas the lowest yield per plant was recorded (17.33 g) from S 3 M 0 2. This result suggests that, exogenous Ca^{2+} can effectively mitigate the detrimental effect of salt stress in chili: 8 dSm^{2+}.

Girija *et al*. (2002) conducted an experiment with Peanut and stated that peanut (*Arachis hypogaea* L.), when exposed to salinity stress produce the osmoticants: proline and glycinebetaine. Calcium ions also play a role in osmoprotection. During germination of peanut seeds subjected to NaCl salinity stress, proline and glycinebetaine concentrations in the embryonic axis increased continuously. A further increase in glycinebetaine concentration was observed with the addition of calcium chloride to the sodium chloride. The effects of sodium and calcium are thus additive in causing accumulation of glycinebetaine. Calcium appears to confer greater osmoprotection to the seedling exposed to salinity in this way. Two enzymes play an important role in controlling the level of proline. Proline oxidase catalyzes the conversion of proline to glutamate, thus reducing the concentration of proline. Another enzyme, γ-glutamyl kinase, plays an important role in the synthesis of proline. Addition of calcium chloride to NaCl-stressed seedlings lowered the proline concentration by increasing the level of proline oxidase and decreasing γ-glutamyl

kinase activities. Salinity stress, in the absence of calcium, increased proline due to reduced proline oxidase activity and increased γ-glutamyl kinase activity both in the cotyledons and embryonic axis of peanut seedlings. Thus calcium ions increase glycinebetaine production but decrease proline levels in NaCl stressed peanut seedlings.

Sabala *et al*. (2003) studied in hydroponic experiments with barley (*Hordeum vulgare* L.) and found that Growth rate and biomass accumulation was significantly lower in salinized roots. In addition to reduction in extension growth, salinity also significantly affected plant developmental processes (for example reduced root hair density and root thickening). Supplemental Ca^{2+} significantly ameliorated those detrimental effects of salinity. Non-invasive, microelectrode ion-flux (MIFE) measurements showed that the onset of salt stress caused rapid and prolonged efflux of H^+, K^+ and NH^{4+} from the root epidermis. This efflux could be significantly reversed, or completely prevented, by the presence of high Ca^{2+} concentration in the bath solution, even after several days of salt stress. Membrane potential measurements in root epidermal cells showed that high Ca^{2+} levels in the bath were able to restore (otherwise depolarised) membrane potential back to control level (–120 to –130 mV). At the same time, no significant impact of Ca^{2+} on net Na^+ uptake in plant roots was found.

Cabanero *et al*. (2004) investigated that, the effects of root-zone temperature on pepper plants (*Capsicum annumm* L.) treated with extra calcium, in order to study the influence of this parameter on water and calcium uptake. The treatments, for plants cultivated hydroponically in a controlled environment chamber, were control, NaCl (50 mM), Ca^{2+} (10 mM) and Ca^{2+} (10 mM) + NaCl (50 mM), all at 25 and 35°C root temperature. After these treatments, it could be seen that salinity reduced the concentration of calcium in roots and leaves, which were restored when calcium was added to saline-stressed plants. This effect of calcium was increased when plants were grown at 35°C root-zone temperature. The effect of both high temperature and extra supply of calcium influenced plant water relations, involving functionality of aquaporins. Therefore, the negative effect of salinity, with respect to tissue calcium concentration and water relations, was mitigated if there was a supply of Ca^{2+}, this effect being greater when the root-zone temperature was increased.

CHAPTER 3

MATERIALS AND METHODS

This chapter illustrates the concerning methodology used in execution of the experiment to study the Calcium induced changes on morpho-physiology, growth and yield of rice (*Oryza sativa*. L) under salt stress. This part comprises a brief description of locations of experimental site, planting materials, climate and soil, seedbed preparation, layout and design of the experiment, pot preparation, fertilizing, transplanting of seedlings, intercultural operations, harvesting, data recording procedure, statistical analysis etc. which are presented as follows:

3.1 Experimental site

This experiment was conducted in the Field of Agroforestry and Environmental Science Department, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh during the period from February 2021 to April 2021. Location of the site is 23°74'N latitude and 90°35'E longitude with an elevation of 8 meter from sea level (Islam, 2020) in Agro-ecological zone of "Madhupur Tract" (AEZ-28). The experimental site is shown in the map of AEZ of Bangladesh in [Appendix 1].

3.2 Climate and soil

Experimental site was located in the subtropical climatic zone, having with plenty of sunshine and moderately high temperature prevails during March to May (Boro season), which is highly suitable for Rice growing in Bangladesh. Information of weather and physiochemical properties of the soil used in pot experiments are given in [Appendix 2 and Appendix 3, respectively].

3.3 Planting materials

BRRI Dhan 28 seed was collected from BRRI, Gazipur on January 2021.

3.4 Treatments of the experiment

The experiment was conducted to evaluate the performance of 1 rice variety under 9 different NaCl and $CaCl_2$ treatments. These treatments are:

1. S0C0: 0mM NaCl and 0 mM $CaCl_2$ (control)
2. S0C1: 0 mM NaCl and 2.5 mM $CaCl_2$
3. S0C2: 0 mM NaCl and 5 mM $CaCl_2$
4. S1C0: 50 mM NaCl and 0 mM $CaCl_2$
5. S1C1: 50 mM NaCl and 2.5 mM $CaCl_2$
6. S1C2: 50 mM NaCl and 5 mM $CaCl_2$
7. S2C0: 100 mM NaCl and 0 mM $CaCl_2$
8. S2C1: 100 mM NaCl and 2.5 mM $CaCl_2$
9. S2C2: 100 mM NaCl and 5 mM $CaCl_2$

Here, 50 mM and 100 mM NaCl was treated as mild and severe stress, respectively.

3.5 Design and layout of the experiment

The experiment was laid out and evaluated during Boro season 2021 in completely randomized design (CRD) using two factors Factor A comprises 1 rice variety and Factor B comprises 9 salt and calcium treatments. The experiment was conducted in 3 replications and total 27 plastic pots were used.

3.6 Pot preparation

The experimental pot size was 35 cm in height, 30 cm in top diameter and 20 cm in bottom diameter. Pots were filled with fertilizer mixed soils for seed sowing and plant growth on February 01, 2021. Before soil filling, weeds and stubbles were completely removed from soil to ensure smooth plant growth. The soil was treated with Formaldehyde (45%) for 48 hours before filling the plastic pots to keep soil free from pathogen. Each pot was filled with 10 kg soil mixed with compost fertilizer.

3.7 Seed sowing and raising of seedlings

Seed sowing was carried out on 3rd February 2021 in the treatment pots. Before sowing, seeds were treated with 70% ethanol for five minutes. Seedlings were raised in the pots using regular nursery practices. Recommended cultural practices were done before and after sowing seeds. When the seedlings become 25 days old on 28th February 2021. Only 10 seedlings were allowed to grow per pot while additional

seedlings were uprooting. Pot preparation, emergence of seedlings, and seedling establishment are shown in figure 3.1.

3.8 Manure and fertilizers application

Soil was well pulverized and dried in the sun and only well decomposed cow dung was mixed with the soil including other required fertilizers. The required amount of fertilizer was calculated for each pot considering the dose required for 1 ha land. Overall Total decomposed cow dung was applied before transplanting the seedlings to plastic pots. On an average, each plastic pot was filled with soil containing 100gm decomposed cow dung (10 tons/ha). Fertilizers (urea, TSP, MoP) were applied in each pot following recommended dose.

3.9 Application of salt treatment

BRRI dhan 28 variety was evaluated under different salt treatments such as S0C0, (control): 0mM NaCl and 0mM $CaCl_2$, S0C1:0mM NaCl and 2.5mM $CaCl_2$, S0C2: 0mM NaCl and 5 mM $CaCl_2$, S1C0: 50mM NaCl and 0 mM $CaCl_2$ S1C1: 50mM NaCl and 2.5 mM $CaCl_2$, S1C2: 50mM NaCl and 5 mM $CaCl_2$, S2C0: 100mM NaCl and 0 mM $CaCl_2$, S2C1: 100mM NaCl and 2.5mM $CaCl_2$, S2C2: 100mM NaCl and 5 mM $CaCl_2$. All the plants were watered equally from the first day after seedling emergence.

Figure 3.1: A and B) Pot preparation C) Emergence of seedlings

3.10 Intercultural operations

Recommended watering and intercultural operations were provided as and when required for different treatments (Figure 3.2). Weeding was performed in all pots at regular interval to keep plants free from weeds. Diseases and pest attack is a limiting factor to rice growth and yield. All the steps of watering and intercultural operations are presented in figure 3.2.

Figure 3.2: Intercultural Operation

3.11 Data recording

Data were recorded from each pot based on growth and yield parameters. Different data recording procedure and growth stages at which time data were recorded are shown in figure 3.3. Data were recorded in respect of the following parameters:

3.11.1 Growth parameters

3.11.1.1 Plant height

Plant height of each plant was measured in cm unit after 4 weeks of seedling transplanting using meter scale and mean was calculated. Plant height measuring procedure of tomato plant is shown in figure 3.3.

3.11.1.2 Relative growth rate

Relative growth rate per plant-on-plant height basis was counted in cm/week unit by using the following formula:

$$\text{Relative growth rate (cm/week)} = \frac{\text{Final plant height} - \text{Initial plant height}}{\text{Time interval between two heights}}$$

Initial plant height (average cm) was measured at the time of seedling transplantation and final plant height for different plants was measured after 4 weeks of transplanting

Figure 3.3: Plant Height Measurement

3.11.1.3 Number of leaf per plant

Number of leaf per plant was calculated and mean was calculated.

3.11.1.4 Flag leaf height

Flag leaf height per plant was measured using Digital meter scale (cm) unit. Later it was converted to centimeter (cm) unit and then mean was calculated for each treatment.

3.11.1.5 Flag leaf width

Flag leaf width per plant was measured using Digital Caliper-515 (DC-515) in millimeter (mm) unit. Later it was converted to centimeter (cm) unit and then mean was calculated for each treatment.

3.11.1.6 Plant fresh weight

Plant fresh weight excluding fruits was counted after uprooting plant using electrical balance machine and mean was calculated.

3.11.1.7 Plant dry weight

Plant dry weight excluding fruits was counted after drying the uprooted plant sample using electrical balance machine and mean was calculated.

3.11.2 Physiological parameters

3.11.2.1 Measurement of relative water content

Relative water content (RWC) was measured according to Barrs and Weatherly (1962). Whole leaf discs were weighed as FW and then turgid weight (TW) was taken after floating the fresh leaves on distilled water. Finally, DW was measured after drying at 80°C for 48 h. Leaf RWC was calculated using the following formula:

$$RWC\ (\%) = [(FW–DW)/(TW–DW)] \times 100$$

3.11.2.2 *SPAD* value of leaf

SPAD (soil plant analysis development) values of leaf was recorded for rice using a portable SPAD 502 Plus meter (Konica-Minolta, Tokyo, Japan) to get assumption about chlorophyll (chl) content under different treatment condition. In every measurement, the SPAD reading was repeated 5 times from the leaf tip to base, and the average was used for statistical analysis.

3.11.3 Statistical analysis

The means for all the treatments were calculated and the analyses of variance for all the characters were performed by LSD test. The analyses were done following the software STATISTIX 10. The significance of the difference among the means was evaluated by the Least Significant Difference Test (LSD) at 5% level of probability.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Plant height

Salt stress significantly decreased the plant height of rice plant with the increase of salt level.

At 25 DAS mild and severe salt stress decrease plant height by 7 and 32.6% respectively. But use of 2.5 mM $CaCl_2$ (C1) restored the plant height by 10.9 and 6.7% under mild and severe stress, respectively. On the other hand 5 mM $CaCl_2$ (C2) restored plant height only under mild salt stress by 6.7% (Figure 4.1).

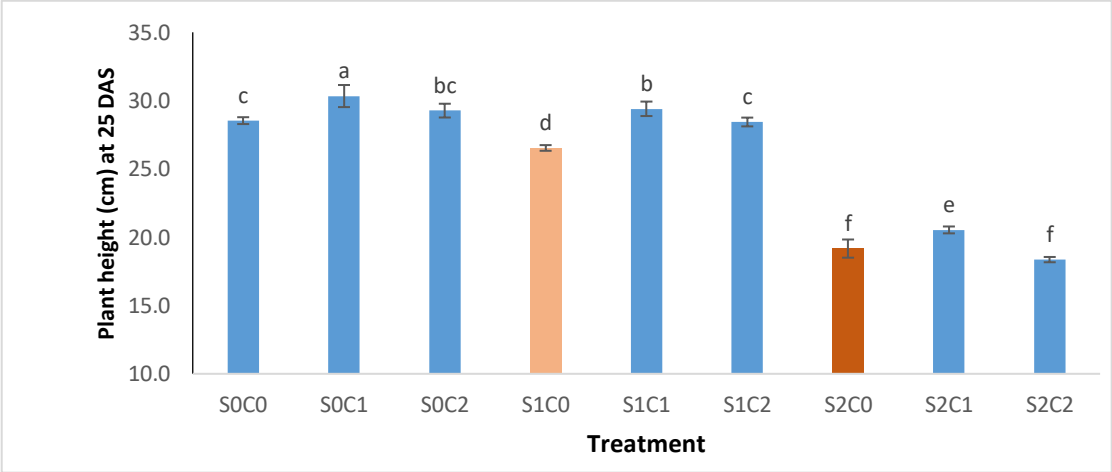

Figure 4.1. Effect of Ca on plant height of rice under salt stress at 25 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test

At 40 DAS mild and severe salt stress decreased plant height by 16.7 and 7.5%, respectively. But use of 2.5 mM $CaCl_2$ (C1) restored the plant height by 12.8 and 7.5% under mild and severe stress, respectively. On the other hand 5 mM $CaCl_2$ (C2) restored plant height only under mild salt stress by 9% (Figure 4.2).

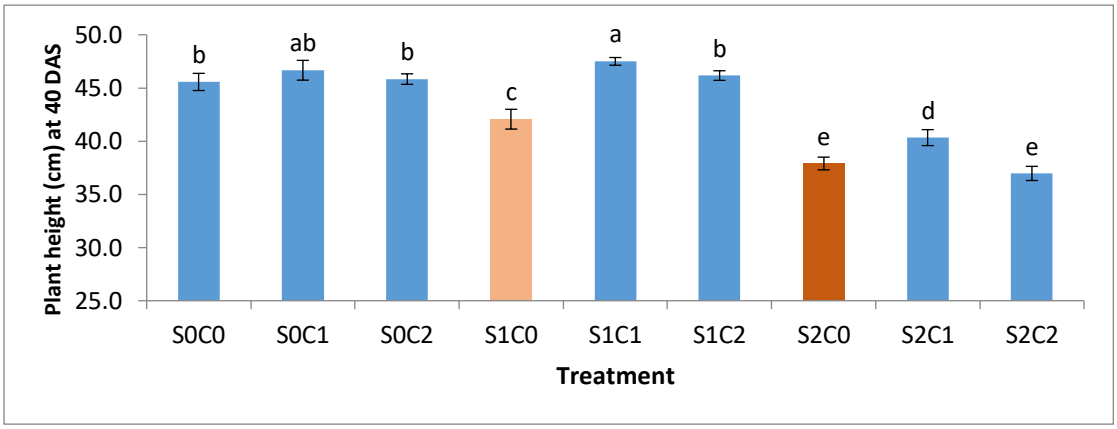

Figure 4.2. Effect of Ca on plant height of rice under salt stress at 40 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test

At 55 DAS mild and severe salt stress decreased plant height by 6.9 and 12.24%, respectively. But use of 2.5 mM $CaCl_2$ (C1) restored the plant height by 7.6 and 5.2% under mild and severe stress, respectively. On the other hand 5 mM $CaCl_2$ (C2) restored plant height only under mild salt stress by 3.4% (Figure 4.3). As a result, reduction of plant height was observed under salt stress condition compared to control. Supplementation of Ca improved the plant height considerably compared to salt treated plant alone. 2.5 mM $CaCl_2$ (C1) showed most significant result for both mild and severe salt stress condition in present experiment.

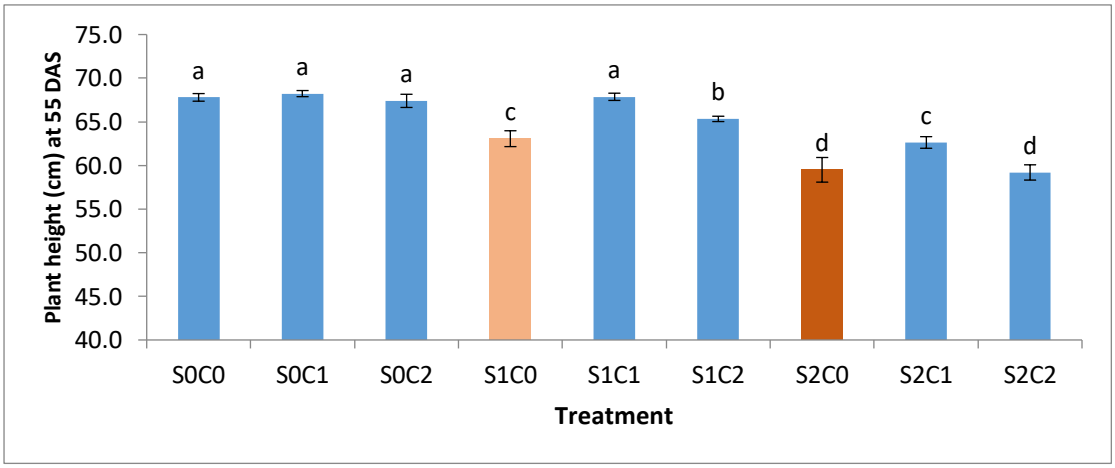

Figure 4.3. Effect of Ca on plant height of rice under salt stress at 55 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test

Similar results were observed by Manivannan *et al*. (2007) and Rahman *et al.* (2016) who stated that salt-affected rice seedlings showed growth inhibition in terms of plant height. In fact under salt stress conditions higher accumulation of Na disrupts ion homeostasis, causes osmotic stress and inhibits growth (Tuncturk *et al*., 2008; Munns, 2011). Moreover salt stress mitigation due to the improved ion homeostasis with Ca supplementation was recorded in many studies. Rahman *et al*. (2016) also found supplementation of calcium to the salt-treated seedlings markedly restored plant growth compared the seedlings treated with salt alone. This mitigation of growth inhibition under salt stress might be due to the improved ion homeostasis with Ca supplementation.

4.2 Relative growth rate (RGR)

Salt stress showed a significant variation in the relative growth rate values on plant height on rice plant. Mild Salt stress decreased relative growth rate by 6.8% on the other hand for severe salt stress did not decrease relative growth significantly. Use of 2.5 mM $CaCl_2$ (C1) restored relative growth rate by 4.9 and 4.5% under mild and severe stress, respectively. On the other hand restoration of relative growth rate for 5 mM $CaCl_2$ (C2) is limited under mild and severe salt stress (Figure 4.4). So it is clear that in RGR value is reduced in case of salt treated plant alone compared to control. Application of Ca improved RGR value both in mild and severe salt stress. 2.5 mM $CaCl_2$ (C1) gave best performance in terms of relative growth rate. Tuncturk *et al*. (2008) and Munns (2011) found almost similar result and said that, estimation of relative growth rate value on plant height basis is totally dependent on the rate of plant height increase or decrease over time.

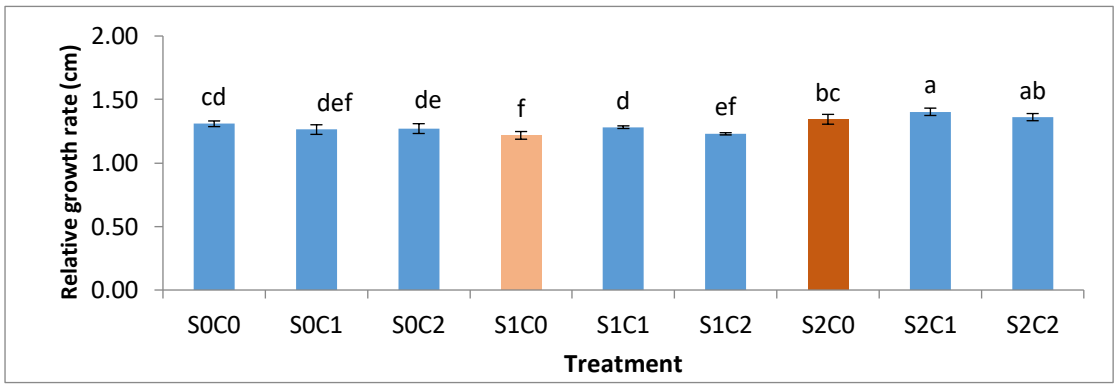

Figure 4.4. Effect of Ca on relative growth rate of rice under salt stress at 40 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test

4.3 Number of leaf per plant

Data taken on different DAS showed slight reduction of leaf number due to salt induced stresses which recovered by calcium supplementation. At 25 DAS mild and severe salt stress decreased leaf number per plant by 21.4 and 23.8%, respectively. But use of 2.5 mM $CaCl_2$ (C1) restored the number of leaf per plant by 21.2 and 15.6% under mild and severe stress, respectively. On the other hand 5 mM $CaCl_2$ (C2) restored number of leaf per plant only under mild salt stress by 12.1% (Figure 4.5).

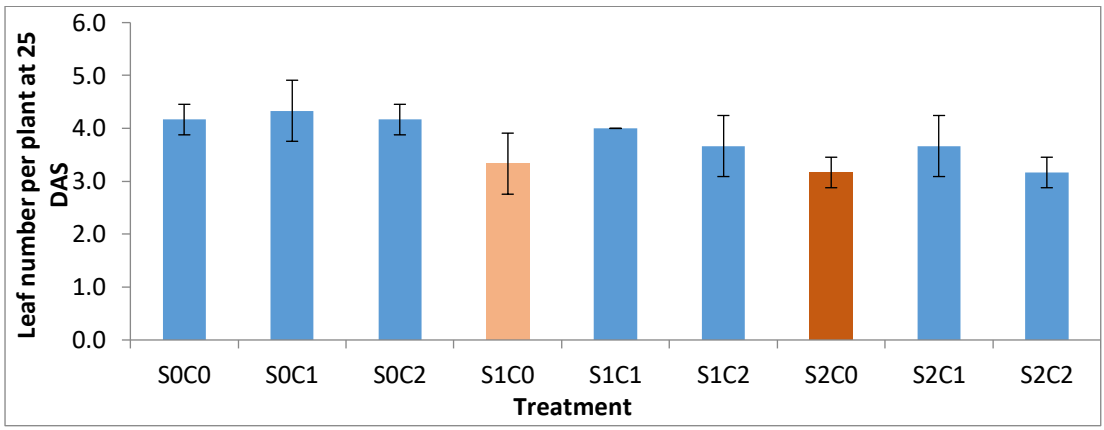

Figure 4.5. Effect of Ca on leaf number of rice under salt stress at 25 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment.

At 40 DAS mild and severe salt stress decreased leaf number per plant by 24.5 and 26.3%, respectively. But use of 2.5 mM $CaCl_2$ (C1) restoresd the number of leaf per plant by 39.5 and 2.3% under mild and severe stress, respectively. On the other hand 5 mM $CaCl_2$ (C2) restored number of leaf per plant only under mild salt stress by 23.2% (Figure 4.6).

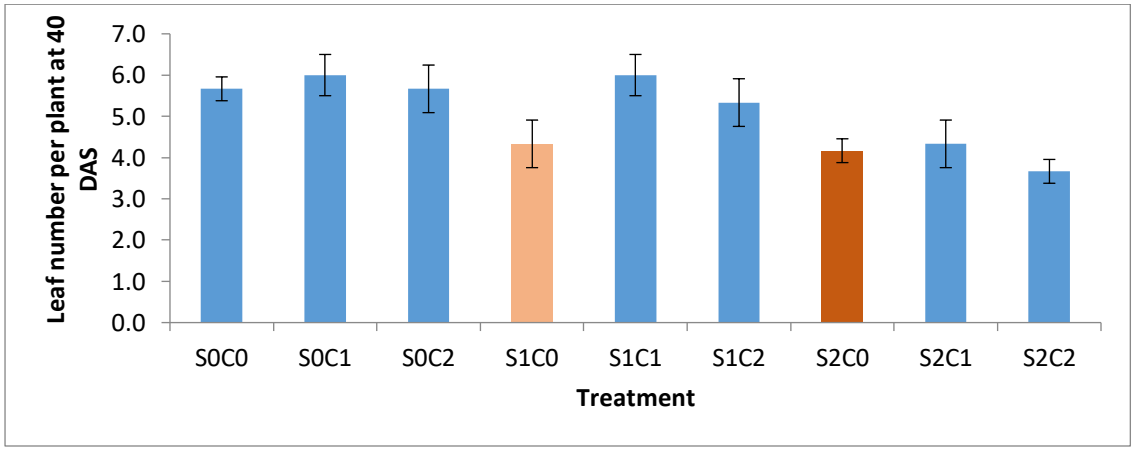

Figure 4.6. Effect of Ca on leaf number of rice under salt stress at 40 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment.

At 55 DAS mild and severe salt stress decreased leaf number per plant by 10 and 16.25%, respectively. But use of 2.5 mM $CaCl_2$ (C1) restored the number of leaf per plant by 11.1 and 4.5% under mild and severe stress, respectively. On the other hand 5 mM $CaCl_2$ (C2) restored number of leaf per plant only under mild salt stress by 1.38% (Figure 4.7). As a result, reduction leaf per plant was observed under salt stress condition compared to plant under control. Supplementation of Ca improves the number of leaf per plant. Furthermore 2.5 mM $CaCl_2$ (C1) showed most significant result for both mild and severe salt stress condition in present experiment. Salinity disturbed seriously the production of leaf, which was depicted in the stiff reduction in leaf number per plant. Green leaves as well as leaf area per plant were reported to be reduced with the increase in soil salinity (Ali *et al*., 2013; Jamil *et al*., 2012) Inhibition of the formation of leaf primordial under salt stress could be the probable reason for low leaf number (Alamgir and Ali, 2006). The decrease of leaf numbers may be due to the accumulation of sodium chloride in the cell walls and cytoplasm of the older leaves whereas Ca reduced the Na accumulation and improves leaf formation (Jamil *et al*., 2012).

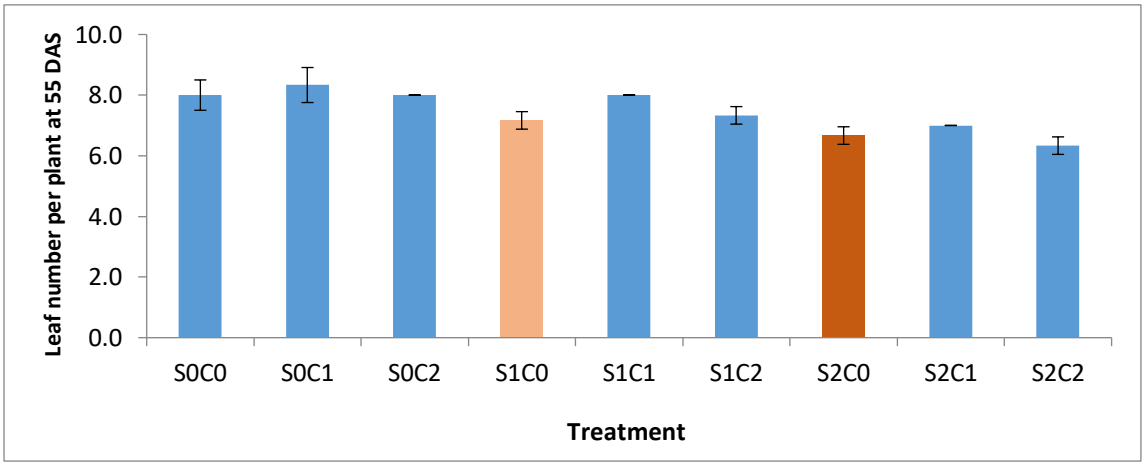

Figure 4.7. Effect of Ca on leaf number of rice under salt stress at 55 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment.

4.4 Length of flag leaf

Flag leaf length was decreased under salt stress in dose dependent manner. At 25 DAS mild and severe salt stress decreased length of flag leaf by 7.5 and 16.7%, respectively. But use of 2.5 mM $CaCl_2$ (C1) restored the length of flag leaf by 14.1 and 1.7% under mild and severe stress, respectively. On the other hand 5 mM $CaCl_2$

(C2) restored number of leaf per plant only under mild salt stress by 9.4% and for severe salt stress the result is non-significant (Figure 4.8).

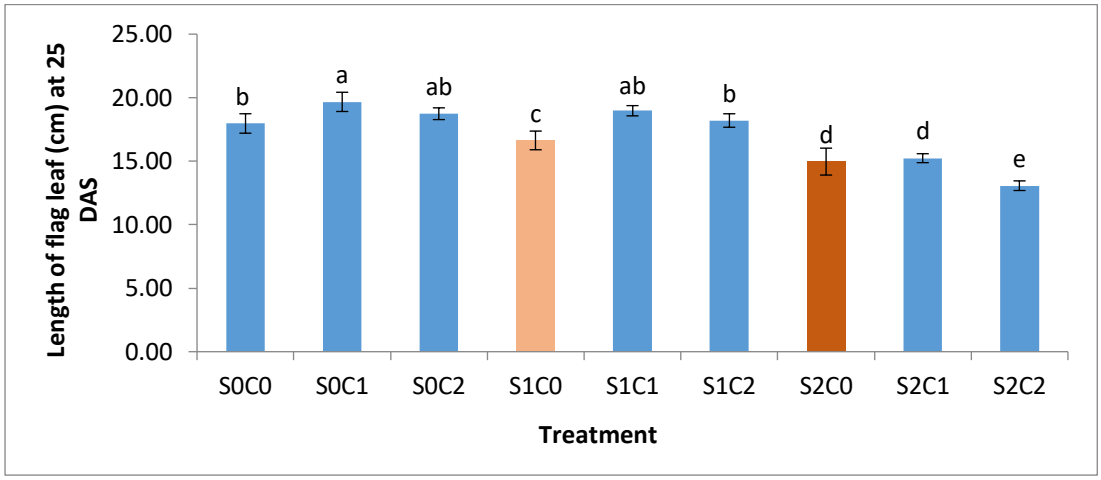

Figure 4.8. Effect of Ca on length of flag leaf of rice under salt stress at 25 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment. Bars with different letters are significantly different at P≤0.05 applying Fisher's LSD test

At 40 DAS mild salt stress result is non-significant for length of flag leaf but severe salt stress decreased length of flag leaf by 6.8%. Use of 2.5 mM $CaCl_2$ (C1) restored the length of flag leaf by 11.1 and 1.3% under mild and severe stress, respectively. On the other hand 5 mM $CaCl_2$ (C2) restored number of leaf per plant only under mild salt stress by 7.1% and for severe salt stress the result is non-significant (Figure 4.9).

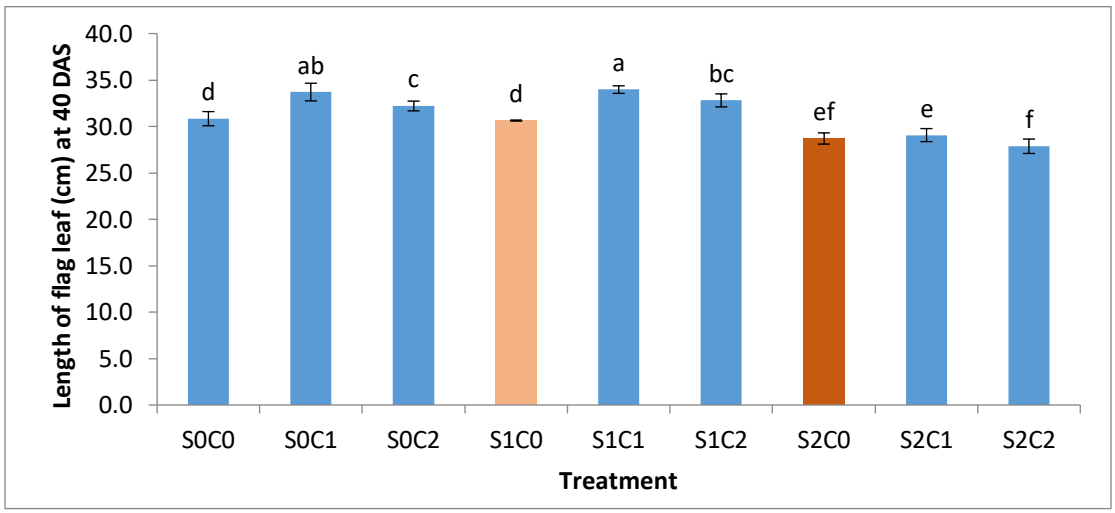

Figure 4.9. Effect of Ca on length of flag leaf of rice under salt stress at 40 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment. Bars with different letters are significantly different at P≤0.05 applying Fisher's LSD test

At 55 DAS mild and severe salt stress decreased length of flag leaf by 5.6 and 10%, respectively. But use of 2.5 mM $CaCl_2$ (C1) restored the length of flag leaf by 10.5 and 4.4% under mild and severe stress, respectively. On the other hand 5 mM $CaCl_2$ (C2) restored number of leaf per plant only under mild salt stress by 3.9% and for severe salt stress the result is non-significant (Figure 4.10). So it is clear that, reduction of flag leaf length was occurred under salt stress condition in comparison with plant under control. Application of Ca increases the length of flag leaf significantly than salt stress plant alone. Moreover 2.5 mM $CaCl_2$ (C1) showed most substantial result for both mild and severe salt stress condition in present experiment. Reduction of flag leaf length is associated with similar reduction of plant height. Many results confirmed such reduction in rice plant which recorded by Manivannan *et al*. (2007) and Rahman *et al.* (2016) where they also showed calcium improve plant growth status.

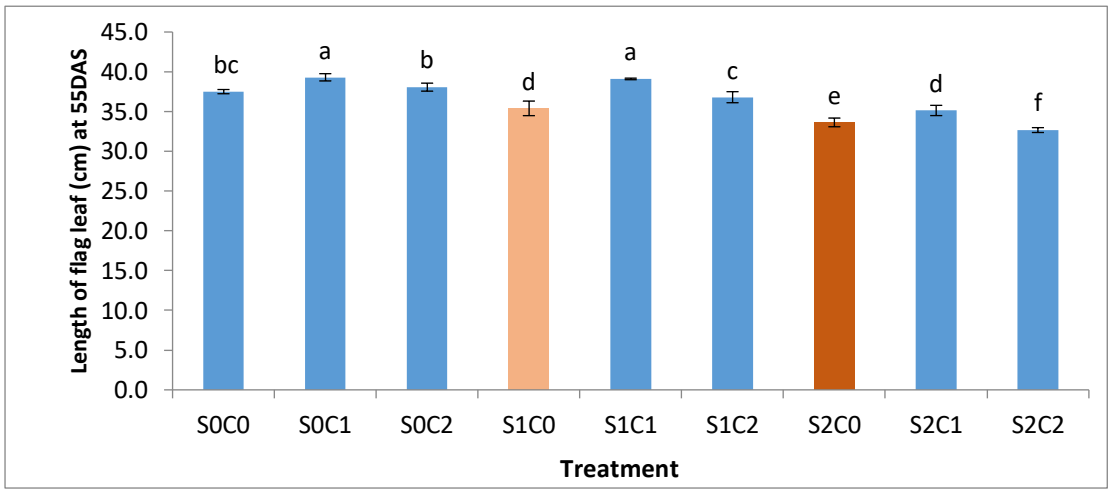

Figure 4.10. Effect of Ca on length of flag leaf of rice under salt stress at 55 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$,respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test

4.5 Width of flag leaf

Width of rice plant did not differ significantly due to salt stress but due to stress width decreased slightly which recovered by Ca supplementation. At 25, 40 and 55 DAS mild and severe salt stress decreased width of flag leaf insignificantly.

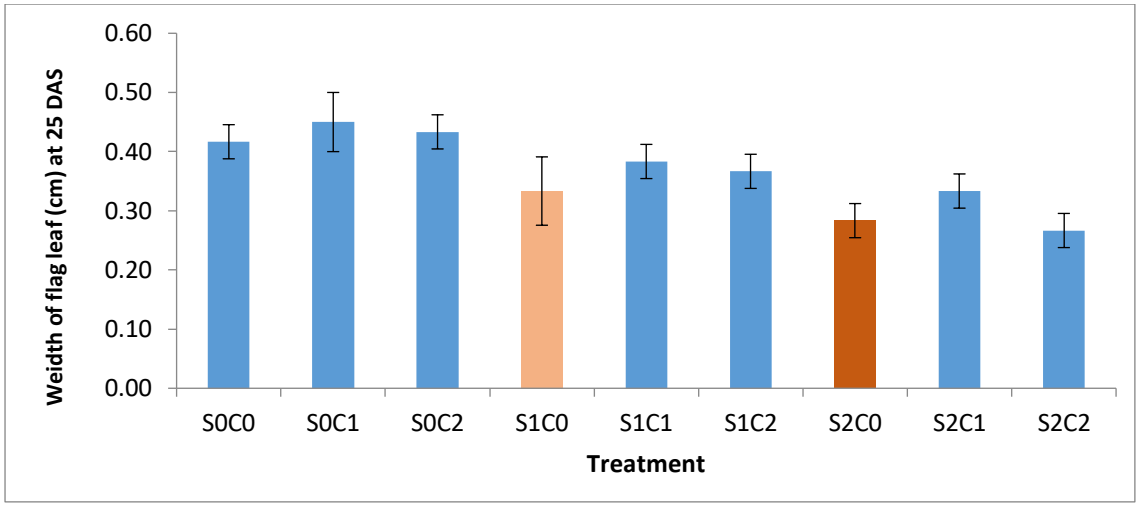

Figure 4.11. Effect of Ca on width of flag leaf of rice under salt stress at 25 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment.

Also use of 2.5 mM $CaCl_2$ (C1) and mM $CaCl_2$ restored the width of flag leaf in a very small scale. So effect of salt stress and Ca treatment on leaf width is non-significant. Result showed in (Figure 4.11), (Figure 4.12) and (Figure 4.13) for 25, 40 and 55 DAS respectively. Here, we found that effect of salt stress is very limited in case of width of flag leaf. But Ca supplementation improved the status. Our findings is indirectly supported by Shabala *et al*. (2003), Khanom *et al*. (2018) who confirmed salt stress decreased flag leaf area of plant.

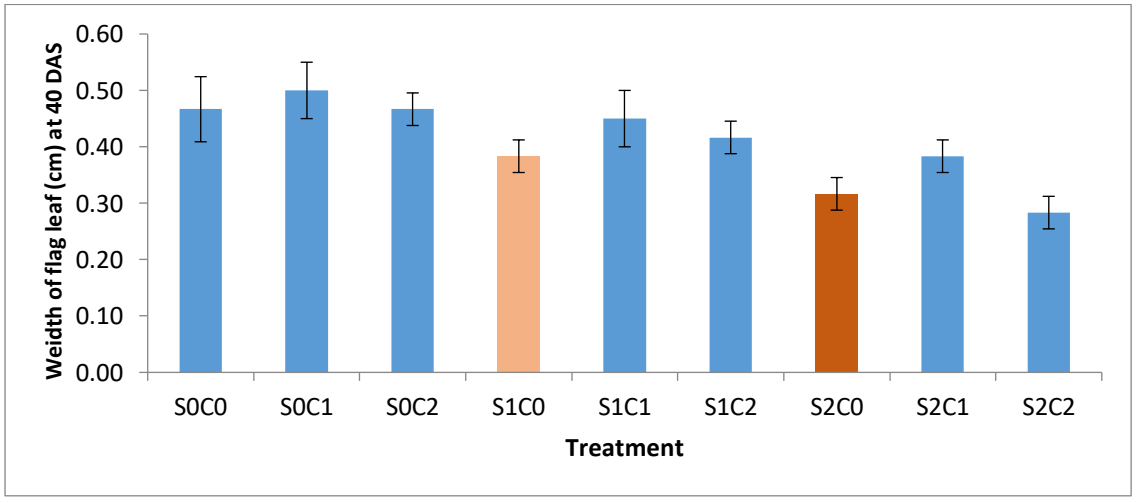

Figure 4.12. Effect of Ca on width of flag leaf of rice under salt stress at 40 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment.

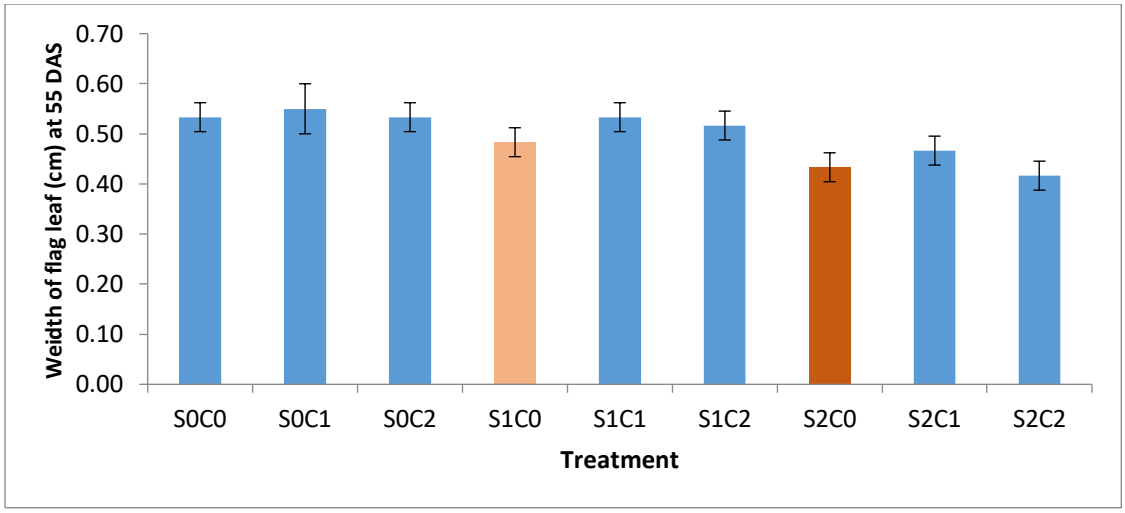

Figure 4.13. Effect of Ca on width of flag leaf of rice under salt stress at 55 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment.

4.6 Fresh weight of plant

Fresh weight of plant directly indicates the growth performance of plant. At 25 DAS mild and severe salt stress decreased fresh weight of plant by 4.8 and 11.3%, respectively. But use of 2.5 mM $CaCl_2$ (C1) restored fresh weight of plant by 10 and 10.9% under mild and severe stress, respectively. On the other hand 5 mM $CaCl_2$ (C2) restored fresh weight of plant only under mild salt stress by 3.3% and for severe salt stress the result is non-significant (Figure 4.14).

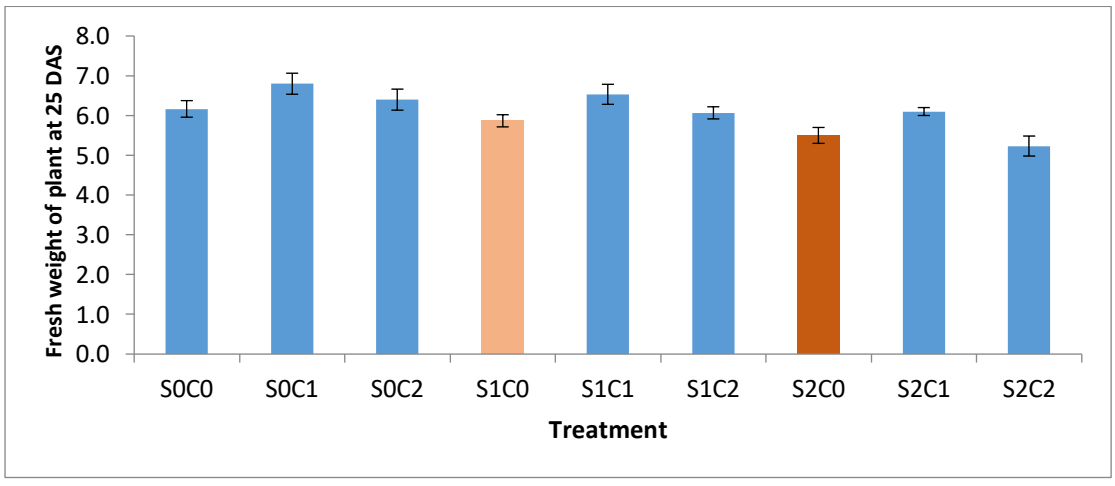

Figure 4.14. Effect of Ca on fresh weight of rice under salt stress at 25 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment.

At 40 DAS mild and severe salt stress decreased fresh weight of plant by 10.2 and 18.8%, respectively. But use of 2.5 mM $CaCl_2$ (C1) restored fresh weight of plant by 18.1 and 15.7% under mild and severe stress, respectively. On the other hand, 5 mM $CaCl_2$ (C2) restored fresh weight of plant only under mild salt stress by 9.5% and for severe salt stress the result is non-significant (Figure 4.15).

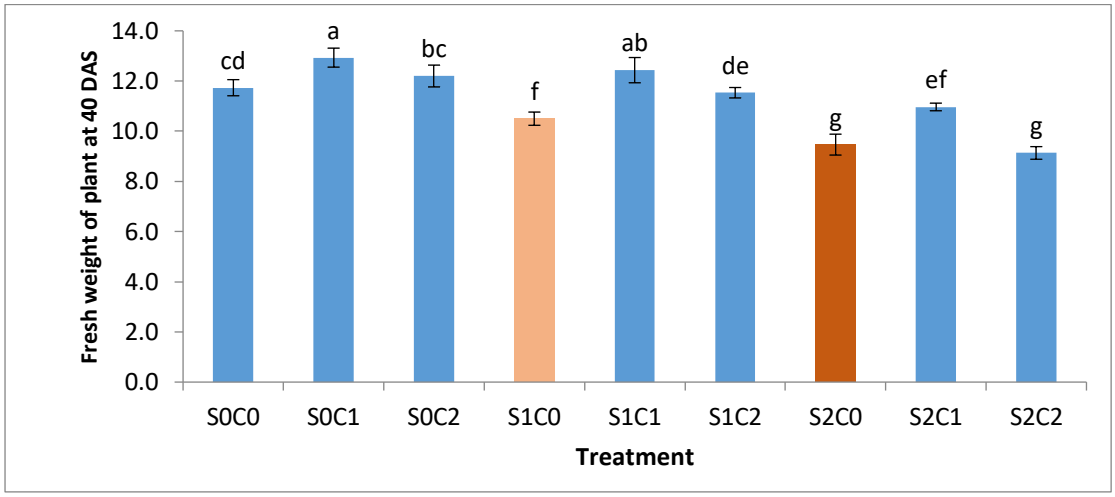

Figure 4.15. Effect of Ca on fresh weight of rice under salt stress at 40 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test

At 55 DAS mild and severe salt stress decreased fresh weight of plant by 12.5 and 18.9%, respectively. But use of 2.5 mM $CaCl_2$ (C1) restored fresh weight of plant by 18.4 and 18.1% under mild and severe stress respectively. On the other hand, 5 mM $CaCl_2$ (C2) restored fresh weight of plant only under mild salt stress by 12.7% and for severe salt stress the result is non-significant (Figure 4.16). Application of Ca markedly restored FW of rice seedlings under salt stress (Rahman *et al.*, 2016). As a result, drop of plant fresh weight was observed under salt stress condition compared to control. Application of Ca restores the fresh weight of plant in both mild and severe salt stress in comparison with salt treated plant alone. 2.5 mM $CaCl_2$ (C1) showed most substantial result for both mild and severe salt stress condition in our experiment. Similar result found as salt-affected rice seedlings showed growth inhibition in terms of seedling fresh weight (Mahmud *et al.*, 2020). Moreover Manivannan *et al.* (2007) and Rahman *et al.* (2016) confirmed that exogenous Ca restored fresh weight of plant under stress conditions.

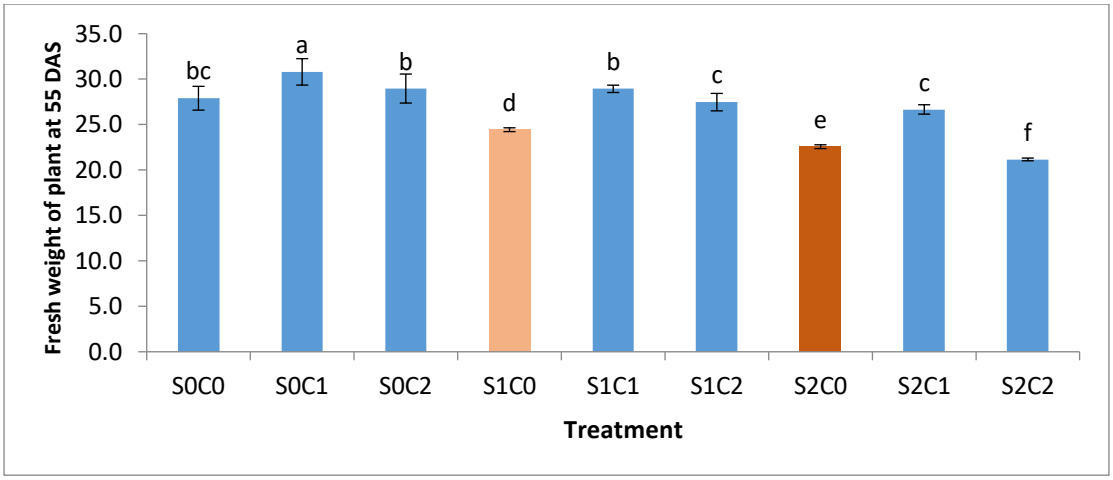

Figure 4.16. Effect of Ca on fresh weight of rice under salt stress at 55 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test

4.7 Dry weight of plant

At 25 DAS mild and severe salt stress decreased dry weight of plant by 5.3 and 10.6%, respectively. But use of 2.5 mM $CaCl_2$ (C1) restored dry weight of plant by 12.1 and 10.8% under mild and severe stress, respectively. On the other hand 5 mM $CaCl_2$ (C2) restores dry weight of plant only under mild salt stress by 3.7% and for severe salt stress the result is non-significant (Figure 4.17).

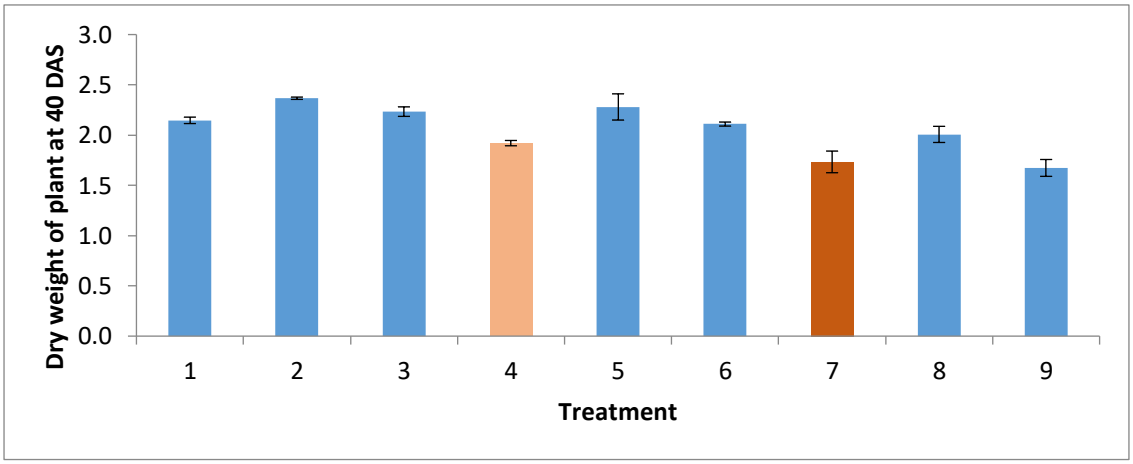

Figure 4.17. Effect of Ca on dry weight of rice under salt stress at 25 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment.

At 40 DAS mild and severe salt stress decreased dry weight of plant by 9.5 and 19%, respectively. But use of 2.5 mM $CaCl_2$ (C1) restored dry weight of plant by 21 and

17% under mild and severe stress, respectively. On the other hand 5 mM $CaCl_2$ (C2) restores dry weight of plant only under mild salt stress by 10% and for severe salt stress the result is non-significant (Figure 4.18).

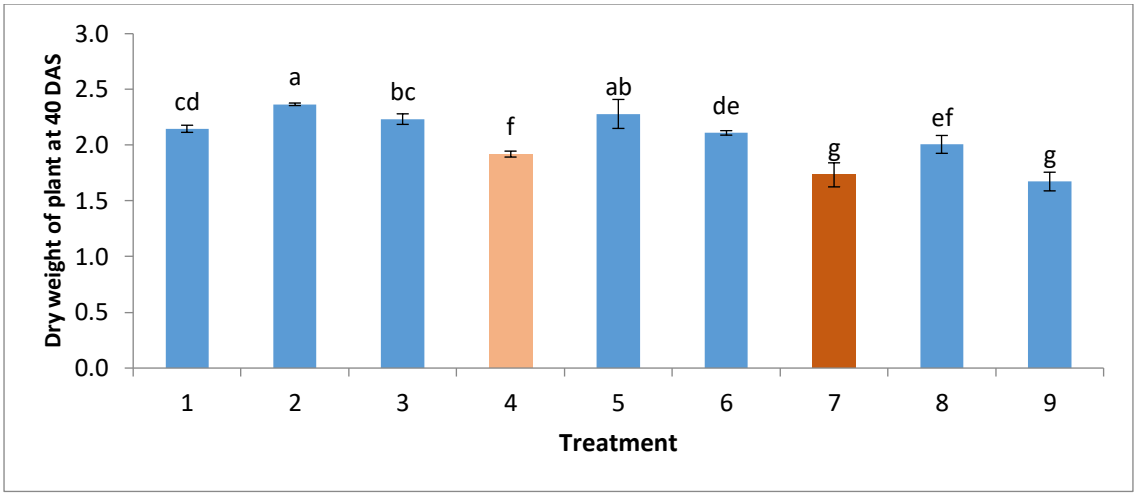

Figure 4.18. Effect of Ca on dry weight of rice under salt stress at 40 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test

At 55 DAS mild and severe salt stress decreased dry weight of plant by 11.7 and 19.6%, respectively. But use of 2.5 mM $CaCl_2$ (C1) restores dry weight of plant by 17.7 and 19.5% under mild and severe stress, respectively. On the other hand 5 mM $CaCl_2$ (C2) restored dry weight of plant only under mild salt stress by 11.1% and for severe salt stress the result is non-significant (Figure 4.19). As a result, reduction of dry weight was detected under salt stress condition compared to control. Supplementation of Ca maintained higher DW in both mild and severe salt stress than salt treated plant alone. And 2.5 Mm $CaCl_2$ (C1) presented most significant result for both mild and severe salt stress condition in my research. Under salt stress conditions higher accumulation of Na disrupts ion homeostasis, causes osmotic stress and inhibits growth (Tuncturk *et al.*, 2008; Munns, 2011). Salt-affected rice seedlings showed growth inhibition in terms of plant dry weight, which were restored with Ca supplementation. Rahman *et al.* (2016) found almost similar result for cadmium stress rice plant treated with Calcium. Dry weight of rice seedlings was reduced by 18 and 31 % when seedlings were treated with 0.25 and 0.5 mM Cd, respectively. However, the Cd-treated seedlings supplemented with Ca maintained higher dry weight than Cd-alone treatment.

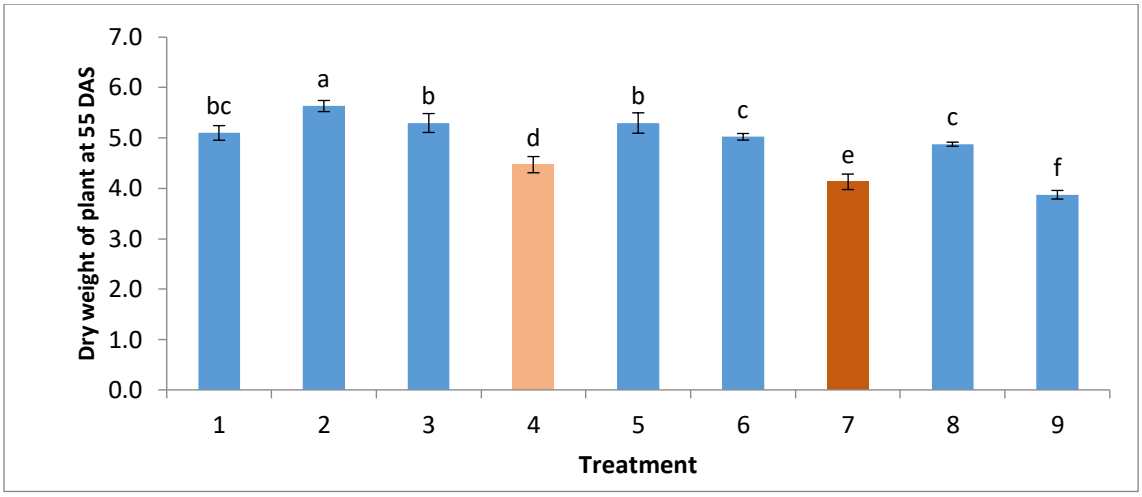

Figure 4.19. Effect of Ca on dry weight of rice under salt stress at 55 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$,respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test

4.8 Leaf relative water content

Mild and severe salt stress decreased leaf relative water content by 4.7 and 7.2%, respectively. But use of 2.5 mM $CaCl_2$ (C1) increase leaf relative water content by 3.8 and 3.5% under mild and severe stress, respectively. On the other hand, 5 mM $CaCl_2$ (C2) increase leaf relative water content only under mild salt stress by 1.4% and for severe salt stress the result is non-significant (Figure 4.20). As end result, reduction of relative water content was detected under salt stress condition compared with control. Supplementation of Ca improves RWC than salt treated plant alone. 2.5 mM $CaCl_2$ (C1) presented most significant result for both mild and severe salt stress condition in my research. Rice seedlings exposed to salt showed lower RWC, which indicated a salt-induced water imbalance and osmotic stress (Mahmud *et al.*, 2020). Similar salt-induced water shortage and Pro accumulation were observed in salt-affected rice seedlings (Hasanuzzaman *et al*., 2014). However, exogenous Ca restored water loss (indicated by increased RWC) and decreased Pro accumulation in the salt-affected rice seedlings. Rahman *et al*. (2016) found that, supplementation of Ca to the salt-stressed seedlings enhanced leaf RWC in contrast to salt stress alone.

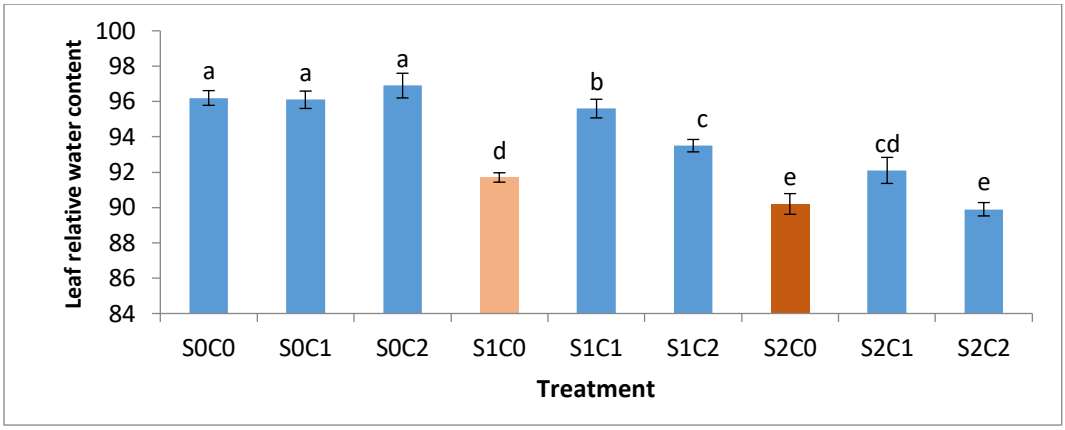

Figure 4.20. Effect of Ca on leaf relative water content of rice under salt stress at 55 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment. Bars with different letters are significantly different at P≤0.05 applying Fisher's LSD test

4.9 SPAD value of plant

SPAD value indicate the photosynthetic pigment content of plant. In contrast to control, mild and severe salt stress decreased SPAD value by 13.9 and 22.5%, respectively. But use of 2.5 mM $CaCl_2$ (C1) increased SPAD value by 11.6 and 13.2% under mild and severe stress, respectively, in contrast to respective stress. On the other hand, 5 mM $CaCl_2$ (C2) increased SPAD value only under mild salt stress by 4.5% (Figure 4.21). In my experiment, it is detected that SPAD value decrease because of mild and severe salt stress compared with control. On the other hand, Ca supplementation restored chl and content of the rice seedlings and improve SPAD value in both mild and severe salt-stress conditions than salt treated plant alone.

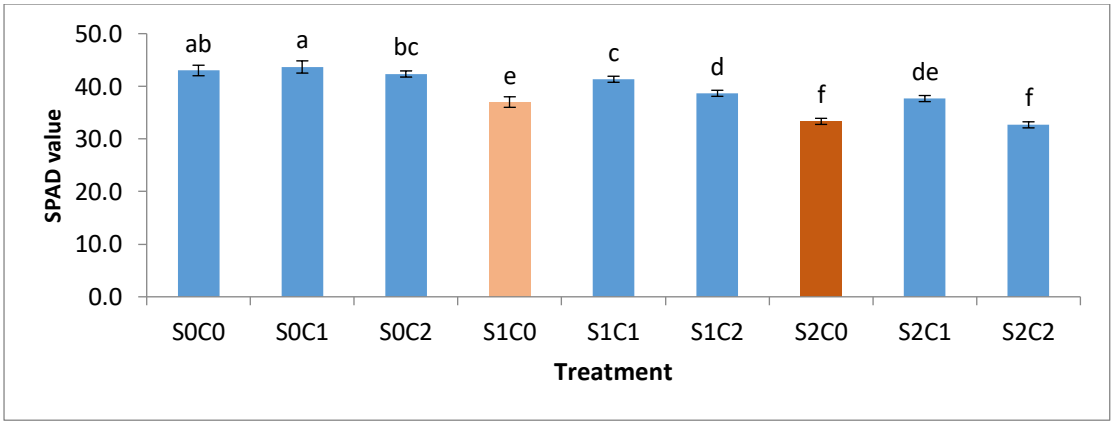

Figure 4.21. Effect of Ca on SPAD value of rice under salt stress at 55 DAS. Here, S0, S1, S2, C0, C1 and C2 indicate 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 0 mM $CaCl_2$, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$, respectively. Bars (±SD) were calculated from three replications (n = 3) for each treatment. Bars with different letters are significantly different at P≤0.05 applying Fisher's LSD test

Similar to other abiotic stresses, salt-induced stress undermines the pigment protein complex and diminishes photosynthetic pigments by escalating the activity of chlorophyllase enzyme and/or overproduction of ROS (Saha *et al*., 2010; Hasanuzzaman *et al*., 2014). In our experiment, we observed that Ca-supplementation restored chl content of the rice seedlings under salt-stress conditions. The restoration of photosynthetic pigment might be due to lower production of ROS with Ca supplementation under salt-stress conditions. This result is in agreement with the findings of previous studies in which Ca supplementation improved chl content under abiotic stress conditions as well as increase food production and growth of plant (Ahmad *et al*., 2016; Rahman *et al*., 2016).

CHAPTER 5
SUMMARY AND CONCLUSION

5.1 Summary

A pot experiment was conducted to observe the changes of morpho-physiology and growth of rice under nine different salt and Ca treatments and to find out the suitable dose of Ca against salinity stress of rice plant. The experiment was conducted at the net house of Agroforestry and Environmental Science, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh, during the period from March 2021 to May 2021. The experiment was conducted to evaluate the performance of 1 rice variety (BRRI dhan 28) under 9 different NaCl and $CaCl_2$ treatments,(S0C0) 0 mM NaCl and 0 mM CaCl2 (control), (S0C1) 0 mM NaCl and 2.5 mM CaCl2 (control), (S0C2) 0 mM NaCl and 5 mM CaCl2 (control), (S1C0) 50 mM NaCl and 0 mM CaCl2 (control), (S1C1) 50 mM NaCl and 2.5 mM CaCl2 (control), (S1C2) 50 mM NaCl and 5 mM CaCl2 (control), (S2C0) 100 mM NaCl and 0 mM CaCl2 (control), (S2C1) 100 mM NaCl and 2.5 mM CaCl2 (control), (S2C2) 100 mM NaCl and 5 mM CaCl2 (control) with 3 replications.

Collected data were statistically analyzed for the evaluation of Ca performance in rice (BRRI dhan 28) under different salt treatments. With the interaction of rice plant and salinity treatment in 25 DAS, 40 DAS and 55 DAS plant height was reduced 7 and 32.6%, 16.7 and 7.5%, 6.9 and 12.24% respectively for mild and severe salt stress. On the other hand 2.5 mM Ca control plant restores 10.9% and 6.7%, 12.8 and 7.5%, 7.6 and 5.2% respectively for mild and severe salt stress. 5 mM Ca control plant show 6.7%, 9% and 3.4% restoration of plant height only for mild stress. In case of relative growth rate, 2.5 mM Ca shows most significant result as salt stress decrease relative growth rate of 6.8% in mild salt stress and 2.5 mM Ca shows 4.9 and 4.5% restoration of relative growth rate. On the other hand 5 mM Ca control plant showed limited effect. Again for number of leaf per plant, mild and severe salt stress decrease 21.4 and 23.8%, 24.5 and 26.3%, 10 and 16.25%. And 2.5 mM Ca control restores 21.2 and 15.6%, 39.5 and 2.3%, 11.1 and 4.5% respectively. 5 mM Ca control only restore in mild stress about 12.1%, 23.2% and 1.38% respectively. In case of length of flag leaf, salt stress reduced 7.5% and 16.7%, 6.8% in severe salt stress, 5.6 and 10%

respectively. 2.5 mM Ca control restore 14.1 and 1.7%, 11.1 and 1.3%, 10.5 and 4.4% respectively on mild and severe stress in 25, 40 and 55 DAS. 5 mM Ca control shows 9.4%, 7.1% and 3.9% only on mild salt stress in regular interval. Width of flag leaf was reduced insignificantly on both salt stress and restoration is also very limited. Fresh weight of plant in 25, 40 and 55 DAS decrease 4.8 and 11.3%, 10.2 and 18.8%, 12.5 and 18.9% respectively for severe and mild stress. 2.5 mM Ca control restores 10 and 10.9%, 18.1 and 15.7%, 18.4 and 18.1% respectively for mild and severe stress. On the other hand 5mM Ca control increases 3.3%, 9.5% and 12.7% only for mild stress. In case of dry weight of plant mild and severe salt stress reduced 5.3 and 10.6%, 9.5 and 19%, 11.7 and 19.6% respectively in 15 days interval. 2.5mM Ca control restores 12.1 and 10.8%, 21 and 17%, 17.7 and 19.5% respectively for mild and severe stress. On the other hand 5 mM Ca increase dry weight of plant 3.7%, 10% and 11.1% for only mild stress in 25, 40 and 50 DAS. Mild and severe salt stress reduced 4.7% and 7.2% respectively and 2.5 mM Ca control restore 3.8% and 3.5% respectively. On the other hand 5mM Ca control restores 1.4% only in mild salt stress. Again for SPAD value of plant mild and severe salt stress reduced SPAD value by 13.9% and 22.5% respectively. 2.5 mM Ca control increase SPAD value by 11.6% and 13.2% for mild and severe salt stress. On the other hand 5mM Ca control increases SPAD value only under mild stress by 4.5%.

Considering all the findings and performance, 2.5 mM $CaCl_2$ control was the best treatment for BRRI dhan 28 under mild and severe salt stress condition.

5.2 Conclusion

Soil salinity is the most critical worldwide problem. In Bangladesh, salinization is one of the major natural hazards obstructing crop production. Coastal area in Bangladesh constitutes 20% of the country of which about 53% are affected by different degrees of salinity. Agricultural land use in these areas is very deprived due to soil salinity stress. Rice, (*Oryza sativa*), edible starchy cerealgrain and the grass plant belongs to the Poaceae family. Rice is the very important cereal crop in Bangladesh and is very sensitive to salt stress. To overcome the salinity problem, suitable soil treatment should be done. Salt stress disturbs the plant physiological activities which have negative effects on growth and other performances of rice plant. As an essential macronutrient, calcium (Ca) plays important roles to mitigate negative effect of soil salt stress. The rice seedlings with salt-induced oxidative damage recovered with Ca supplementation. Considering all the findings of the experiment it is very clear that addition of 2.5 mM $CaCl_2$ under all levels of salt stress had positive impact on plant growth and physiology. But addition of 5 mM $CaCl_2$ showed good result under 50 mM NaCl stress only. So, addition of 2.5 mM $CaCl_2$ in fertilization process could be a solution for rice production under salt stress. This dose of $CaCl_2$ might be applied in large scale in rice production for mitigating salinity stress.

RECOMMENDATIONS

- Furthermore growth and yield based researches on this similar topic should be done in future to get more accurate results.
- More researches on physiological, biochemical and molecular mechanisms of salt stress should be undertaken.

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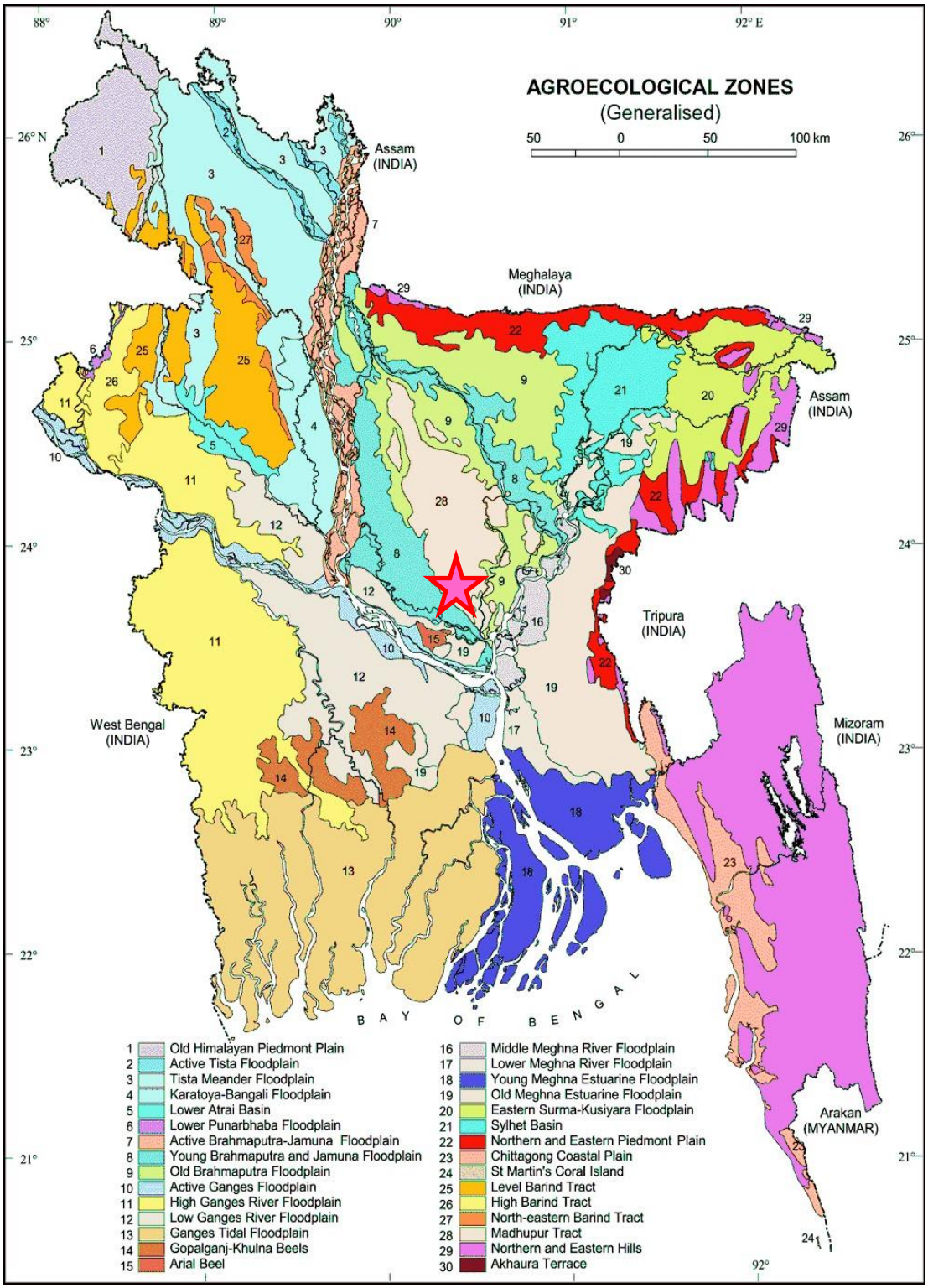
APPENDICES

Appendix 1. Map display the experimental site under the experiment



Shows the experimental site

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Appendix 2. The mechanical and chemical characteristics of soil of the experimental site as observed prior to experimentation (0 -15 cm depth).

A. Morphological properties of the soil

Morphological features	Characteristics
Location	Agroforestry farm , SAU, Dhaka
AEZ	Madhupur Tract (28)
General Soil Type	Shallow red brown terrace soil
Land type	High land
Soil series	Tejgaon
Topography	Fairly leveled

B. Physical properties of the soil

Particle size analysis	Results
Sand (%) (0.0-0.02 mm)	21.75
Silt (1%) (0.02-0.002 mm)	66.60
Clay (%) (<0.002 mm)	11.65
Soil textural class	Silty loam
Color	Dark grey
Consistency	Grounder

Source: Soil Resources Development Institute (SRDI), Dhaka

Appendix 3. Monthly record of air temperature, relative humidity and rainfall of the experimental site during the period from February 2021 to May 2021

Month	*Air temperature (^{0}C)	*Relative humidity (%)	Rainfall (mm) (total)
February, 2021	21.7	59	01
March, 2021	26.6	58	29
April, 2021	28.8	72	90
May, 2021	29.4	76	110

* Monthly average

* Source: Bangladesh Meteorological Department (Climate & weather division), Agargaon, Dhaka.

Appendix 4. Mean values of plant height on rice plant under control and salt stress treatment

Treatment	25 DAS	40 DAS	55 DAS
S0C0	28.5	45.6	67.8
S0C1	30.3	46.7	68.2
S0C2	29.3	45.8	67.4
S1C0	26.5	42.1	63.1
S1C1	29.4	47.5	67.9
S1C2	28.4	46.2	65.3
S2C0	19.2	37.9	59.5
S2C1	20.5	40.3	62.6
S2C2	18.4	37.0	59.2

SO: No salt stress; S1: 50mM NaCl; S2: 100 mM NaCl; C0: No control; C1: 2.5mM $CaCl_2$; C2: 5mM $CaCl_2$

Appendix 5. Mean values of Relative on rice plant under control and salt stress treatment

Treatment	Mean
S0C0	1.31
S0C1	1.26
S0C2	1.27
S1C0	1.22
S1C1	1.28
S1C2	1.23
S2C0	1.34
S2C1	1.40
S2C2	1.36

SO: No salt stress; S1: 50mM NaCl; S2: 100 mM NaCl; C0: No control; C1: 2.5mM $CaCl_2$; C2: 5mM $CaCl_2$

Appendix 6. Mean values of leaf number per plant on rice plant under control and salt stress treatment

Treatment	25 DAS	40 DAS	55 DAS
S0C0	4.2	5.7	8.0
S0C1	4.3	6.0	8.3
S0C2	4.2	5.7	8.0
S1C0	3.3	4.3	7.2
S1C1	4.0	6.0	8.0
S1C2	3.7	5.3	7.3
S2C0	3.2	4.2	6.7
S2C1	3.7	4.3	7.0
S2C2	3.2	3.7	6.3

SO: No salt stress; S1: 50mM NaCl; S2: 100 mM NaCl; C0: No control; C1: 2.5mM $CaCl_2$; C2: 5mM $CaCl_2$

Appendix 7. Mean values of Length of flag leaf on rice plant under control and salt stress treatment

Treatment	25 DAS	40 DAS	55 DAS
S0C0	17.97	30.8	37.5
S0C1	19.67	33.7	39.3
S0C2	18.73	32.2	38.1
S1C0	16.63	30.6	35.4
S1C1	18.97	34.0	39.1
S1C2	18.20	32.8	36.8
S2C3	14.97	28.7	33.6
S2C1	15.23	29.1	35.1
S2C2	13.07	27.9	32.7

SO: No salt stress; S1: 50mM NaCl; S2: 100 mM NaCl; C0: No control; C1: 2.5mM $CaCl_2$; C2: 5mM $CaCl_2$

Appendix 8. Mean values of Width of flag leaf on rice plant under control and salt stress treatment

Treatment	25 DAS	40 DAS	55 DAS
S0C0	0.42	0.47	0.53
S0C1	0.45	0.50	0.55
S0C2	0.43	0.47	0.53
S1C0	0.33	0.38	0.48
S1C1	0.38	0.45	0.53
S1C2	0.37	0.42	0.52
S2C3	0.28	0.32	0.43
S2C1	0.33	0.38	0.47
S2C2	0.27	0.28	0.42

SO: No salt stress; S1: 50mM NaCl; S2: 100 mM NaCl; C0: No control; C1: 2.5mM $CaCl_2$; C2: 5mM $CaCl_2$

Appendix 9. Mean values of Fresh Weight of Plant on rice plant under control and salt stress treatment

Treatment	25 DAS	40 DAS	55 DAS
S0C0	6.2	11.7	27.9
S0C1	6.8	12.9	30.8
S0C2	6.4	12.2	29.0
S1C0	5.9	10.5	24.4
S1C1	6.5	12.4	28.9
S1C2	6.1	11.5	27.5
S2C0	5.5	9.5	22.6
S2C1	6.1	11.0	26.7
S2C2	5.2	9.1	21.2

SO: No salt stress; S1: 50mM NaCl; S2: 100 mM NaCl; C0: No control; C1: 2.5mM $CaCl_2$; C2: 5mM $CaCl_2$

Appendix 10. Mean values of Dry Weight of Plant on rice plant under control and salt stress treatment

Treatment	25 DAS	40 DAS	55 DAS
S0C0	1.13	2.1	5.1
S0C1	1.24	2.4	5.6
S0C2	1.17	2.2	5.3
S1C0	1.07	1.9	4.5
S1C1	1.20	2.3	5.3
S1C2	1.11	2.1	5.0
S2C0	1.01	1.7	4.1
S2C1	1.12	2.0	4.9
S2C2	0.96	1.7	3.9

SO: No salt stress; S1: 50mM NaCl; S2: 100 mM NaCl; C0: No control; C1: 2.5mM $CaCl_2$; C2: 5mM $CaCl_2$

Appendix 11. Mean values of Leaf Relative Water Content on rice plant under control and salt stress treatment

Treatment	Mean
S0C0	96.5
S0C1	96.7
S0C2	96.9
S1C0	91.9
S1C1	95.4
S1C2	93.2
S2C0	89.5
S2C1	92.7
S2C2	89.5

SO: No salt stress; S1: 50mM NaCl; S2: 100 mM NaCl; C0: No control; C1: 2.5mM $CaCl_2$; C2: 5mM $CaCl_2$

Appendix 12. Mean values of SPAD Value rice plant under control and salt stress treatment

Treatment	Mean
S0C0	43.0
S0C1	43.7
S0C2	42.3
S1C0	37.0
S1C1	41.3
S1C2	38.7
S2C0	33.3
S2C1	37.7
S2C2	32.7

SO: No salt stress; S1: 50mM NaCl; S2: 100 mM NaCl; C0: No control; C1: 2.5mM $CaCl_2$; C2: 5mM $CaCl_2$

Appendix 13. Factorial ANOVA Table for all the growth parameters rice under control and salt stress treatment

Factorial ANOVA Table for plant height at 25 DAS

Source	DF	SS	MS	F	P
Rep	2	0.116	0.058		
Salt	2	536.878	268.439	1140.84	0.0000
Calcium	2	19.145	9.572	40.68	0.0000
Salt*Calcium	4	5.775	1.444	6.14	0.0034
Error	16	3.765	0.235		
Total	26	565.679			

Grand Mean 25.618
CV 1.89

Factorial ANOVA Table for plant height at 40 DAS

Source	DF	SS	MS	F	P
Rep	2	1.236	0.618		
Salt	2	316.649	158.324	341.81	0.0000
Calcium	2	40.936	20.468	44.19	0.0000
Salt*Calcium	4	27.276	6.819	14.72	0.0000
Error	16	7.411	0.463		
Total	26	393.507			

Grand Mean 43.222
CV 1.57

Factorial ANOVA Table for plant height at 55 DAS

Source	DF	SS	MS	F	P
Rep	2	1.425	0.713		
Salt	2	254.259	127.129	228.34	0.0000
Calcium	2	39.565	19.783	35.53	0.0000
Salt*Calcium	4	17.768	4.442	7.98	0.0010
Error	16	8.908	0.557		
Total	26	321.925			

Grand Mean 64.559
CV 1.16

Factorial ANOVA Table for plant relative growth rate

Source	DF	SS	MS	F	P
Rep	2	0.00181	0.00090		
Salt	2	0.07574	0.03787	44.94	0.0000
Calcium	2	0.00453	0.00227	2.69	0.0984
Salt*Calcium	4	0.01158	0.00289	3.43	0.0330
Error	16	0.01348	0.00084		
Total	26	0.10713			

Grand Mean 1.2980
CV 2.24

Factorial ANOVA Table for leaf number at 25 DAS

Source	DF	SS	MS	F	P
Rep	2	0.24074	0.12037		
Salt	2	3.62963	1.81481	9.39	0.0020
Calcium	2	0.96296	0.48148	2.49	0.1143
Salt*Calcium	4	0.25926	0.06481	0.34	0.8501
Error	16	3.09259	0.19329		
Total	26	8.18519			

Grand Mean 3.7407
CV 11.75

Factorial ANOVA Table for leaf number at 40 DAS

Source	DF	SS	MS	F	P
Rep	2	0.0185	0.00926		
Salt	2	13.9074	6.95370	26.82	0.0000
Calcium	2	2.5741	1.28704	4.96	0.0210
Salt*Calcium	4	2.5926	0.64815	2.50	0.0839
Error	16	4.1481	0.25926		
Total	26	23.2407			

Grand Mean 5.0185
CV 10.15

Factorial ANOVA Table for leaf number at 55 DAS

Source	DF	SS	MS	F	P
Rep	2	0.0741	0.03704		
Salt	2	9.4630	4.73148	43.03	0.0000
Calcium	2	1.6852	0.84259	7.66	0.0046
Salt*Calcium	4	0.3704	0.09259	0.84	0.5186
Error	16	1.7593	0.10995		
Total	26	13.3519			

Grand Mean 7.4259

CV 4.47

Factorial ANOVA Table for flag leaf length at 25 DAS

Source	DF	SS	MS	F	P
Rep	2	0.654	0.3270		
Salt	2	96.383	48.1915	112.74	0.0000
Calcium	2	11.210	5.6048	13.11	0.0004
Salt*Calcium	4	10.001	2.5004	5.85	0.0043
Error	16	6.839	0.4275		
Total	26	125.087			

Grand Mean 17.048

CV 3.84

Factorial ANOVA Table for flag leaf length at 40 DAS

Source	DF	SS	MS	F	P
Rep	2	1.561	0.7804		
Salt	2	87.370	43.6848	112.51	0.0000
Calcium	2	21.787	10.8937	28.06	0.0000
Salt*Calcium	4	9.984	2.4959	6.43	0.0028
Error	16	6.213	0.3883		
Total	26	126.914			

Grand Mean 31.085

CV 2.00

Factorial ANOVA Table for flag leaf length at 55 DAS

Source	DF	SS	MS	F	P
Rep	2	0.889	0.4444		
Salt	2	96.842	48.4211	173.79	0.0000
Calcium	2	28.667	14.3333	51.45	0.0000
Salt*Calcium	4	6.624	1.6561	5.94	0.0040
Error	16	4.458	0.2786		
Total	26	137.480			

Grand Mean 36.400

CV 1.45

Factorial ANOVA Table for flag leaf width at 25 DAS

Source	DF	SS	MS	F	P
Rep	2	0.00241	0.00120		
Salt	2	0.08685	0.04343	33.20	0.0000
Calcium	2	0.00963	0.00481	3.68	0.0484
Salt*Calcium	4	0.00315	0.00079	0.60	0.6668
Error	16	0.02093	0.00131		
Total	26	0.12296			

Grand Mean 0.3630
CV 9.96

Factorial ANOVA Table for flag leaf width at 40 DAS

Source	DF	SS	MS	F	P
Rep	2	0.00352	0.00176		
Salt	2	0.10241	0.05120	35.39	0.0000
Calcium	2	0.01852	0.00926	6.40	0.0091
Salt*Calcium	4	0.00593	0.00148	1.02	0.4247
Error	16	0.02315	0.00145		
Total	26	0.15352			

Grand Mean 0.4074
CV 9.34

Factorial ANOVA Table for flag leaf width at 55 DAS

Source	DF	SS	MS	F	P
Rep	2	0.00519	0.00259		
Salt	2	0.04796	0.02398	29.18	0.0000
Calcium	2	0.00574	0.00287	3.49	0.0551
Salt*Calcium	4	0.00259	0.00065	0.79	0.5492
Error	16	0.01315	0.00082		
Total	26	0.07463			

Grand Mean 0.4963
CV 5.78

Factorial ANOVA Table for fresh weight at 25 DAS

Source	DF	SS	MS	F	P
Rep	2	0.09407	0.04704		
Salt	2	3.29852	1.64926	36.69	0.0000
Calcium	2	2.21407	1.10704	24.63	0.0000
Salt*Calcium	4	0.28593	0.07148	1.59	0.2251
Error	16	0.71926	0.04495		
Total	26	6.61185			

Grand Mean 6.0741
CV 3.49

Factorial ANOVA Table for fresh weight at 40 DAS

Source	DF	SS	MS	F	P
Rep	2	0.1067	0.0533		
Salt	2	27.6867	13.8433	109.65	0.0000
Calcium	2	11.6156	5.8078	46.00	0.0000
Salt*Calcium	4	1.9178	0.4794	3.80	0.0235
Error	16	2.0200	0.1262		
Total	26	43.3467			

Grand Mean 11.211

CV 3.17

Factorial ANOVA Table for fresh weight at 55 DAS

Source	DF	SS	MS	F	P
Rep	2	6.207	3.1033		
Salt	2	151.229	75.6144	128.16	0.0000
Calcium	2	72.327	36.1633	61.29	0.0000
Salt*Calcium	4	21.204	5.3011	8.98	0.0005
Error	16	9.440	0.5900		
Total	26	260.407			

Grand Mean 26.544

CV 2.89

Factorial ANOVA Table for dry weight at 25 DAS

Source	DF	SS	MS	F	P
Rep	2	0.00987	0.00494		
Salt	2	0.10739	0.05369	39.30	0.0000
Calcium	2	0.07463	0.03731	27.31	0.0000
Salt*Calcium	4	0.00997	0.00249	1.82	0.1735
Error	16	0.02186	0.00137		
Total	26	0.22372			

Grand Mean 1.1126

CV 3.32

Factorial ANOVA Table for dry weight at 40 DAS

Source	DF	SS	MS	F	P
Rep	2	0.03336	0.01668		
Salt	2	0.92416	0.46208	123.96	0.0000
Calcium	2	0.39349	0.19674	52.78	0.0000
Salt*Calcium	4	0.06422	0.01606	4.31	0.0149
Error	16	0.05964	0.00373		
Total	26	1.47487			

Grand Mean 2.0522

CV 2.98

Factorial ANOVA Table for dry weight at 55 DAS

Source	DF	SS	MS	F	P
Rep	2	0.06976	0.03488		
Salt	2	5.03607	2.51803	146.80	0.0000
Calcium	2	2.42809	1.21404	70.78	0.0000
Salt*Calcium	4	0.70271	0.17568	10.24	0.0003
Error	16	0.27444	0.01715		
Total	26	8.51107			

Grand Mean 4.8556

CV 2.70

Factorial ANOVA Table for leaf relative water content (RWC)

Source	DF	SS	MS	F	P
Rep	2	0.245	0.1226		
Salt	2	170.010	85.0048	297.30	0.0000
Calcium	2	25.094	12.5470	43.88	0.0000
Salt*Calcium	4	14.017	3.5043	12.26	0.0001
Error	16	4.575	0.2859		
Total	26	213.941			

Grand Mean 93.581

CV 0.57

Factorial ANOVA Table for SPAD value

Source	DF	SS	MS	F	P
Rep	2	2.074	1.037		
Salt	2	321.185	160.593	299.03	0.0000
Calcium	2	56.074	28.037	52.21	0.0000
Salt*Calcium	4	19.481	4.870	9.07	0.0005
Error	16	8.593	0.537		
Total	26	407.407			

Grand Mean 38.852

CV 1.89